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FARM-, FIELD-, AND PLANT-SCALE EFFECTS ON EUROPEAN CORN BORER OVIPOSITION

A Dissertation in

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ABSTRACT

New technologies and strategies in commodity agriculture result in higher yields and quality harvests. Corn, one of the most economically important crops in the United States, is a crucial source of food, fuel, and consumer products. The European corn borer (*Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae)) threatens producers in the U.S. and Canada with more than $1 billion in annual crop damage. Many studies have investigated various aspects of European corn borer biology; however, little is known about female corn borer movement, which directly impacts where larval damage will occur. Temporal and spatial aspects of dispersal also affect pesticide resistance management and population gene flow. The development of current knowledge also requires more insight into female corn borer oviposition (egg-laying) decision-making.

This dissertation research uses results from a range of experimental scales to synthesize the description of factors that influence female European corn borer oviposition behavior. Previous research has shown that female corn borers prefer taller, more mature plants when laying eggs. Individual plant-based experiments suggested that plant leaf area is a major plant maturity factor influencing female oviposition choice. However, no solid evidence indicated that females respond to plant height when laying eggs.

First-generation European corn borers tended to oviposit on the earliest-planted corn, while second-generation females preferred later plantings. During the second-generation peak, these late plantings were in late vegetative or early reproductive stages; earlier plantings were less succulent and near physiological maturity. Although female European corn borers exhibit general plant maturity preferences, egg infestation predictions based on comparisons among available planting dates are not accurate. Infestation predictions may only be feasible with exhaustive sampling, which is impractical.

Field- and farm-scale dispersal studies showed that many newly-emerged females flew out of the immediate area before laying egg masses. These results suggested that many females fly over 500 meters, though some mate and lay eggs less than 50 meters from their emergence point. Oviposition decay by distance, a function of dispersal, is apparent within 1 acre of a mass emergence site. Beyond this local scale, however, dispersal patterns are less evident.

Analysis of larval survival among various plant growth stages suggested European corn borer oviposition preference follows optimal oviposition theory. Offspring success generally corresponded with oviposition preference. Larval survival was highest on plants infested at early reproductive stages; these were found to be most attractive to ovipositing females in the other experiments. Survival rates and larval weights were lowest in the youngest vegetative plantings. Within-plant egg mass position also affected larval success; larvae were significantly more likely to survive on plants infested at the ear zone than those placed on higher leaves.

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CHAPTER 1.

Part I: Statement of General Purpose and Motivation for Research

Recent developments in transgenic crop technology and the use of corn as fuel have led to increased research interest in one of the most economically important pests of corn, the European corn borer (*Ostrinia nubilalis* (Hübner)). While the larvae damage corn plants by chewing leaves, infesting ears, and stalk tunneling, adult females are also important components of the pest cycle because they choose where eggs are laid and subsequent damage occurs. Pest management efforts would arguably improve with increased precision in predictive modeling of European corn borer oviposition.

Spangler and Calvin (2000, 2001) found that ovipositing corn borers are choosy when offered plants in different growth stages; females also prefer certain leaves within a given plant. A natural progression from this research would move to explore the plant maturity factors that govern oviposition choice, female spatial dispersal due to oviposition site search, and the rationale behind female decision-making. This research is needed help growers improve integrated pest management programs and add to the current record of European corn borer behavior knowledge. Additional information about corn borer dispersal will help researchers fine-tune resistance management models. With the increasing adoption of transgenic *Bt* corn targeting European corn borer larvae, research efforts have focused on predicting the evolution of resistance to transgenic crops. Population gene flow, a critical component of resistance models, is directly influenced by insect movement and mating patterns.

Prior experimental designs aiming to qualify corn borer movement have depended almost solely on trapping data, which may artificially affect behavior and skew dispersal results. In addition, none of these studies addressed the role of oviposition behavior in female dispersal. As yet, no experiments or surveys have documented European corn borer egg mass dispersion on a

landscape scale of many heterogeneous fields. Moreover, the influence of field corn growth stages on oviposition preference, relative to the distance and direction from adult emergence sites, has never been addressed.

The role of individual plant maturity factors in oviposition is also poorly understood. While various plant chemicals have documented effects on European corn borer egg-laying choice, this information is based on research using extracts and leaf components; very few choice experiments have utilized whole plants. Finally, although researchers have described the nutritional requirements of European corn borer larvae, no one has ventured to relate larval development with oviposition site selection. Many of the knowledge gaps and experimental drawbacks from previous studies limit the application of research conclusions to real-world predictions of European corn borer behavior. Therefore, there is a need for more field-based, applied behavior research.

This body of work is the synthesis of several experiments to further clarify female European corn borer movement and choices related to oviposition. The aims of this research were to describe within- and among-field female movement, determine whether or not European corn borer female oviposition preference is distinct enough to predict based on plant- or field-wide factors, and establish a link between preferred plants and superior offspring development.

Therefore, the following objectives were used to address current issues in European corn borer oviposition site selection and behavior:

1. Determine which plant traits (using whole plants) and moth sensory systems influence egg mass placement
2. Track female movement across areas within and among corn fields
3. Determine if oviposition can be predicted based on the available array of corn plant stages
4. Compare plant maturity traits such as height and leaf area to see if they influence egg-laying behavior
5. Examine optimal oviposition theory and how it relates to corn borer preference

Part II: Review of Current Literature

Background and Life History

Classification and host plants. The European corn borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae), is one of the most economically significant agricultural pests in North America. Over 200 plant species are hosts of the European corn borer (ECB), including corn, peppers, cotton and snap beans (Lewis 1975, Hudon and LeRoux 1986). However, given the overwhelming impact on one crop species, this review will restrict further discussion of host plants to corn (*Zea mays* L.).

Historical background: Introduction to the United States. Vinal (1917) first reported the incidence of European corn borer moths, which were discovered in Massachusetts in 1914. Corn borer populations were also found in western New York and Ontario within the same decade (Felt 1919, Hudon and LeRoux 1986). Shipments of broomcorn from southern Europe seem to explain the introduction of the pest into eastern North America (Caffrey and Worthley 1927).

Expansion and geographic distribution. Another European corn borer population was discovered in Ohio in 1921 (Huber et al. 1928). Corn borer expansion moved westward over the next thirty years, with populations reported in Illinois in 1939, Iowa in 1942, and South Dakota in 1946 (Mason et al. 1996). Additional populations appeared in Minnesota, North Dakota, Manitoba, and Saskatchewan; others were found in southern states, such as Missouri, Arkansas, and Georgia.

Currently, the European corn borer is established throughout most of the United States and Canada. Other populations are documented in parts of Europe, northern Africa, and southwestern Asia (Hudon and LeRoux 1986).

Voltinism. Though most initial corn borer populations were reported to be univoltine, the rapid movement of the pest coincided with reports of populations with more than one generation per year (Huber et al. 1928, Hudon and LeRoux 1986, Hoard and Weiss 1995). Early work

attributed voltinism to a population's response to temperature and photoperiod, though inter-population differences in diapause induction were evident (Mutchmor and Beckel 1959, Beck and Apple 1961). More recent genetic studies suggest that sex-linked (Z-linked) genes control diapause induction (Glover et al. 1992). These Z-linked genes, along with temperature and photoperiod cues, seemingly influence voltinism (Coates et al. 2004).

Other authors have grouped populations based on voltinism characteristics (i.e., the number of degree-days required for pupation) into European corn borer "bio-" or "eco-types" (Showers 1979, Coates et al. 2004). Currently, three biotypes exist in North America: a group of univoltine corn borer populations spreading through the upper states and lower Canadian provinces; bivoltine populations extending from the mid-Atlantic states west to Nebraska and Kansas; and multivoltine populations with three or four generations per year in the lower southern states from the Carolinas to Oklahoma and northern Texas (Chiang et al. 1968, Showers 1979, Hudon and LeRoux 1986, Mason et al. 1996, Coates et al. 2004).

The boundaries between voltinism establishment are not clearly defined, and biotype ranges overlap in some areas (McLeod 1976, Mason et al. 1996). In Pennsylvania, Calvin and Song (1994) reported a mixture of univoltine and bivoltine populations in several counties. The authors noted that coexistence of two biotypes can result in continual pest emergence over the growing season and may also increase crop damage in some areas. Roelofs et al. (1985) suggested that the asynchrony of univoltine and bivoltine emergence and mating periods may reduce gene flow between the two populations.

Sex pheromone differences and genetic types. Laboratory bioassays determined that further population differentiation could be made by males' response to specific pheromone blends. Klun and Brindley (1970) first identified the European corn borer sex pheromone as *cis*-11-tetradecenyl acetate. However, other authors discovered that male corn borers responded to a pheromone blend using both *cis*- and *trans*- isomers; additionally, the ratio of *cis*- (Z) and *trans*-

(E) isomers determined male response between two populations (Cardé et al. 1975). European corn borer populations are now classified according to the pheromone strain that females produce: the *Z* race (3 percent E, 97 percent Z) and the *E* race (99 percent E, 1 percent Z).

The *Z* type predominates in corn borer populations west of Pennsylvania; individuals of the *E* race there are rare (Mason et al. 1996). In the Eastern United States, both *E* and *Z* races are present. The *Z* and *E* ranges often overlap, as shown in New York, North Carolina and several other states' populations (Roelofs et al. 1985, Glover et al. 1991, Sorenson et al. 1992, Sorenson et al. 2005). Coates and Hellmich (2003) cite evidence that hybridization between pheromone races is more common than between voltinism biotypes. Host preferences do differ between races (Pelozuelo et al. 2004), with *E* individuals found in non-corn host crops (e.g., potato and wheat) more often than *Z* individuals (Mason et al. 1996).

Life cycle and seasonal development. Each spring, overwintering European corn borer larvae respond to warmer temperatures and longer day lengths by pupating within corn stalk stubble and debris. After adult females emerge and mate, they disperse to find host plants on which to lay eggs. Once a suitable corn plant is chosen, a female will lay an egg mass of approximately five to sixty eggs on the underside of a leaf (Mason et al. 1996). After about three to seven days, the eggs hatch and neonate larvae emerge. Early instar larvae feed on leaf tissue near the leaf axil or whorl, while later instars bore into the stalk and continue their life cycle consuming the plant pith (Huber et al. 1928, Mason et al. 1996). After reaching the fifth instar, a corn borer larva either prepares for pupation or enters diapause. Diapause induction depends on biotype, as well as changes in temperature and photoperiod. Larvae from univoltine populations, which only complete one generation per year, initiate diapause when temperatures drop and day lengths shorten in the autumn. Bi- or multi-voltine individuals continue development through pupation if temperatures and photoperiods are above a threshold specific to their climate; at the

end of the growing season, however, larvae will prepare for diapause in response to cue thresholds.

Degree-day development models. Insect developmental rates depend on external abiotic factors, including minimum temperature thresholds, accumulated temperature units (degree-days), photoperiod, and humidity requirements. By documenting the physiological effects of these factors on laboratory-raised corn borers, researchers can develop reasonably accurate adult emergence predictions and growth models. Many of these models are based on degree-days, a measure of heat units accumulated above a developmental base temperature threshold. Thresholds range from 8.7°C to 14.9°C across corn borer populations and developmental stages (Calvin et al. 1991), but a value of 12.5°C is commonly used (Tollefson and Calvin 1994, Spangler et al. 2003). Degree-days are then calculated by subtracting the base threshold temperature from each mean daily temperature.

In the spring, diapausing corn borer larvae resume development when temperatures exceed the base threshold for development (Mason et al. 1996). Degree-day accumulations after 1 January then can be used in models to predict initial adult emergence, larval growth, and interactions with host plant phenology. Models already developed include climate-based phenology models for field scouting, larval growth curves, life stage development time models, and yield reduction predictions based on crop maturity (Calvin et al. 1988a, Calvin et al. 1988b, Got et al. 1996, Spangler et al. 2003). Other authors have also incorporated ecological interactions, such as predation and parasitism, into population dynamics models (Onstad 1988).

Current Issues and Pest Impact

Overall value of European corn borer pest management. Corn growers face substantial losses from crop injury, reduced yields, and control costs due to corn borer larval damage. Mason et al. (1996) estimated the annual economic impact of the European corn borer at $1 billion in North America. Recent interest in the use of corn in ethanol production continues to

raise corn acreage and prices (USDA 2006); therefore, the significance of corn borer damage is expected to increase with rising corn and ethanol demands. At the same time, care must be taken to ensure that environmental and ecological health (e.g., water quality, natural enemy diversity, etc.) do not deteriorate with pest control measures.

Traditional corn borer management tactics. European corn borer management using traditional methods is challenging; the adult moths are highly mobile, overlapping ecotype ranges can result in continual adult emergence throughout the summer, and larvae are protected from most chemical treatments after they bore into the plant (Betz et al. 2000). Historically, cultural control methods such as planting date adjustments, damage-tolerant hybrids, crop residue destruction and intercropping have been recommended (Huber 1941, Hudon and LeRoux 1986, Hudon et al. 1989); however, these can be costly and do not guarantee protection. Other tactics, including chemical and biological control methods, were developed as well.

Chemical treatments include both broad-spectrum pyrethroids and more selective insecticides. In sweet corn, selective insecticides (e.g., spinosad, indoxacarb) are generally preferred to reduce negative effects on natural enemy communities (Musser and Shelton 2003, Musser et al. 2006), but they offer reduced levels of protection. Another treatment option is to release corn borer egg parasitoids *Trichogramma* spp., (Losey et al. 1995), particularly in higher-value crops such as fresh market sweet corn (Musser et al. 2006). However, some of these methods are cost-prohibitive for large areas of field corn production.

Currently, common pest management practices in corn include pest scouting, alternating pesticides, and crop residue destruction (USDA-NASS 2001). Planting date adjustments and beneficial organism releases were used on less than 10 percent of field corn acreage in 2005 (USDA-NASS 2006). Broad-spectrum insecticide use was also rather low in 2005; less than 15 percent of all field corn acreage was treated with pesticides recommended for corn borer control (USDA-NASS 2006).

***Bt* transgenic hybrids.** Corn growers have an additional pest control option: transgenic hybrids. In 1996, transgenic corn producing the insecticidal *Bacillus thuringiensis* (*Bt*) toxin was introduced for European corn borer control. Transgenic *Bt* varieties have been hailed as safe, pest-specific and highly effective methods for insect control (Betz et al. 2000).

Transgenic corn use offers many advantages. First, hybrids expressing the Cry1Ab, Cry1Ac, or Cry1F protein toxins provide over 99% control against first-generation corn borer larvae in whorl-stage corn (Ostlie et al. 1997, EPA 2005a, EPA 2005b). Season-long expression of the *Bt* toxin in the plant allows long-term pest suppression, unlike chemical sprays that must be timed to coincide with pest activity. While *Bt* toxin activity is aimed at neonate larvae, it can also kill some fourth- and fifth-instar larvae that burrow into the corn stalk and would otherwise be protected from spray applications (Betz et al. 2000). Corn growers benefit from higher crop yields while reducing insecticides that can jeopardize environmental health.

The Cry protein toxins expressed by transgenic *Bt* corn plants are also highly selective, killing only the target pest with a low risk for natural enemies (Romeis et al. 2006). Therefore, beneficial insects can still inhabit transgenic fields and help control secondary pests (Betz et al. 2000). In addition, *Bt* corn appeals to many growers because it is easy to use and saves time. No additional equipment, treatments or techniques beyond basic cropping guidelines are required to grow transgenic varieties. Finally, *Bt* corn is often economical for both large- and small-scale farms. Calvin (1995) argues that the cost of planting transgenic corn is farm-size neutral, since seed cost per acre is the same regardless of the number of acres planted. Using European corn borer pest pressure data and current pricing information, modelers have even developed a "*Bt* Evaluation Tool" to help growers determine their projected economic returns from transgenic corn crops (Hellmich et al. 2005).

Although transgenic technology promises to improve target pest suppression, there are concerns that non-target organisms will be affected, particularly monarch butterflies (Losey et al.

1999, Dively et al. 2004). In addition, cross-contamination with non-*Bt* varieties through wind pollination is a concern. Researchers also warn that unforeseen environmental and human health hazards may arise; however, current toxicology data show no significant risks (Betz et al. 2000).

Resistance management. Many recent studies do address the one major concern with transgenic technology: the evolution of insect resistance. Because of the high efficacy of *Bt* corn, widespread adoption could lead to a high degree of selection pressure for *Bt* resistance in corn borer populations (Tabashnik 1994). This is compounded further by short generation times, which will likely speed the evolution of resistance in areas with bi- and multivoltine biotypes (Ostlie et al. 1997).

Importance of managing insecticide resistance. Genes for resistance are likely to be rare (Ostlie et al. 1997); however, just one resistant individual is capable of producing future populations that are unaffected by *Bt* toxins. If the evolution of resistance is not prevented, pest managers could lose an extremely valuable tool. Furthermore, crop production and yield pressures continue to increase with corn grain ethanol production and growing world food requirements. New pest management techniques require years of development and significant costs. Researchers anticipated *Bt* resistance from the initial introduction of transgenic technology, and numerous studies have been conducted to predict and model resistance evolution (Onstad and Gould 1998, Guse et al. 2002, Ives and Andow 2002).

Refugia regulations. One of the key factors in delaying resistance evolution is to keep resistant alleles rare (Tabashnik et al. 2003); this can be done by maintaining a susceptible portion of the population. Matings between susceptible and resistant individuals can result in offspring that are still killed by the *Bt* toxin, provided that insecticide-resistant alleles are recessive. However, if large areas are planted with transgenic crops, susceptible individuals will not be able to survive and mate with resistant genotypes. To ensure resistance infrequency in a population, therefore, a high dose/refuge strategy is recommended (FIFRA 1998, Gould 1998). A high *Bt*

toxin dose (25 times the concentration needed to kill susceptible larvae) is imposed on individuals developing in stands of transgenic corn; theoretically, the dose will cause resistance alleles to be functionally recessive (Tabashnik and Croft 1982, FIFRA 1998). Meanwhile, an adjacently-planted non-*Bt* corn refuge will sustain a population of susceptible individuals that may mate with potential resistant individuals. Both tactics working together should reduce the frequency of resistant alleles in the population, and this is thought to be effective for most cases of *Bt* crop deployment against lepidopteran pests (Tabashnik et al. 2003).

The Environmental Protection Agency (EPA) has jurisdiction over genetically engineered crops that produce pesticides. EPA guidelines mandate a minimum 20% non-*Bt* refuge requirement for acreage planted with *Bt* transgenic corn targeting the European corn borer (EPA 2005a). The refuge must be planted in an adjacent block, perimeter strip, or in-field strips (EPA 2005a). A separate refuge area ensures that larvae cannot easily move between non-*Bt* and *Bt* plants or choose which plants to consume.

Future resistance management research. The success of a resistance management program depends on grower commitment and compliance (Bourguet et al. 2005). For that reason, researchers and agricultural extension personnel have designed surveys to gauge the overall use, perception, and observance of regulations regarding transgenic crops (Pilcher et al. 2002, Wilson et al. 2005). Their objective is to supplement information for integrated pest management and resistance management programs.

Another priority for resistance management is the monitoring of localized population resistance to *Bt* corn. Various authors have continuously tested natural corn borer populations for resistance to Cry proteins since the development of transgenic corn (Andow et al. 1998, Marçon et al. 1999, Andow et al. 2000, Venette et al. 2000, Alves et al. 2006, Stodola et al. 2006).

Other studies conducted include behavioral assays to determine if adults or larvae can perceive differences between transgenic and isoline varieties (Hellmich et al. 1999). Corn borer

dispersal studies are also important for tracking the movement of potentially resistant populations. In addition, information from short- and long-range dispersal studies will help researchers assess mating patterns and gene flow between *Bt*-resistant and susceptible moths (Marçon et al. 1999, Caprio 2001, Bailey et al. 2007). Gene flow among populations is also being assessed using molecular markers (Reardon and Sappington 2007).

Sensory Systems and Behavior

Sensory systems: Visual. Literature regarding vision in the European corn borer is limited; however, Foster and Frérot (1994) report evidence of male corn borer moths using visual stimuli from vertical objects for landing after mate searching. Rothschild and Schoonhoven (1977) also showed that visual cues play a role in oviposition in other species of Lepidoptera.

Auditory. European corn borer moths have a pair of tympanal organs on the abdomen (Agee 1969); these are ostensibly used for avoiding echolocating bats (Belton and Kempster 1962). Agee and Webb (1969) demonstrated that corn borer moths avoided light traps emitting pulsed ultrasonic waves. Belton and Kempster (1962) also found fewer egg masses in corn plots set up with ultrasonic transducers.

Olfactory. Many herbivores utilize plant chemicals for host recognition. Although specialist herbivores depend on specific phytochemicals to find their preferred hosts, generalists like the European corn borer also use chemical stimuli to locate plants suitable for larval growth (Udayagiri and Mason 1995). Corn borer females should be selective during oviposition site searches, as their offspring develop on plants within 100 cm of egg mass placement (Ross and Ostlie 1990).

European corn borers respond to both volatile and non-volatile chemical compounds (Marion-Poll et al. 1992). Volatile chemical cues seem to play a role in host selection during flight or shortly after landing (Schurr and Holdaway 1970). Schurr and Holdaway (1970) found

that vapors from both mechanically-injured and corn borer-damaged plants repelled ovipositing females. This suggests females search for healthier plants for their offspring.

Specific volatile compounds affecting oviposition include farnesene and humulene (oviposition attractants) and farnesal and farnesol (oviposition deterrents) (Binder et al. 1995, Binder and Robbins 1997). Dittrick et al. (1983) also found oviposition-deterring chemicals in larval frass. Results from these assays indicate that two volatile mechanisms may act as deterrents: (1) short-term volatiles released by damaged plants and (2) long-term, low-volatile emissions from larval frass (Dittrick et al. 1983).

Gustatory. Once a female corn borer lands on a potential host plant, she examines the leaf surface with her tarsi and ovipositor; these are packed with mechanoreceptors and contact chemoreceptors (Marion-Poll et al. 1992). The mechanoreceptors likely examine leaf surface structure, while contact chemoreceptors assess leaf exudates, microorganisms, and various chemical cues present in the epicuticle (Marion-Poll et al. 1992, Martin et al. 1993, Udayagiri and Mason 1997). Epicuticular chemicals found to stimulate oviposition include several *n*-alkanes as well as pentane extracts of corn leaf, tassel, and silk tissue (Udayagiri and Mason 1995, Udayagiri and Mason 1997). Derridj et al. (1986, 1989) also provide evidence that carbohydrates on the leaf surface influence egg-laying. Finally, pheromones present in corn borer egg masses also deter conspecific females, apparently limiting intraspecific competition between their offspring (Thiéry and Le Quéré 1991).

Hygroreception. Adult moths may also be able to detect changes in relative humidity, though no investigations regarding potential hygroreceptors have been conducted for the corn borer. Hunt et al. (2001) observed corn borer preferences for irrigated field corn, and many other researchers share anecdotal experience with relative humidity and moisture influencing European corn borer movement. Godfrey and Holtzer (1991) noted a strong relationship between egg hatch

and relative humidity. Therefore, by preferentially laying eggs in more humid fields, females may increase offspring survival.

Flight Behavior and Movement

Mating patterns. Once adult moths emerge from their pupation sites in corn stalks, stubble, or debris, they fly to areas of dense vegetation near or within cornfields (Showers et al. 1976, DeRozari et al. 1977, Sappington 2005). Researchers refer to these locations as action or aggregation sites (Showers et al. 1980, Sappington and Showers 1983). DeRozari et al. (1977) cite the presence of dew (i.e., free water) in dense weedy areas as a stimulus for female sexual activity. Greater numbers of females emit sex pheromone at higher relative humidity levels, and for longer periods of time (Webster and Cardé 1982, Royer and McNeil 1991).

Mating activity peaks around midnight (Showers et al. 1976), though males require low levels of light and slight wind to find "calling" (i.e., sex pheromone-emitting) females (Loughner and Brindley 1971, DeRozari et al. 1977). Groups of males seem to find calling females better than single males (Klun and Graf 1997); the authors explain this behavior as heightened competition when limited numbers of females are present.

Even though previously-mated males confer smaller spermatophores (resulting in reduced fecundity and fertility for their successive mates) (Royer and McNeil 1993), females prefer non-virgin males as mates (Schlaepfer and McNeil 2000). Schlaepfer and McNeil (2000) attribute this choice to evidence that such males are actually higher-quality mates. If a female initially selects a non-virgin mate, she may choose to mate with other males, as multiple matings improve female fecundity and fertility (Fadamiro and Baker 1999).

Dispersal. After mating, females fly out in search of potential host plants for oviposition. Once the moths leave an action site, they rarely return (Showers et al. 1976, Sappington and Showers 1983, Reardon et al. 2006); however, males may stay a bit longer, due to the lingering sex pheromone from unmated females (Sappington and Showers 1983).

Most authors studying European corn borer flight have concluded that adult moths disperse long distances, often hundreds of meters or more in a single night (Belton and Kempster 1962, Showers et al. 2001, Qureshi et al. 2005, Bailey et al. 2007, Reardon et al. 2007). Showers et al. (1995) also claim that bivoltine populations in the Midwest can move at least 32 km in a single year.

Oviposition site search and host choice. On warm nights, female moths search for oviposition sites from dusk until midnight; oviposition activity peaks around 10 PM (Schurr and Holdaway 1966). Females may use various visual and chemical cues (e.g., plant height, leaf reflectivity, or volatile profiles) to orient toward suitable corn plants. Females may also be able to sense relative humidity levels, as Hunt et al. (2001) reported a preference for irrigated corn.

Some of the earliest literature on host preference cited taller plants as more attractive to ovipositing females (Huber 1939, Everly 1959). However, this preference may simply be a response to plant maturity, as maturity and height are naturally correlated (Beard 1943, Spangler and Calvin 2000). Corn borer females even discriminate between sites on a single plant, as most egg masses are distributed just around ear level (Sorenson et al. 1993, Spangler and Calvin 2001).

Other authors investigated the effects of intra- and interspecific competition and tillage practices on corn borer egg-laying choices. Ovipositing females avoid plants infested with aphids (Harmon et al. 2003), conspecific egg masses (Thiéry and Le Quéré 1991) and corn borer larval frass (Dittrick et al. 1983). Females are probably choosy to help their offspring avoid competition. Microclimate effects also seem to play a role in oviposition choice, as suggested by Andow and Ostlie (1990). The authors observed an oviposition response to various tillage methods, which may be explained by differential temperature and/or humidity in the plant canopy.

Optimal oviposition. European corn borer oviposition preference for various plant and field factors has been well-documented, but the reasons behind egg-laying choices are not always clear. Optimal oviposition theory, or the preference-perfomance hypothesis (Jaenike 1978) is

based on the premise that females choose host plants that are most suitable for larval development. In the absence of parental care, choice of an ideal plant can increase female fitness by providing offspring with a nutritious food source and/or protection from natural enemies and pathogens. Oviposition choice may additionally depend on female age, egg load, and the probability of finding a better plant. Leather and Awmack (2002) also presented evidence that some species can adjust egg size relative to plant suitability.

Gaps in Current Research. Females' oviposition site choice is an important aspect in the eventual spatial distribution of egg masses within a corn plant, field, and landscape. Females make choices individually, yet egg-laying preferences common across a population can shape spatiotemporal egg deposition trends. This has been shown in other lepidopteran species. A study by Wiesenborn and Trumble (1988) examined oviposition by the corn earworm, *Helicoverpa zea*, between several large fields of varying plant maturity. The authors used stepwise regression to show the impact of various field effects (size, maturity, field separation distance, acres of available older corn, etc.) on egg mass densities. A different group also constructed an ovipositional preference matrix for *H. zea* based on egg-laying responses to different host species and phenological stages (Johnson et al. 1975).

Similar studies on European corn borer egg mass dispersion in a landscape would aid researchers in examining oviposition site selection on a larger scale, as well as help farm managers more precisely predict areas susceptible to crop damage. Previous research has examined the impact of individual factors (e.g., transgenic varieties, planting date, irrigation), but no studies have compared European corn borer egg deposition across a large configuration of fields of varying plant traits. To fully understand this, adult emergence sites and their relationship with the spatial pattern of egg masses at different scales should also be studied.

Insecticide resistance management models could be improved considerably with information regarding oviposition choices relative to landscape structure. Additionally, typical

female flight patterns are unclear; this has been tested with light trap captures, but flight range in relation to oviposition can help IRM modelers determine how refuges should be structured in relation to *Bt* plantings, and clarify the effect local and landscape movement has on population resistance to transgenic crops.

Oviposition behavior could also be manipulated to act as a substitute mode of action if corn borer resistance to transgenic crops seems imminent. Onstad and Buschman (2006) assert that oviposition deterrence acts as a "toxin" because it causes the loss of eggs. Alternatively, planting dates or field treatments that are deemed attractive to moths could be used in refugia to draw in ovipositing females and remove the resistance pressure found in *Bt* fields. However, more precise information is needed about the strength of attractive factors across multiple fields and how oviposition is affected by different plant maturities over time and space.

Other gaps in knowledge about European corn borer oviposition choice involve the reasons behind female decision-making. Corn borers tend to choose more mature plants, particularly when only vegetative stages are available. However, it is unclear which plant traits (e.g., height, leaf area) impact oviposition choice. Finally, no studies have examined the preference-performance hypothesis in the European corn borer and determined if oviposition choice follows plant suitability for their offspring.

Part III: Summary of Thesis Objectives

European Corn Borer Dispersal and Oviposition Choice Effects on Egg Mass Density at Different Scales

The purpose of this research was to characterize European corn borer oviposition choices across fields with different traits. Studies were conducted at plant-, field-, and farm-level scales to determine how females compare oviposition sites and how dispersal trends shape egg distribution patterns over time and space. Egg deposition across several planting dates was compared with an oviposition preference model to see if egg mass densities follow predictable patterns. Choice

experiments were also used to determine which plant maturity traits (height, leaf area) affect oviposition behavior. Finally, measures of larval success (e.g., weight, survival) were analyzed from various plant maturities and vertical leaf placement treatments to discover whether European corn borer oviposition choices follow optimal oviposition theory.

Chapter 2: Field-level analysis of egg mass deposition. The first study was designed to quantify the relative importance of corn growth stage, distance from emergence site, and direction from a release site at a small plot scale. Dispersal was measured via egg mass deposition by marked moths that were released from a central action site. The surrounding study site was designed so the distance and directionality of observed egg masses could be tested. To confirm plant maturity preferences shown by Spangler and Calvin (2000), three planting dates were used in the plot layout. The spatial aspect of dispersal and oviposition choice was also studied. Wind direction effects on dispersal were examined; egg mass deposition was also tested for spatial association. The data were then fitted to an exponential-decay dispersal model.

Results from this chapter examined local egg deposition trends in relation to plant maturity and distance from an emergence or aggregation site. The data also provided insight on the directionality and flight range of ovipositing females. This will help future insecticide resistance research and modeling by providing more detailed information about flight propensity for refugia guidelines; currently, the EPA requires refuge plantings within 800 m of transgenic *Bt* corn. If the spatial range of a typical female is estimated to be significantly less or greater than 800 m, then refuge requirements could be changed to ensure adequate gene flow between *Bt* and non-*Bt* fields.

Chapter 3: Landscape-level analysis of oviposition choice. After exploring small-scale dispersal effects in corn borer oviposition, similar questions were asked at a larger scale. This chapter summarizes results from a larger farm-wide study that examined the spatial distribution of egg masses within a heterogeneous landscape. The data gathered show which fields in a

landscape are most attractive to ovipositing corn borers. Factors such as field size, plant maturity, plant height, and leaf area were considered. The spatial structure of the field layout was also important in determining if inter-field distances have a meaningful effect on corn borer dispersal and subsequent egg-laying. These data were also used, in combination with a smaller planting date study, to assess the predictive power of an oviposition preference model developed by Steve Spangler and Dennis Calvin.

Chapter 4: Plant-level factors influencing oviposition. Several field cage, growth chamber, and field plot experiments were conducted to investigate maturity-based factors that seem to influence oviposition. Plant height and leaf area naturally increase with plant maturity, which confounds studies that assume one factor solely affects female preference. The purpose of this chapter was to help clarify individual plant-based effects that may attract females: plant height and leaf area, or visually imperceptible attributes like changing volatile profiles and microclimate effects. Data from a field plot study also addressed the effect of plant maturity, leaf area, and density on oviposition at a larger scale.

Chapter 5: Oviposition choice as part of the optimal oviposition theory. This chapter aimed to relate field data to the preference-performance hypothesis. Two experiments were designed to test plant maturity effects on larval survival and weight. One study additionally investigated whether or not vertical egg mass placement (i.e., number of leaves above and below the ear) affected larval development. Other factors, such as larval level, time to complete development, and parasitism rates were compared between planting date and leaf level treatments.

Additional chapters. The final chapter (6) serves as a summary of the implications of the thesis objectives and relevant corn borer literature on applied entomology and integrated pest management.

Appendix A reports results from observations of lab-reared, dyed corn borer moths. Internally dyed corn borer females have been used in a type of “mark-release-recapture egg mass”

method. Although the dye used has not been shown to affect adult vigor or flight (Ostlie et al. 1984), there is a concern that dyed egg mass color intensity may decrease with female age; that is, egg masses laid later may be lighter in color and less distinguishable from white masses laid by wild moths. The results may help researchers gauge the success of recaptures in future field experiments.

Appendices B and C are included as companions to Chapters 3 and 4. Appendix B summarizes calculations made from an oviposition preference model created by Steve Spangler and Dennis Calvin. Appendix C is a collection of regressions of whole-plant and individual leaf areas which were used in analyses of plant leaf area effects on oviposition. Sample statistical code for selected analyses is also included in Appendix D.

CHAPTER 2.

Local Dispersal and Oviposition Choice of Marked European Corn Borer Females

Introduction

The European corn borer, *Ostrinia nubilalis* (Lepidoptera: Crambidae), causes severe damage to North American corn crops. Estimated losses from European corn borer damage exceed $1 billion annually in the United States and Canada (Mason et al. 1996). In 1996, a major tool for European corn borer pest management was introduced: transgenic corn expressing the toxin *Bacillus thuringiensis* kurstaki (*Bt* corn). *Bt* corn has been widely grown because of its high efficacy, ease of use, and apparent environmental and human safety (Betz et al. 2000). However, researchers have expressed concern about the possible effects of excessive *Bt* corn usage on insect resistance to the *Bt* toxin.

In areas outside of the Cotton Belt, such as Pennsylvania, an Insecticide Resistance Management (IRM) plan governed by the Environmental Protection Agency (EPA) mandates a minimum 20% non-*Bt* corn refuge planting within 800 meters of a *Bt* cornfield (EPA 2005a). This IRM strategy aims to slow the spread of resistance to the *Bt* toxin by offering a refuge for the survival of susceptible insects, in the hope that they will mate with resistant individuals that may arise from the transgenic corn. Theoretically, this plan should delay European corn borer resistance provided that resistance genes are rare and recessive, and plant-expressed *Bt* toxin levels are sufficiently high to kill partially resistant individuals. However, the success of this IRM plan ultimately hinges on dispersal and mating patterns; if resistant insects fail to mate with susceptible individuals, instead pairing with other *Bt*-resistant insects, population resistance could develop much more quickly. Post-mating dispersal is also an important consideration in

resistance models, as gravid females choose where their progeny will develop (i.e., *Bt* versus non-*Bt* corn).

Several research teams have created models to predict the onset of resistance in European corn borer populations; for examples, see Onstad and Gould (1998), Caprio (2001), Guse et al. (2002), and Ives and Andow (2002). Other groups are continually testing feral moth populations for possible resistant individuals or genes (see Andow et al. 1998, Marçon et al. 1999, Andow 2000, Venette et al. 2000, Alves et al. 2006, Stodola et al. 2006). Numerous major gaps in current knowledge, and therefore resistance model design, involve European corn borer mating patterns, dispersal, and spatial dynamics. No published studies have definitively confirmed the distribution of ranges that European corn borer females move to mate or oviposit. Consequently, flawed model assumptions made about such movement could result in highly inaccurate predictions about the rate of *Bt* resistance development.

Much of the recent published literature describing individual European corn borer movement has utilized traps (e.g., blacklight and pheromone) or sweep net/flush bar sampling to determine flight range or settling preferences (Showers et al. 2001, Qureshi et al. 2005, Reardon et al. 2006). A danger lies in trapping methods, as artificially luring an insect via light or sex pheromone may cause it to deviate from normal flight behavior. Furthermore, sex pheromone traps cannot be used to track female European corn borer movement. One method that can show female spatial dynamics is the mark and release of lab-raised, colored moths. The moths are fed a diet impregnated with oil-soluble dye, which then stains the eggs of adult females (Qureshi et al. 2004a) and allows in-field detection of released female oviposition behavior.

After emerging as adults, European corn borer females and males fly to weedy "aggregation" sites where they congregate and/or mate (Showers et al. 1976, DeRozari et al. 1977, Sappington and Showers 1983). These humid areas of dense vegetation also provide free water to drink, which is essential for egg maturation (Kira et al. 1969). Once mated, females fly in search

of suitable plants on which to oviposit. Observed egg mass distribution and density at a site could be attributable to many factors: population numbers, the number of eggs laid by females, sampling frequency, length of time between oviposition and egg hatch, host plant characteristics, microclimate/weather effects, interactions with other animals, and females' flight behavior (Lee 1988, Andow and Ostlie 1990, Spangler and Calvin 2000, Hunt et al. 2001, Harmon et al. 2003).

In sweet corn, females generally prefer more mature plants for oviposition (Beard 1943, Andrew and Carlson 1976, Spangler and Calvin 2000). A study by Hunt et al. (2001) also concluded that females prefer to oviposit and settle in irrigated fields, a preference likely due to higher humidity levels. As yet, no preference between *Bt* versus non-*Bt* corn has been reported (Hellmich et al. 1999).

Currently, much is unknown about local (i.e., within-habitat) and long-range female European corn borer dispersal. This study was designed to improve knowledge about local movement and host selections at a field scale. A mass release of colored moths was used to evaluate female dispersal, measured via oviposition, as influenced by plant maturity, distance, and wind.

Materials and Methods

Released Insect Colony. Dyed pupae were provided by the USDA-ARS Corn Insects and Crop Genetics Research Unit in Ames, IA. Their European corn borer colony was established by collecting adults using black light traps in areas surrounding Ames, IA in 2003 and 2004. Trapped adults were second-generation, bivoltine moths typical to the Midwest (i.e., *Z*-type individuals producing/responding to a pheromone ratio of 97:3 *cis*:*trans*-11-tetradecenyl acetate). The colony was reared using methods similar to those described by Raun (1961) and Guthrie et al. (1965).

To facilitate identification of released moths' egg masses in the field, the larvae were raised on diet containing blue or red dye. After consumption, the dyes (Sudan Blue II, C.I. 306436 and Sudan Red 7B, C.I. 201618; Aldrich, Milwaukee, WI) stain the fat bodies of the larvae; dyed individuals retain coloration through adulthood (Qureshi et al. 2004a). Ostlie et al. (1984) reported reduced European corn borer larval survival and/or delayed development on diets impregnated with Sudan Blue and Red dyes. However, the authors found no negative dye effect on adult longevity or flight duration. Hunt et al. (2000) experimented with lower concentrations of Sudan red and blue dyes and found that the resulting egg masses were satisfactorily stained; no adverse effects on larval survival, development time, or adult life span resulted from the dyes. Therefore, dye treatments appear suitable for adult European corn borer dispersal studies.

Dye concentrations recommended by Hunt et al. (2000) were used with a base wheat germ meridic diet preparation detailed by Lewis and Lynch (1969). After hatching, larvae were transferred to dishes containing the colored diet and reared in an environmentally controlled chamber at 27°C and 80% relative humidity with a photoperiod of 16:8 (L:D). Waxed corrugated cardboard rings were laid over the dishes of feeding larvae. After their final molt, the larvae crawled up inside the cells in the cardboard rings to pupate. Once colonized with pupae, the rings were shipped to Pennsylvania via overnight courier. Each ring contained between 500 and 1000 pupae.

Upon delivery, the cardboard rings were placed in a growth chamber (Percival Scientific, Boone, IA) set at 25°C with a photoperiod of 16:8 (L:D) until the field release the following day. One ring was placed separately in a 25 cm x 25 cm x 25 cm wood-frame, wire mesh cage. A cotton wick was placed in a plastic vial filled with a honey-water mixture and hung in the cage as a moisture and energy source for the adult moths. The cage was sprayed with distilled water, wrapped in clear plastic sheeting and placed in the growth chamber. Daily counts of emerging females and males were taken to determine an approximate sex ratio for the released moths.

Several mated, two-day-old females were also removed from the communal cage and isolated in individual cylindrical wire mesh cages to track variation in females' egg mass coloration. See Appendix A for details and analysis of intra- and inter-individual variation in egg color intensity.

2004 Experiment. A 0.7-hectare (1.72-acre) experimental field site was selected at the Russell Larsen Experimental Field Station at Rock Springs, Pennsylvania. The field was located in an area of Andover variant loam soil previously in sod, adjacent to a fenced-in strip of forest at the base of Tussey Mountain. A 0.5-acre portion of the southwestern section of the field had been cultivated the previous year and planted with peppers. The mountain was located SSW-S-SSE-SE in relation to the field, with all local fields extending west-, north-, and eastwards. Most of the surrounding area consists of Hagerstown silt loam soils.

The field was marked out as a hexagonal configuration of seventy-two plots (Fig. 2.1). A hexagon was used to approximate a circular field for directional analysis of moth dispersal and wind data. The plot layout formed a split-split plot design with six directions, four distances and three planting dates. Each direction constituted a block; within each block, there were four distance factors (sub plot). One of three planting dates (sub-sub plot) was randomly assigned to a plot within each distance and direction (Fig. 2.1). The directional blocks were assigned letters A, B, C, D, E, and F. The distance factors were grouped into four "rings", each identified by its distance from the field center (15, 24, 34, or 43 m; mean distance measured from plot centroids). Plot dimensions and planting specifications are summarized in Table 2.1.

A center hexagonal section of 290 m^2 was uncultivated and left as a mix of weedy grasses, such as quackgrass (*Agropyron repens*), barnyardgrass (*Echinochloa crusgalli*), and foxtail (*Setaria* spp.); the grassy area was intended to act as an aggregation site to facilitate mating before the released moths flew out from the central release. A 0.6 m x 0.3 m x 0.6 m covered, wood-frame release cage was set in the center of the grassy release area. The corrugated cardboard rings of marked pupae were hung on a polyvinyl chloride pipe that was suspended in the interior of the

cage. The sides and bottom of the release cage were covered with 1.25 cm open-hole plastic mesh to protect the rings from predators while allowing dispersal of moths from the cage. A weather station was also set at the center of the release site (Vantage Pro2; Davis Instruments, Hayward, CA), with its anemometer set at 3 m. The weather station unit was programmed to record median temperature, relative humidity, wind speed, wind direction and precipitation measurements at 15-minute intervals.

On 28 April, herbicides (glyphosate + 2,4-D LVE + nonionic surfactant) were applied at respective rates (3.5 + 1.2 + 0.6 liters/ha) to burn down the existing sod in preparation for no-tillage planting. Starter fertilizer (10-30-10 at 112.0 kg/ha) was applied in-furrow as 0.76 m (30-inch) rows were marked on 7 May; each plot contained 12 rows. A 101-day, non-*Bt* field corn hybrid (DeKalb DKC51-43) was planted on 7 May in all plots assigned to the first planting date. Corn was planted using a six-row planter for distance factors B, C and D and hand planters for distance factor A. Seeds were planted at a density of 28,531 per acre (70,472 per ha) and a depth of 1.5 inches (3.8 cm). Table 2.1 gives the approximate plant populations for each plot. Plots assigned to planting dates 2 and 3 were planted on 23 May and 4 June, respectively. All plots also received a preemergence application of herbicides with a urea-ammonium nitrate carrier at 205.7 liters/ha (1.8 + 1.8 + 1.8 + 0.6 liters/ha of s-metolachlor 78.2%/flumetsulam 3.2%+ atrazine + paraquat + nonionic surfactant) on 11 May.

Six Hartstack wire-mesh cone pheromone traps were set 3 m from the field perimeter to monitor directional male European corn borer movement. One trap was centered at the edge of each directional block, with the base set 1 m from the ground. *Z*-type European corn borer synthetic pheromone was prepared in the laboratory with *cis*- and *trans*-11-tetradecenyl acetate (C.I. P6040-95, P6042-80; Bedoukian Research, Danbury, CT) according to instructions from Mason (1995). Rubber stoppers (Wheaton red 5mm, C.I. 1780-K02, Thomas Scientific, Swedesboro, NJ) were impregnated with a 100 µg load of pheromone per stopper. One stopper

was secured at the base of each cone trap; lures were changed every two weeks, and each trap was checked twice each week.

The laboratory-raised, colored European corn borer moths were released on 12 June, 2 July and 3 August 2004. Releases were made by hanging eight or nine rings of pupae (ca. 4000 to 8000 insects) in the release cage. Each release group alternated between red and blue. After all viable moths emerged, the rings were removed and torn apart, and empty pupal cases left in the cells were counted to determine adult emergence (Table 2.2).

Beginning on 17 June 2004, plants in all plots were sampled twice per week (when possible), with a two or three day sampling interval. Sampling continued until 26 August, for a total of 18 days. On each sampling date, twenty plants per plot were selected at random (one or two plants per row) and examined thoroughly for European corn borer egg masses. The number of eggs per mass, plant stage, vertical leaf location (leaf number from bottom, 1-20) and mass color were recorded. Each egg mass was circled and dated with a permanent marking pen to ensure masses were not inadvertently re-counted.

Five plants from each planting date were flagged; these were used to determine the median plant stage for each planting date treatment on each sampling day. Plant phenological stages were classified according to characteristics described by Ritchie et al. (1992). Vegetative stages (V1-18) were identified by the number of leaves with visible leaf collars until full tassel exposure (VT). Reproductive stages were characterized by ear development from silk emergence (R1), blister-type kernels (R2), milky-white kernels (R3), doughy kernels (R4), and dented kernels (R5).

2005 Experiment. The same field site was used in 2005, though planting date assignments were re-randomized in each directional block and distance factor (Fig. 2.1). Block and distance letter designations remained the same. All plots received preemergence herbicide applications on 29 April (glyphosate at 2.3 liters/ha) and 6 May (s-metolachlor + isoxaflutole +

compatibility agent + nonionic surfactant; urea-ammonium nitrate solution carrier). Plots were marked with in-furrow fertilizer applications (10-30-10 at 112.0 kg/ha) on 2 May. The planting rate and depth were the same as those used in 2004. Planting dates 1, 2 and 3 were on 5 May, 19 May and 2 June 2005, respectively. A soil insecticide (tefluthrin) was also applied to all plots at planting to reduce damage from western and northern corn rootworm larvae (*Diabrotica virgifera virgifera* Leconte; *D. barberi* Smith & Lawrence).

Dyed moths were released on 19 June, 13 July, 29 July and 12 August 2005. Plants were sampled on 13 dates from 21 June to 22 August. Wire pheromone traps were replaced with white plastic mesh cone traps; however, these data are not included because male captures from the new traps were extremely low. All other planting, weather data collection, egg mass sampling, and laboratory methods were unchanged from those used in 2004.

Statistical Analysis. In the laboratory, European corn borer females tend to exhibit local movement and dispersal relating to oviposition behavior in the early evening hours between dusk and midnight, with activity peaking around 10pm (Schurr and Holdaway 1966). Assuming similar timing of females' oviposition site searching in the field, only daily wind direction data between 8pm and 12am were used. The median wind speed at the corn canopy during this period was 0.9 mph (1.4 kph) over both years, and rarely exceeded 10 mph (16 kph). One-minute wind direction data from the Rock Springs Automated Surface Observing System (ASOS) (All Weather Inc.; Sacramento, CA) were substituted on days when the weather station failed to record data (11 and 20 days in 2004 and 2005, respectively). The ASOS was located 1.5 km northeast of the in-field weather station. Wind direction degrees were re-oriented with 0° as north and 90° as west. Daily mean wind directions were determined using circular statistics methods described by Batschelet (1981). Days with insufficient winds to determine wind direction (<0.4 mph) were discounted. Wind direction differed between the ASOS and in-field station by an average of 29.5° (median difference of sample daily mean directions collected at both sites; $N = 34$). A Rayleigh test

(Batschelet 1981) was also applied to each year's wind direction data to determine if wind direction differed significantly from randomness. The same tests were used for analysis of egg mass directionality within the hexagonal site. Egg masses were identified by their plot locations, which were divided into 18 20-degree sectors (three sectors per directional block).

A log-link Poisson regression model was used to determine the effects of planting date, direction, and distance from center release on egg mass counts (PROC GENMOD, SAS Institute 2004; GLM function, R Development Core Team 2008). Due to the sparseness of egg mass data, yearly total mass counts for each plot were used. A zero-inflated Poisson model was also fitted to the data using the *zicounts* function in the R statistical program (Mwalili 2008). The fits and coefficients of both models were similar, so a zero-inflated model was not necessary; moreover, the residual deviance for the Poisson model showed no evidence of overdispersion. Separate models were fitted to the following 7 groups: 2004 colored, uncolored and combined (colored + uncolored) egg masses; 2005 colored, uncolored and combined masses; all combined masses from both years. Mean wind direction was also incorporated into the Poisson regression models to explain directionality of egg mass deposition. In this case, the plot direction factor was replaced by a downwind covariate, which consisted of the proportion of days each plot spent downwind from the center (i.e., proportion of days that the mean daily wind direction flowed from the center and over the plots in each 20-degree sector). The same analyses were also done with an upwind covariate (i.e., the proportion of days that the mean daily wind direction flowed from the outer plot edge toward field center).

Combined (colored + uncolored) egg mass data from each year were also used as a response in analyses examining the effects of planting date on oviposition at different times of the season. Mass counts were summed within each planting date across days from the periods of 17 June to 7 July (early-season), 8 July to 30 July (mid-season), and 31 July to 26 August (late-season). A contingency table was constructed with mass totals partitioned according to year, time

of season (early, mid, or late) and planting date. PROC FREQ (Stokes et al. 1991, SAS Institute 2004) was used to obtain the Mantel-Haenszel correlation statistic for the association between planting date and time of season. A three-way saturated log-linear model was also fitted with year, time of season, and planting date explaining variation in combined egg mass counts (PROC CATMOD & PROC GENMOD, SAS Institute 2004). The saturated model was used due to significant interactions and poor model fits from more parsimonious models. Cumulative logits of counts by planting date were also modeled with an ordered logistic regression using the proportional odds model (PROC LOGISTIC, Stokes et al. 1991).

To model the effect of distance on oviposition, exponential decay curves were fitted to egg mass distances from the release point. To do this, a grid of plot corner coordinates was first plotted in R. Next, the plot centroid coordinates were taken from all plots and used to calculate the distance from each plot center to the moth release point. Specific within-plot egg mass locations were not available, so masses were identified according to their respective plot centroid's distance from the release. An exponential decay model of the form described by Frampton et al. (1942)

$$u = ae^{-br} + \varepsilon \qquad (2.1)$$

where
a = number of egg masses in plot at $r = 0$
$-b$ = rate of exponential decay
r = plot distance from center (m)
u = predicted number of egg masses present in each plot

were estimated using PROC NLIN in SAS (Freund and Littell 2000). PROC NLIN uses Gauss-Newton iterative methods to calculate least squares estimates of nonlinear model parameters (SAS Institute 2004). Initial coefficient estimates were made visually with a scatterplot of egg mass counts at each plot distance. Observations with residual absolute values greater than 5 were eliminated to improve model predictions and obtain more conservative estimates for curve intercepts.

Two analyses were used to determine if plot adjacencies affect plot mass counts (i.e., if larger continuous areas of the same planting date are more attractive than small, isolated patches). The first used a log-link Poisson regression (PROC GENMOD, SAS Institute 2004) to assess the effect of plot adjacency layout on egg mass deposition. Adjacent plots were identified as those that were part of a group of two or more plots of the same planting date sharing a common border. Total plot egg mass count was used as a response variable in the model, with year and number of adjacent plots (n = 0, 1, 2, 3, 4, or 6) serving as explanatory variables. The second analysis used the Moran's I statistic, an indicator of spatial association among lattice data (Banerjee et al. 2004). Moran's I multiplies a covariance term from neighboring plot values by an adjacency matrix **W**. The statistic is generally supported on the interval [-1,1], with positive and negative values indicating positive and negative spatial autocorrelation, respectively (O'Sullivan and Unwin 2003). The weights matrix **W** was constructed with all possible plot adjacencies. If plots i and j were adjacent (as defined above), $w_{ij} = 1$; if not, $w_{ij} = 0$.

Results

An approximate sex ratio of 0.82 (female/male) was observed from the sample rings kept in the growth chamber; this was used to estimate the number of adult females released in the field (Table 2.2). The total estimated number of released females approached 7400 in 2004 and 9400 in 2005. In 2004, only 61 colored egg masses were found on the 25,920 plants examined during the season. Forty-nine colored masses were found on the 18,720 plants sampled in 2005. Recaptures were low from all moth releases. Colored mass observations from the first, third, and fourth releases in 2005 were particularly low; the moths from these cohorts must have died, flown away, or chosen not to oviposit in the field.

Table 2.2 also summarizes extrapolations of the total egg mass "recapture rates" from each moth release. Based on the number of females released and the estimated total number of egg masses that were potentially laid by each group, the entire experimental field "recaptured" between 0.05 and 2.1% of all egg masses produced from each release. However, the actual "recapture" rate is probably a bit higher, due to colored females that do not express dye in their eggs, inviable adults and/or premature death of individuals. If the proportion of dyed females laying colored eggs is as high as 30% (Appendix A), then the field recapture rate estimate could be as high as 3.0%.

Fig. 2.2 shows Rose diagrams generated in R with the rose.diag function (CircStats package, Agostinelli 2007) superimposed over the hexagonal plot outline. Each dot (as part of lines radiating from the diagram edge) represents one day's mean wind direction. Season-wide mean wind directions from 2004 and 2005 were 210˚ (SSE) and 173˚ (S), respectively. Rayleigh *r* statistics (representing the length of the mean wind direction vector) indicated that season-wide mean wind directions from 2004 and 2005 were significantly oriented from SSE and S, respectively (Table 2.3). Although daytime winds at Rock Springs are generally westerly, cool air descending along the side of Tussey Mountain in the early evening probably contributed to the southerly winds at the field site. In 2004, direction block D contained the fewest number of colored egg masses, while the most were found in direction A (Fig. 2.3). Interestingly, colored egg mass orientation in 2005 was reversed, with fewest found in A plots and most found in blocks C and D. However, a Rayleigh test of the egg mass counts showed no significant orientation among any group (colored, uncolored, or combined) in either year. Counts of males between pheromone traps were significantly different ($\chi^2 = 75.03$; df = 5; $P < 0.00001$). Most of the males were found in Direction B & F traps (Table 2.4),

Median plant stage among planting dates ranged from V2 to R4 during the 2004 sampling period and V4 to R5 in 2005 (Fig. 2.4). The proportional odds assumption of the log-linear model

was not contradicted, indicating that the log cumulative odds were proportional to the distance between the explanatory variable values ($\chi^2 = 6.722$; df = 5; $P = 0.2422$). The full model was used, with year, time of season, and year*time interaction included; the likelihood ratio supported the model fit ($\chi^2 = 156.25$; df = 5; $P < 0.0001$). Controlling for year, there was a significant association in egg mass deposition (colored + uncolored) between planting date treatment and the time of season ($Q = 104.8$; df = 1; $P < 0.0001$). Time of season, time*planting date, and year*time*PD interactions were all significant factors in the loglinear model (Time: $\chi^2 = 88.8$; df = 2; $P < 0.0001$; Time*PD: $\chi^2 = 76.67$; df = 4; $P < 0.0001$; Year*Time*PD: $\chi^2 = 37.22$; df = 4; $P < 0.0001$). Type III analysis of effects indicated that the time factor and year*time interaction were significant in the logistic regression model (Time: $\chi^2 = 14.73$; df = 2; $P = 0.0006$; Year*Time: $\chi^2 = 32.63$; df = 2; $P < 0.0001$).

Females showed more distinct planting date preferences in 2004 than 2005 (Fig. 2.5). Across both years, most of the early-season egg masses were laid in the planting date 1 plots. Relative to the late part of the season, females were 4 times more likely to lay an egg mass in planting date 1 than planting date 3 in the early part of the season ($\chi^2 = 10.45$; df = 1; $P = 0.0012$) and nearly 3 times more likely to lay in planting date 1 than the other two planting dates combined (Wald $\chi^2 = 12.03$; df = 1; $P = 0.0005$). Planting date 2 was 2.7 times more likely to receive an egg mass than planting date 3 when comparing early to late season counts ($\chi^2 = 5.64$; df = 1; $P = 0.0176$).

Conversely, most late season egg masses were laid in planting date 3. Relative to the early part of the season, late-season planting date 3 plots were 2.3 times more likely to receive an egg mass than planting dates 1 and 2 combined. When comparing late-season and mid-season counts, females were 2.7 and 1.6 times more likely to lay egg masses in planting date 3 than 1 and 1+2 plots, respectively (PD 3 vs. 1: $\chi^2 = 8.39$; df = 1; $P = 0.0038$; PD 3 vs. 1+2: Wald $\chi^2 = 3.50$;

df = 1; $P = 0.0615$). Finally, combined egg mass counts from planting dates 2 and 3 in the late season were significantly higher than those in planting date 1, relative to mid-season counts; egg masses were 2.2 times more likely to be laid in the younger plants (Wald $\chi^2 = 10.12$; df = 1; $P = 0.0015$).

Season-wide colored egg mass numbers from 2004 were greater in planting date 1 than 3 plots ($\chi^2 = 4.59$; df = 1; $P = 0.032$; Fig. 2.6), though this was probably due to two sampling days that were missed later in the season. Uncolored mass counts from the same year showed significantly higher numbers in planting date 1 versus 2 ($\chi^2 = 4.22$; df = 1; $P = 0.040$). When the 2004 colored and uncolored data were combined, planting date comparisons ceased to be significant (1 vs. 2: $\chi^2 = 3.21$; df = 1; $P = 0.073$; 1 vs. 3: $\chi^2 = 2.98$; df = 1; $P = 0.084$). No season-wide planting date effects were significant in 2005; furthermore, no comparisons showed significance when all data from both years were combined.

Directional differences in egg mass deposition varied according to year and egg mass group (Fig. 2.7). In 2004, significantly more colored egg masses were found in directional block A than D ($\chi^2 = 3.91$; df = 1; $P = 0.048$); no other blocks were significantly different. Fewer uncolored masses were found in 2004 directional block B than C ($\chi^2 = 5.65$; df = 1; $P = 0.018$), E ($\chi^2 = 3.75$; df = 1; $P = 0.053$), and F ($\chi^2 = 5.24$; df = 1; $P = 0.022$). When combined, the 2004 data only showed a significant difference between directions B and C ($\chi^2 = 4.25$; df = 1; $P = 0.039$). In 2005, fewer uncolored masses were found in direction C than F ($\chi^2 = 4.08$; df = 1; $P = 0.043$). Over all the combined data, the only directional comparison retaining significance was F versus B ($\chi^2 = 4.85$; df = 1; $P = 0.028$).

A cursory comparison of the wind and colored egg mass direction Rose diagrams from 2004 suggested that females may have flown or been displaced downwind from the center into the A directional block. To test a possible effect of wind direction on egg mass deposition, the

directional plot factor was replaced with the downwind covariate described above. With the covariate, a Poisson regression of the colored 2004 data resulted in a similar model fit as found using the original regression (Original model: residual deviance = 61.892, df = 61; Model with covariate: residual deviance = 61.906, df = 65). Downwind covariates were substituted in all of the other previously generated models and generally resulted in good model fits. However, directional differences in egg mass counts were generally minor, contributing only negligible improvements to each model. In fact, all models were generally satisfactory, regardless of downwind covariate inclusion. Furthermore, wind and egg mass comparisons from 2005 did not support a hypothesis of downwind displacement. If the hypothesis regarding downwind moth displacement held true, then colored mass numbers should have been higher in direction blocks A and B, which were located downwind (Fig. 2.2). Directional analysis of the data did not bear this out. The result from 2004 may have been an artifact, though higher observed colored mass numbers might have led to more conclusive results regarding wind direction.

Since male moths fly upwind in response to pheromone sources, it follows that females may fly upwind when they encounter attractive odorants. Therefore, an upwind covariate was used to test whether plot positions upwind from the release attracted more ovipositing females. However, the covariate showed no effect whatsoever on the distribution of any egg mass subgroup (colored, uncolored, or combined from either year).

Plot distance effects on egg mass counts were generally significant (Table 2.5). Plots located 24, 34, and 43 meters from the center in 2004 contained lower densities of colored and uncolored masses than those located 15 m from the plot center. In 2004, colored egg mass deposition showed a 2.4-, 3-, and 11-fold decrease from 15m to 24m, 34m, and 43m plots, respectively. Some distance factor comparisons in 2005 failed to be significant, though a general trend of decreasing egg density with increasing distance from the field center was apparent (Fig. 2.8). When all data were combined, plants in the 15m plots had 1.5 times more egg masses than

24m or 34m and 1.7 times more than 43m plots. However, combined egg mass numbers showed no difference when comparing 24m, 34m, and 43m distance factors.

Models fitted to the colored data show a moderate exponential decay curve, while the uncolored egg masses exhibit a near-linear decay in density with increasing distance from the release point (Fig. 2.9). Table 2.6 summarizes parameter estimates for data fitted to Equation 2.1. Quantitative comparisons between the decay models and natural field egg densities have little applicable value, as a mass emergence of adults from a small area (i.e., >7000 moths from 300 m^2) are unlikely in the real world. However, the models imply several important trends. First, a proportion of the released females showed a tendency to oviposit very near the aggregation/release site, rather than redistribute themselves evenly among plants at varying distances. In addition, those females apparently mated and stayed in the aggregation site until they were ready to lay eggs, though it is possible that some females returned to the field center after flight elsewhere. Finally, the decay in uncolored egg mass deposition suggests that (1) feral moths were attracted to the central weedy area, and/or (2) a number of released moths laid uncolored eggs. The second explanation was almost certainly contributing to distance decays of egg mass numbers, as seen in the distance decay models of uncolored masses (Fig. 2.9).

Neither the Poisson regression of plot adjacencies nor the Moran's *I* analysis suggested any effects of contiguous planting dates on oviposition. The regression adjacency factor was not significant ($\chi^2 = 0.63$; df = 1; $P = 0.427$); therefore, the number of adjacent plots of the same planting date had no measurable effect on the number of observed egg masses. Furthermore, the Moran's *I* statistics from combined colored and uncolored mass numbers each year were close to zero (2004: $I = 0.061$, $\Sigma_{i \neq j} w_{ij} = 106$, $n = 44$; 2005: $I = 0.141$, $\Sigma_{i \neq j} w_{ij} = 64$, $n = 41$). No evidence exists for any spatial correlation in egg masses between neighboring plots of the same planting date. This suggests that larger plots in a small-scale matrix of several plant maturities do not attract a disproportionate number of ovipositing females.

Discussion

Hunt et al. (2000) reported that 84% of the egg masses laid by blue-dyed moths were colored, compared with 99% of masses from red individuals. However, egg mass coloration tracked in the laboratory (Appendix A) revealed that up to 30% of dyed moths lay white eggs. Therefore, a significant proportion of egg masses identified as "uncolored" were probably laid by the released moths, but these were indistinguishable from white masses laid by wild females.

Overall, a relatively low number of colored egg masses were observed from released females. Estimated field-wide colored egg masses numbered about 1890 (2004) and 1520 (2005), from a released female population of about 7,000 to 10,000 moths. Released females chose to lay up to 3% of their egg load within a 150 meter radius, barring death or inviability. Clearly, a large proportion left the local field to oviposit elsewhere. What is interesting, however, is the fact that several hundred females likely mated and remained within the field to lay their first eggs; this contrasts with some authors' suggestion that female European corn borers exhibit an obligate dispersal phase after emerging as adults (Reardon et al. 2006, Dorhout et al. 2008). However, the majority of females left the field and laid eggs elsewhere, suggesting that ovipositing females may follow different dispersal strategies.

Wind was not a good predictor of egg mass placement among plots, which may suggest that female moths move locally under their own flight power or on winds too light or variable to be detected by a standard anemometer. When considering previous European corn borer dispersal studies, however, it seems that females may displace themselves in multiple ways. Small inter-plant movements may be used between resting periods or local plant examination for oviposition sites. Alternatively, some moths may choose to take advantage of stronger winds in order to exit a field and fly to a new area. Variation in dispersal propensity among individuals of a given

population could act as a bet-padding strategy, especially if European corn borers cannot assess host plant quality or maturity from a substantial distance.

Ovipositing female European corn borers show a strong preference for the most phenologically advanced corn early in the season, with preference shifting toward younger corn later in the summer (Huber et al. 1928, Everly 1959, Spangler and Calvin 2000). Preference changes are reported to generally coincide with the two bivoltine flights (Beard 1943, Spangler and Calvin 2000), and the results from this study agreed with those trends. Early-season females laid the most eggs on planting date 1; conversely, planting date 3 recruited the most egg masses in the late part of the summer. Egg mass deposition peaked during the middle part of the season in both years, with no planting date receiving significantly more masses than another.

Planting time recommendations to avoid European corn borer damage vary according to hybrid, regional voltinism and expected moth population sizes (Mason et al. 1996). In this study, plant maturity was clearly a factor in egg mass recruitment. Over the entire season, however, egg mass counts in each planting date were not necessarily different. To reduce European corn borer damage, pest managers should consider planting timing carefully; if the bulk of oviposition occurs when plants are most vulnerable, yield losses could be substantial. Proportional yield loss per larva for each phenological stage varies by hybrid, but plants largely incur the most damage from whorl stages (after V6) through kernel fill (Mason et al. 1996). During the early-season portion of this study, plant stages ranged from V6 to V12 in planting date 1, V4 to V8 in 2, and V2 to V7 in 3. Late-season corn phenological stages ranged from R2 to R5, R1 to R4, and V17 to R4 in planting dates 1, 2, and 3, respectively. By and large, oviposition coincided with plant stage susceptibility. Oviposition preferences may also reflect plant suitability for larval development, the "preference-performance" hypothesis (Jaenike 1978).

Most of the captured males were found in two traps, though the reason for this is unknown. Neither trap was located upwind from the field center for a significant portion of the

season (Fig. 2.2), even though the male moths were expected to fly upwind toward pheromone sources. An irrigation pump near the F trap may have rendered it more attractive, either due to higher humidity levels or higher grass surrounding the pump. Both factors may have resulted in an alternative aggregation site, as high humidity levels enhance female calling behavior and mating frequency (Loughner and Brindley 1971, Webster and Cardé 1982, Royer and McNeil 1991). Attempts to identify dyed males from the traps were fruitless, as their abdomens faded and dried out quickly during the interval between trap capture and collection. Thus, the percentage of released males recaptured in the traps cannot be estimated.

Approximate moth densities in the center aggregation site were noted throughout the season; about 15 to 30 could be lightly disturbed on a given day. It is possible that the center site re-attracted colored moths that already dispersed away from the field, but this is unlikely due to frequent redistribution of individuals among grassy sites (Sappington and Showers 1983, Sappington 2005). The hexagon center also could have attracted wild moths in search of mates, as a conventional aggregation site (as described in the Midwest) is something of an anomaly at the Rock Springs research farm. In Iowa, European corn borer often congregate in ~ 150 m^2 areas of dense, tall grass (Sappington and Showers 1983). This scenario was approximated in the center of the hexagon. However, no other aggregation sites were found in the surrounding area. The farm staff keeps all grass along the field margins well-mowed, even in ditches. Most female moths appeared to rest within cornfields, as Lee (1988) observed in Alberta, Canada. Other European corn borers were occasionally seen in various alfalfa and soybean fields around the farm. Rock Springs offers a heterogeneous mix of smaller fields, not unlike larger mixed-vegetable and row crop farms in the Mid-Atlantic. The spatial array of plant varieties and microclimates may therefore lead to different European corn borer flight and oviposition trends than those in the Corn Belt.

Nevertheless, these results reflect some of those seen in the Midwest. The highly significant plot distance effects and decay models reveal that oviposition is associated with distance from aggregation site, as suggested by Derrick and Showers (1990). Qureshi et al. (2005) also used exponential decay curves to relate trap counts to dispersal distance of dyed and released European corn borer moths. Unfortunately, the results from the wind analyses were too ambiguous to make definitive conclusions about wind effects on female flight and oviposition. Other authors proposed that European corn borer population displacement follows downwind directionality of light surface airflow (Chiang 1972, Showers et al. 1995, Showers et al. 2001).

Colored egg masses in 2004 were generally distributed downwind (northwards) from the moth release site, implying either flight or displacement from winds descending from the top of Tussey Mountain. Alternatively, females may be able to perceive nearby cornfields via olfaction. All cornfields in the vicinity were located westwards, northwards, and eastwards from the hexagonal field, with most of the closest fields situated about 150 to 450 m directly north of the release. As part of a separate study in 2005, nearly all of the cornfields within an 800 meter semicircular radius of the moth release point were also sampled. Colored masses were found as far away as 805 and 681 meters northwest of the hexagonal field. Other colored masses were observed on plants 605 (N), 496 (NNE) and 298 (NNW) meters away. Four of these masses were found the week of 18 July. Based on the number of plants sampled that week and plant populations in those fields, the number of colored egg masses within 800 meters may have totaled over 1,800.

The exponential decay of egg mass density with increasing distance from the release could be explained in two ways: (1) of the proportion of moths that stay in the field, some do not fly very far and tend to stay near the aggregation site, and (2) moth numbers, and therefore egg masses, are diluted by increased plot area and plant numbers at increasing distances from the center (Turchin 1998). Clearly, the second explanation must hold in this case. First, observed

moth numbers in the aggregation site and field plots never approached total release numbers. Furthermore, the colored egg masses found in surrounding fields indicated that many moths flew outside of the study site. Another explanation is the possibility of two disperser types: some females may stay near the location where they mated, while others may immediately fly away (Qureshi et al. 2005). Alternatively, female age or mating status may determine dispersal tendencies. Flight mill experiments conducted by Dorhout et al. (2008) revealed decreasing dispersal tendencies with increasing female borer age, though this was unaffected by mating status. Most females in their study were capable of and had a tendency to fly long distances (frequently 1-2 km; less commonly 20+ km), particularly virgin females. Translating these results to the field could prove difficult, as net moth displacement due to long-range directed flight versus local, meandering movement cannot be ascertained.

Showers et al. (2001) also reported the ability of males and females to fly long distances; most notable were several individuals flying 14 km within 100 minutes, and a female recovered nearly 50 km from their release site. Qureshi et al. (2005) described more local dispersal from trap data collected within 800 m of a colored moth release point. Qureshi et al. (2005) suggest a more moderate typical female range than Showers et al. (2005) and Dorhout et al. (2008). Reardon et al. (2006) argue that most moths disperse at least 300 m before mating. While the results here may agree with their assertion to some extent, enough colored egg masses were found in the field plots to demonstrate that a proportion of females do stay within 150 m of their natal field for at least a few days. Caffrey and Worthley (1927) and Xiangquan et al. (2004) acknowledge a preoviposition period of 3 to 5 days before female European corn borers start to oviposit. Therefore, reports of near-complete female departure from aggregation sites after 2 days postmating (Showers et al. 1976, Sappington and Showers 1983, Reardon et al. 2005) are not completely consistent with the colored egg deposition found within the hexagon. Male flight behavior was not quantified here, but it is not unreasonable to assume that male and female flight

patterns differ (Reardon et al. 2006). Theoretically, male flight is directed at finding multiple mates, while females search for optimal plants on which to oviposit. Dorhout et al. (2008) found a lower flight propensity in virgin males than virgin females, though this increased with age.

European corn borer dispersal studies may be valuable for refining IRM theory and predictions. The data imply a range of dispersal distances from a moth emergence point, and if local conditions are favorable for mating, some females will lay eggs very near (i.e., within 150 m of) their natal field. This could be advantageous for the current *Bt* corn IRM strategy if susceptible females mate with resistant males and stay near or within refugia to lay eggs. However, the small field and large numbers of moths utilized in this study were extremely artificial, unless natural European corn borer populations are very high and the refuge area is large and conducive to retaining females (e.g., favorable microclimate or nearby mating/aggregation sites). The most likely scenario is oviposition within 800 m for a large proportion of females; this was demonstrated by the egg-laying of colored moths in the cornfields surrounding the hexagon field site.

The success of IRM is certainly dependent on mating patterns between resistant and susceptible males and females, but very little is known about dispersal effects on mating interactions. Assuming random mating, EPA guidelines requiring refugia plantings within 800 m of *Bt* fields seem very reasonable. As this study suggests, researchers have much to learn about European corn borer mating, dispersal and oviposition patterns. More quantitative studies regarding the timing and percentage of populations engaging in long-distance flights would help determine planting requirements and adjust IRM model assumptions.

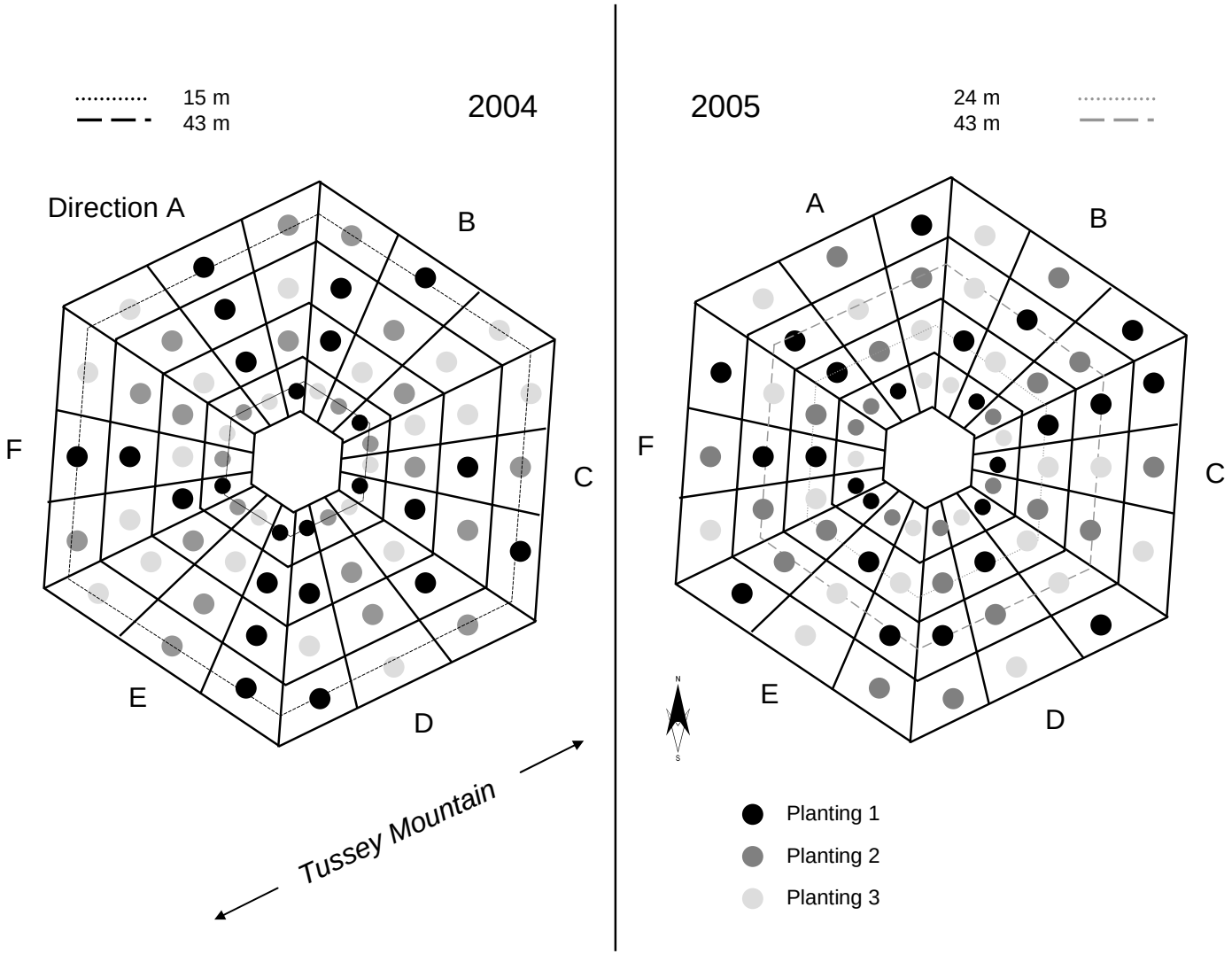


Fig. 2.1. Schematic of hexagonal plot configuration in 2004 and 2005.

Table 2.1. Plot area, plant population, percentage of plants sampled, and number of colored masses found in each distance factor plot.

Plot distance factor	Individual plot area (m^2)	Plant population (per plot)	Total plant population	Percentage of total plants sampled (per day)	Number of colored masses *2004*	*2005*
15 m	48.3	310	5580	6.5	33	18
24 m	80.4	517	9306	3.9	14	14
34 m	112.6	724	13032	2.8	11	7
43 m	144.8	930	16740	2.2	3	10
		Total	44658	---	61	49

Table 2.2. Adult emergence and colored egg mass counts for each marked European corn borer moth release in 2004 and 2005. The final column estimates the percentage of colored egg masses laid by each release group in the experimental field, out of all possible colored egg masses that could have been laid by the group.

	Release date	Emerged adults	Dead adults	Approximate number of emerged females*	Estimated number masses produced**	Release color	Colored egg masses observed	Estimated oviposition within field (%)***
2004	12 June	4431	473	1994	54,117	Blue	23	1.3
	2 July	6212	649	2795	75,856	Red	20	0.8
	3 August	5748	411	2587	70,211	Blue	18	0.8
2005	19 June	4487	539	2019	54,795	Red	1	0.05
	13 July	5253	600	2364	64,159	Blue	43	2.1
	29 July	5554	827	2500	67,850	Red	3	0.1
	12 August	5560	781	2502	67,904	Blue	2	0.1
	Total	37245	4280	16761	454,892	---	110	---

*Estimated from sex ratio observed from samples across all releases.
**Total potential number of egg masses produced by females from each release. From Zaman (2008).
***Estimated percentage of all masses from each release that were laid within the immediate 1.72-acre field area. The total number of colored egg masses observed from each release was divided by the total number of plants sampled each day (1440) to give the percentage of plants infested with eggs from released moths. This quantity was then multiplied by the total estimated number of plants within the field (44,658) to calculate the total number of colored egg masses within the field, then divided by the total estimated number of egg masses produced by females from the release (**).

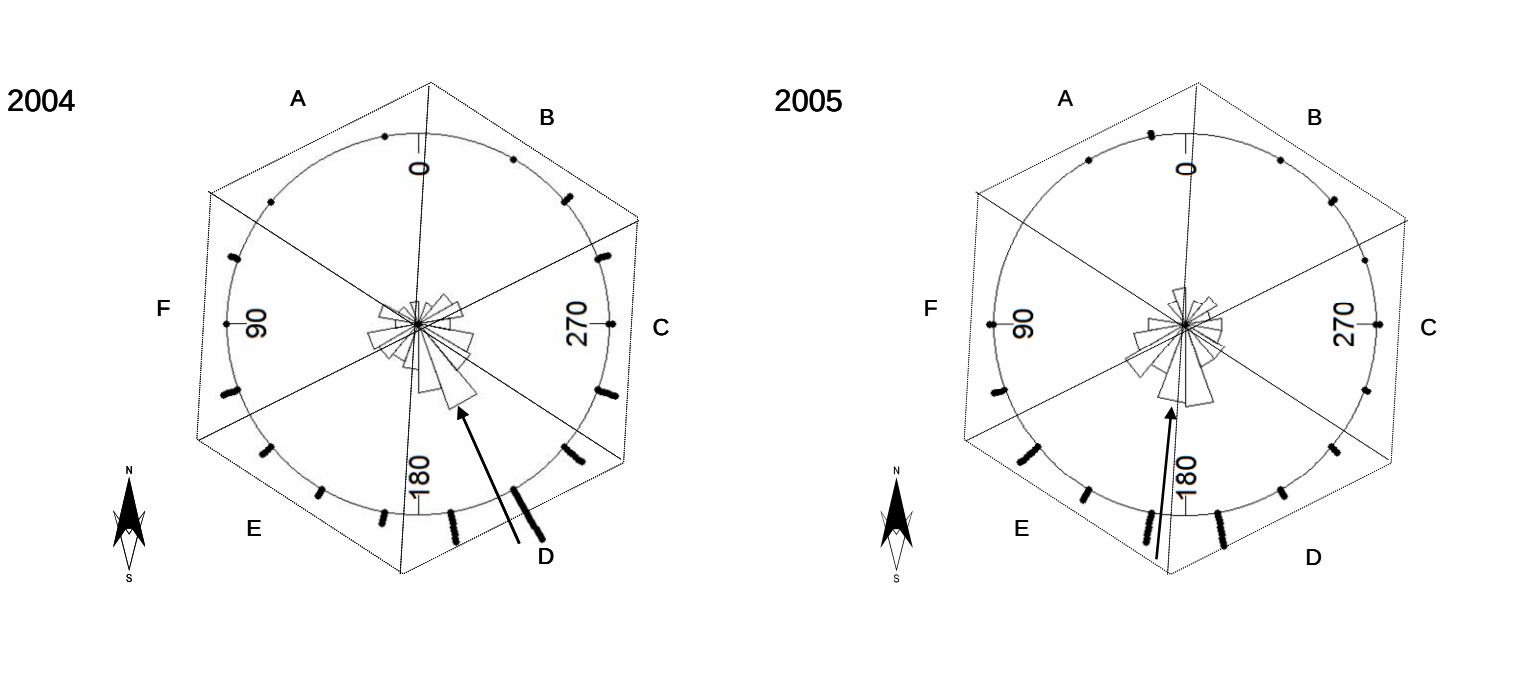


Fig. 2.2. Rose diagrams of daily mean wind directions for the sampling periods during the 2004 and 2005 field seasons. Data were oriented with 90° representing west. Wind flows from the direction indicated by the dots to field center. Each dot represents the mean direction on one day from 8pm to 12am, and letters denote each directional block. The area of each sector at center is proportional to the group frequency. Arrows show the path of each season's mean wind flow.

Table 2.3. Mean wind and egg mass directionality and Rayleigh *r*- and *z*-values from 2004 and 2005.

	Year	Egg mass group	Mean Direction†	Cardinal Direction	*n*	*r*	*z*
Wind Direction	2004	---	201°	SSE	70	0.527	19.46*
	2005	---	173°	S	53	0.536	15.22*
Egg Mass Direction	2004	Colored	354°	N	61	0.165	1.67**
	2004	Uncolored	160°	SSW	317	0.052	0.87**
	2004	Combined	140°	SW	378	0.019	0.14**
	2005	Colored	232°	SE	49	0.115	0.65**
	2005	Uncolored	70°	WNW	243	0.095	2.17**
	2005	Combined	75°	WNW	292	0.061	1.07**

*p < 0.001, **p > 0.10, †*Note:* Direction oriented with 0°=N, 90°=W.

Table 2.4. Weekly mean ± SE pheromone trap captures of European corn borer males in 2004.

Trap direction	*N*	Mean	SE
A	9	0.00	±0.00
B	9	3.44	±0.88
C	9	0.44	±0.29
D	9	0.56	±0.29
E	9	0.78	±0.36
F	9	3.33	±0.85

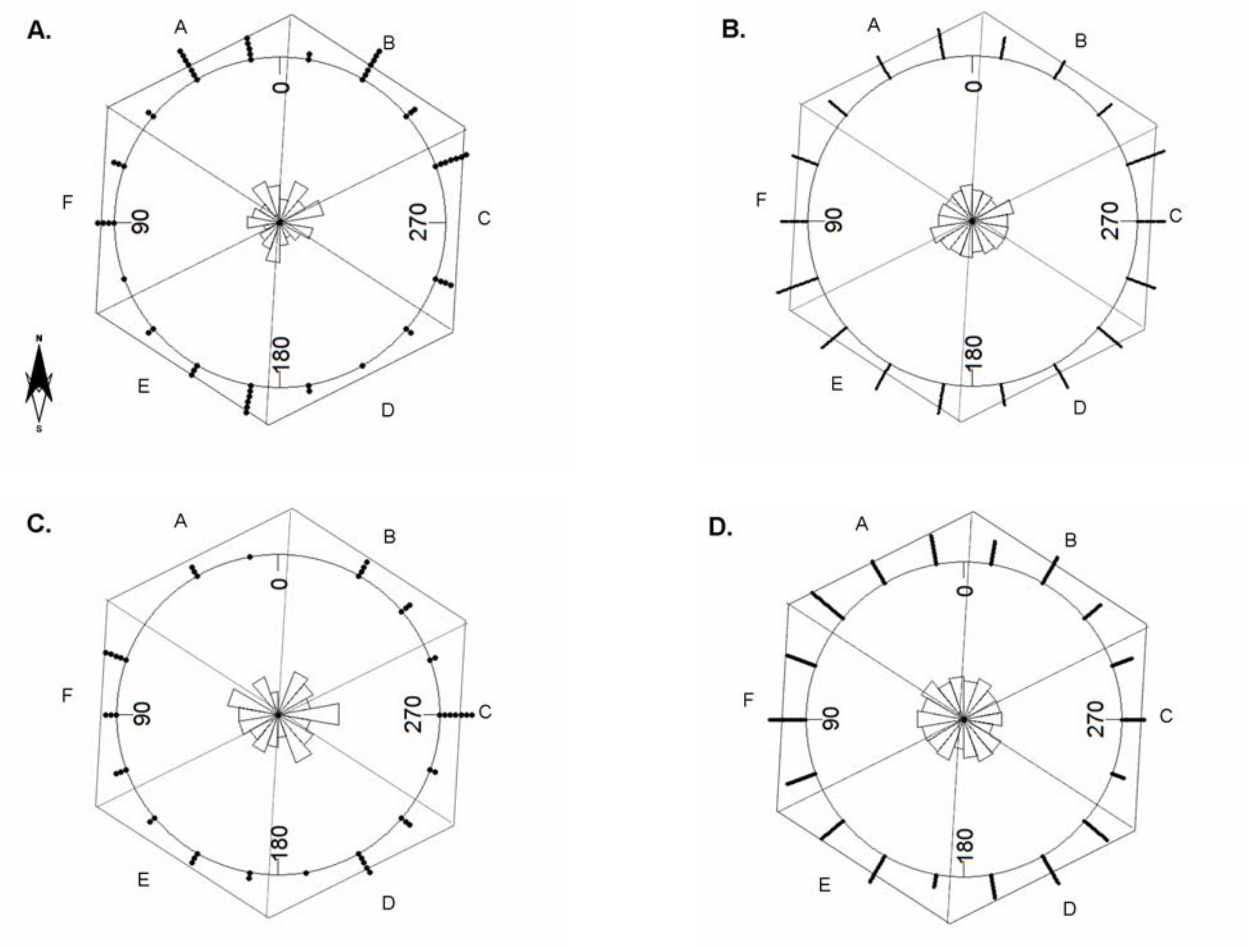
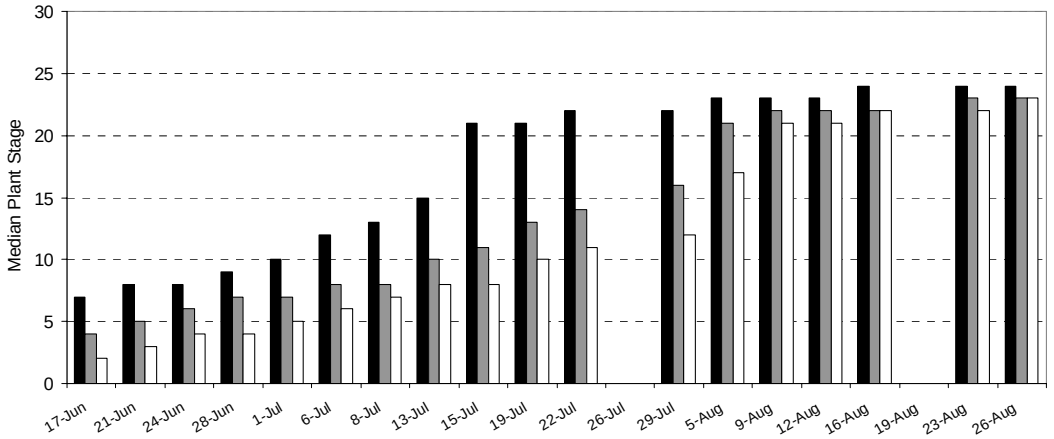


Fig. 2.3. Directionality of colored (2004 (A), 2005 (C)) and uncolored (2004 (B), 2005 (D)) egg masses. Masses were identified by their plot locations, which were divided into 18 20-degree sectors (three sectors per directional block). Each dot represents one egg mass.

(a) 2004



(b) 2005

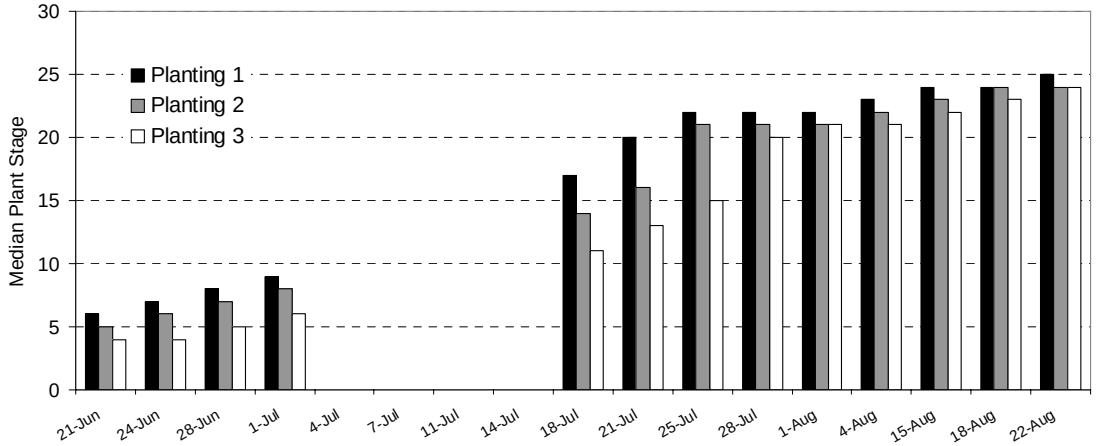
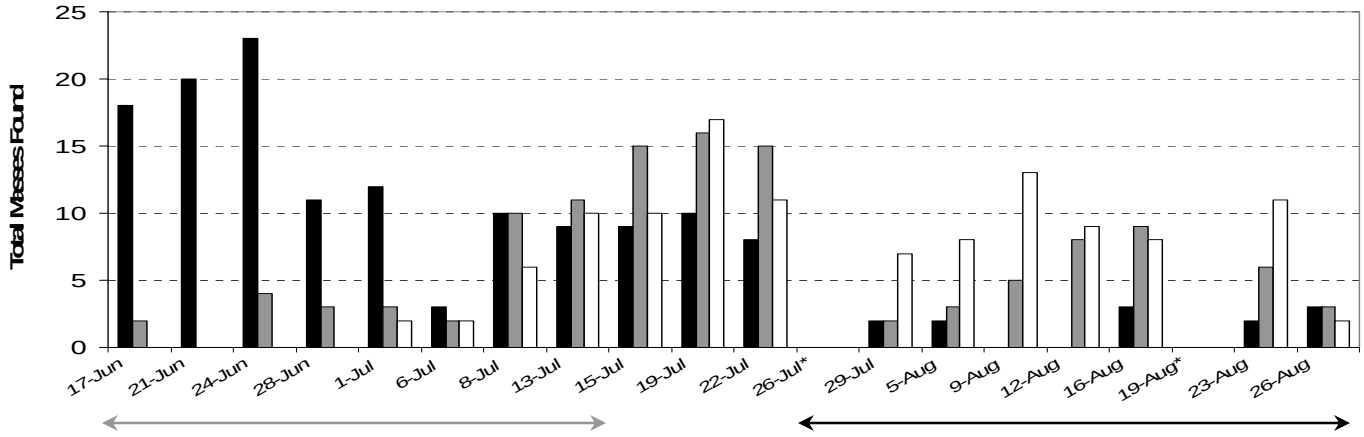


Fig. 2.4. Median plant stage in each of three planting dates over (a) 2004 and (b) 2005. Y-axis values follow vegetative stage numbers through V19 (1-19 = V1-V19). Stage 20 = VT, 21 = R1, 22 = R2, etc. Plots were not sampled on missing dates.

(a) 2004



(b) 2005

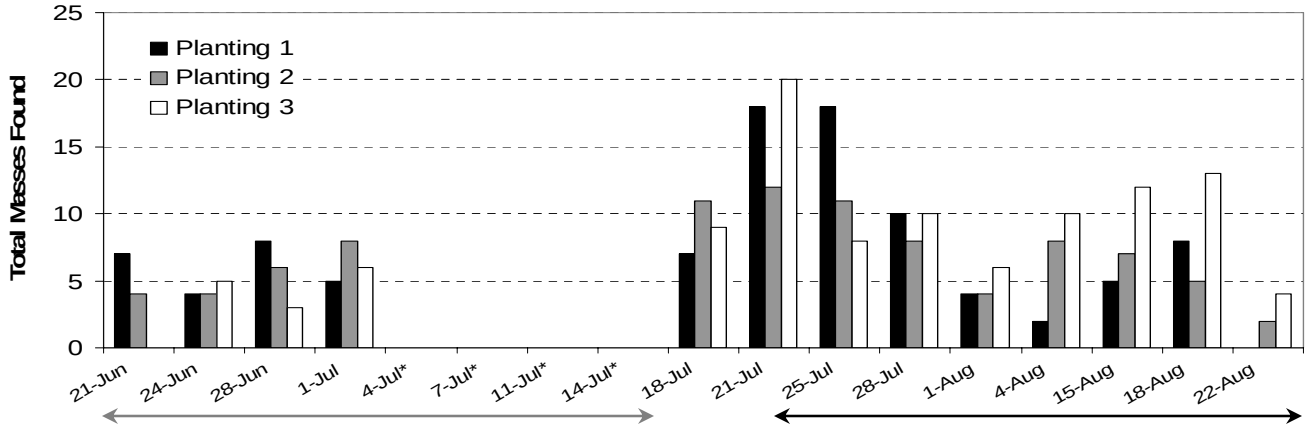
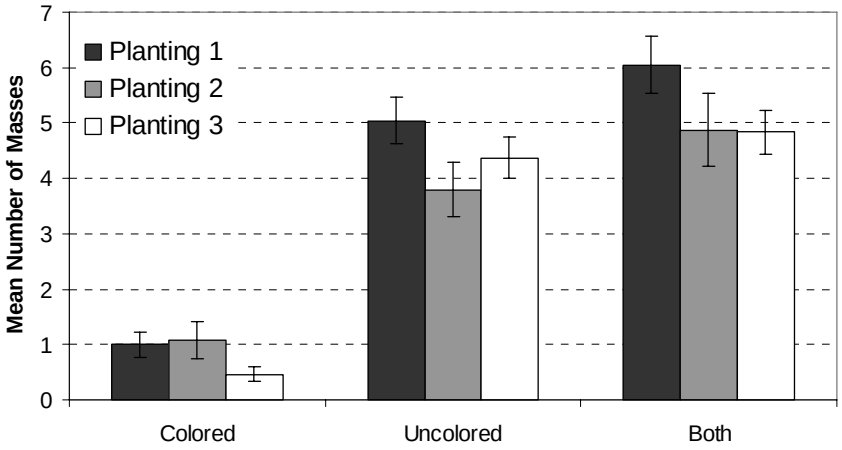
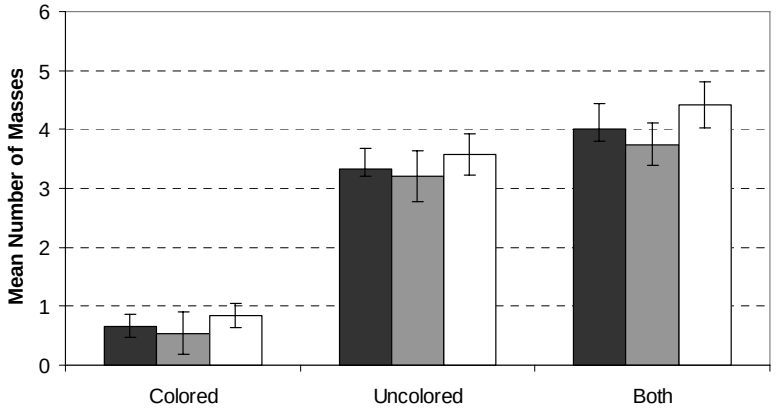


Fig. 2.5. Total number of colored + uncolored egg masses found in each planting date factor in (a) 2004 and (b) 2005. Plants were not sampled on starred dates. Approximate first and second European corn borer generations are indicated by the gray and black arrows.

(a) 2004



(b) 2005



(c) Both Years

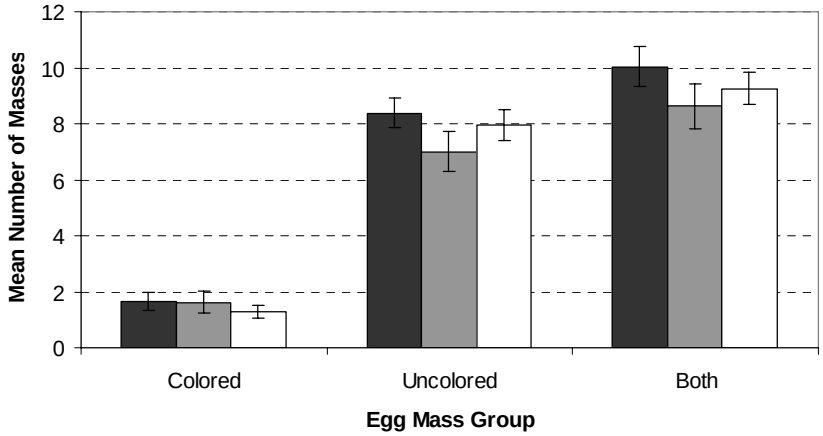
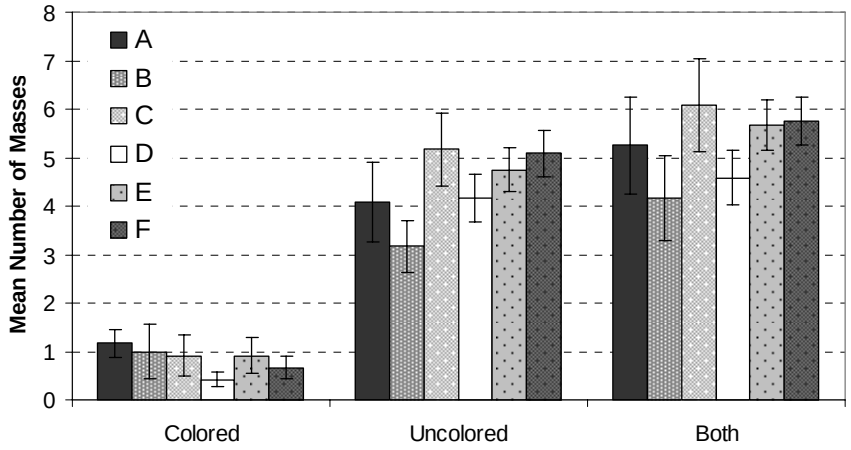
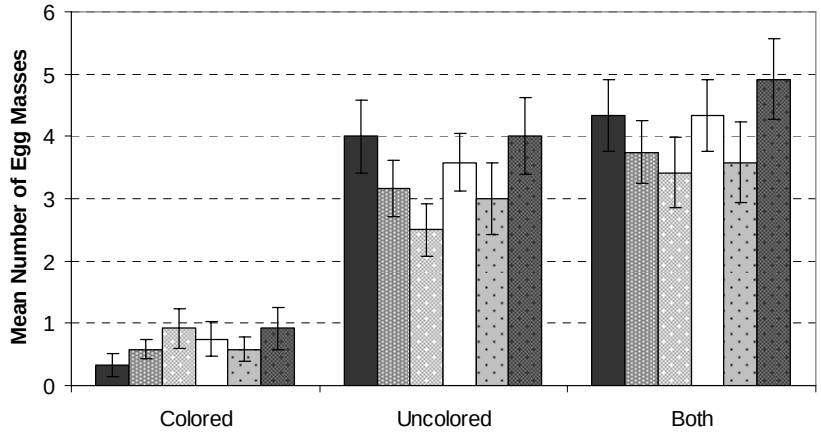


Fig. 2.6. Mean number of colored and uncolored egg masses found per plot of each planting date factor (1, 2, or 3) in (a) 2004 (b) 2005 and (c) 2004 & 2005. Bars represent the standard error of the mean.

(a) 2004



(b) 2005



(c) Both Years

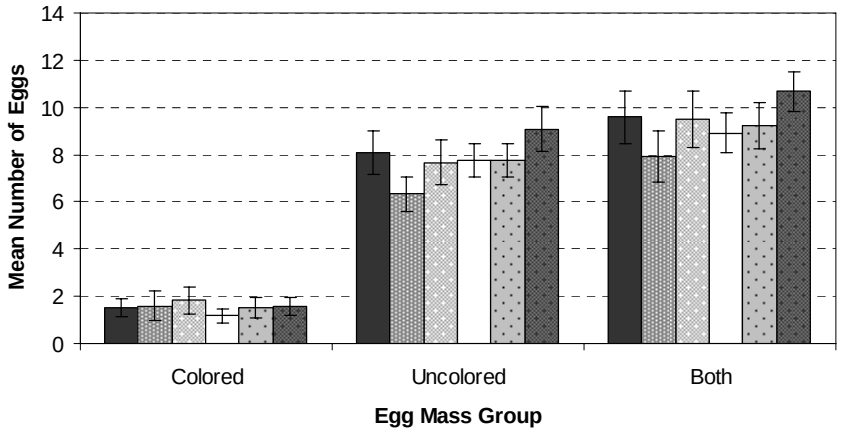
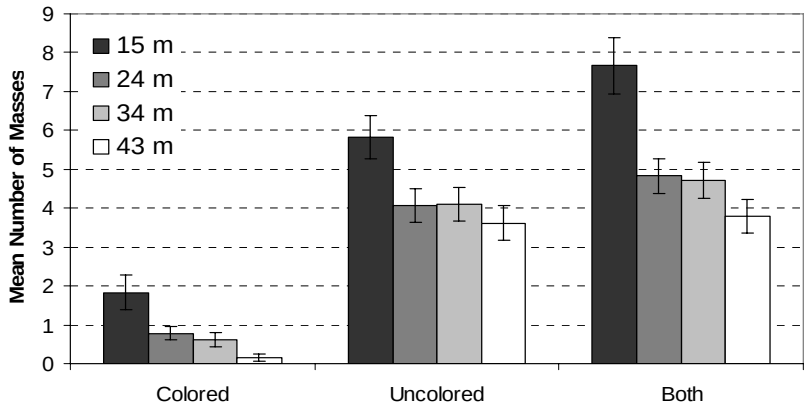


Fig. 2.7. Mean number of colored and uncolored egg masses found per plot for each directional block (A, B, C, D, E or F) in (a) 2004 (b) 2005 and (c) 2004 & 2005. Bars represent the standard error of the mean.

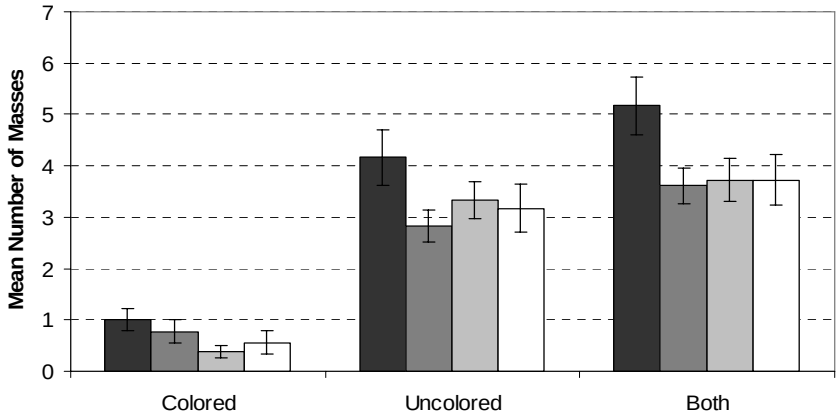
Table 2.5. Poisson regression coefficients for plot distance comparisons of egg mass counts.

Year	Egg mass group	Plot distance comparison	df	Regression coefficient	(SE)	χ^2	*P*
2004	Colored	15m vs. 24m	1	0.858	(0.319)	7.23	0.0072
		15m vs. 34m	1	1.099	(0.348)	9.96	0.0016
		15m vs. 43m	1	2.398	(0.603)	15.81	<0.0001
	Uncolored	15m vs. 24m	1	0.364	(0.152)	5.69	0.0171
		15m vs. 34m	1	0.350	(0.152)	5.31	0.0212
		15m vs. 43m	1	0.480	(0.158)	9.23	0.0024
	Combined	15m vs. 24m	1	0.461	(0.137)	11.36	0.0008
		15m vs. 34m	1	0.485	(0.138)	12.35	0.0004
		15m vs. 43m	1	0.708	(0.148)	22.82	<0.0001
2005	Colored	15m vs. 24m	1	0.251	(0.356)	0.50	0.4807
		15m vs. 34m	1	0.945	(0.445)	4.50	0.0340
		15m vs. 43m	1	0.588	(0.394)	2.22	0.1361
	Uncolored	15m vs. 24m	1	0.386	(0.182)	4.52	0.0336
		15m vs. 34m	1	0.223	(0.173)	1.66	0.1976
		15m vs. 43m	1	0.274	(0.176)	2.44	0.1183
	Combined	15m vs. 24m	1	0.358	(0.162)	4.91	0.0267
		15m vs. 34m	1	0.328	(0.160)	4.19	0.0407
		15m vs. 43m	1	0.328	(0.160)	4.19	0.0407
	All	15m vs. 24m	1	0.419	(0.104)	16.06	<0.0001
		15m vs. 34m	1	0.419	(0.104)	16.06	<0.0001
		15m vs. 43m	1	0.537	(0.108)	24.58	<0.0001

(a) 2004



(b) 2005



(c) Both Years

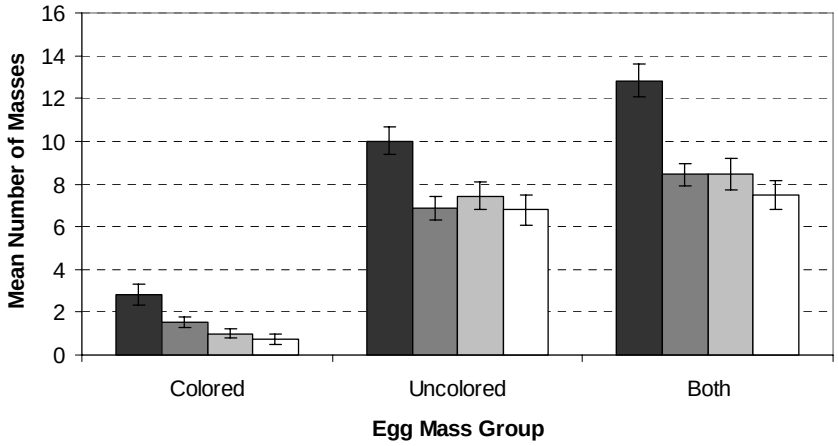


Fig. 2.8. Mean number of colored and uncolored egg masses found per plot of each plot distance factor (15, 24, 34, or 43 m) in (a) 2004 (b) 2005 and (c) 2004 & 2005. Bars represent the standard error of the mean.

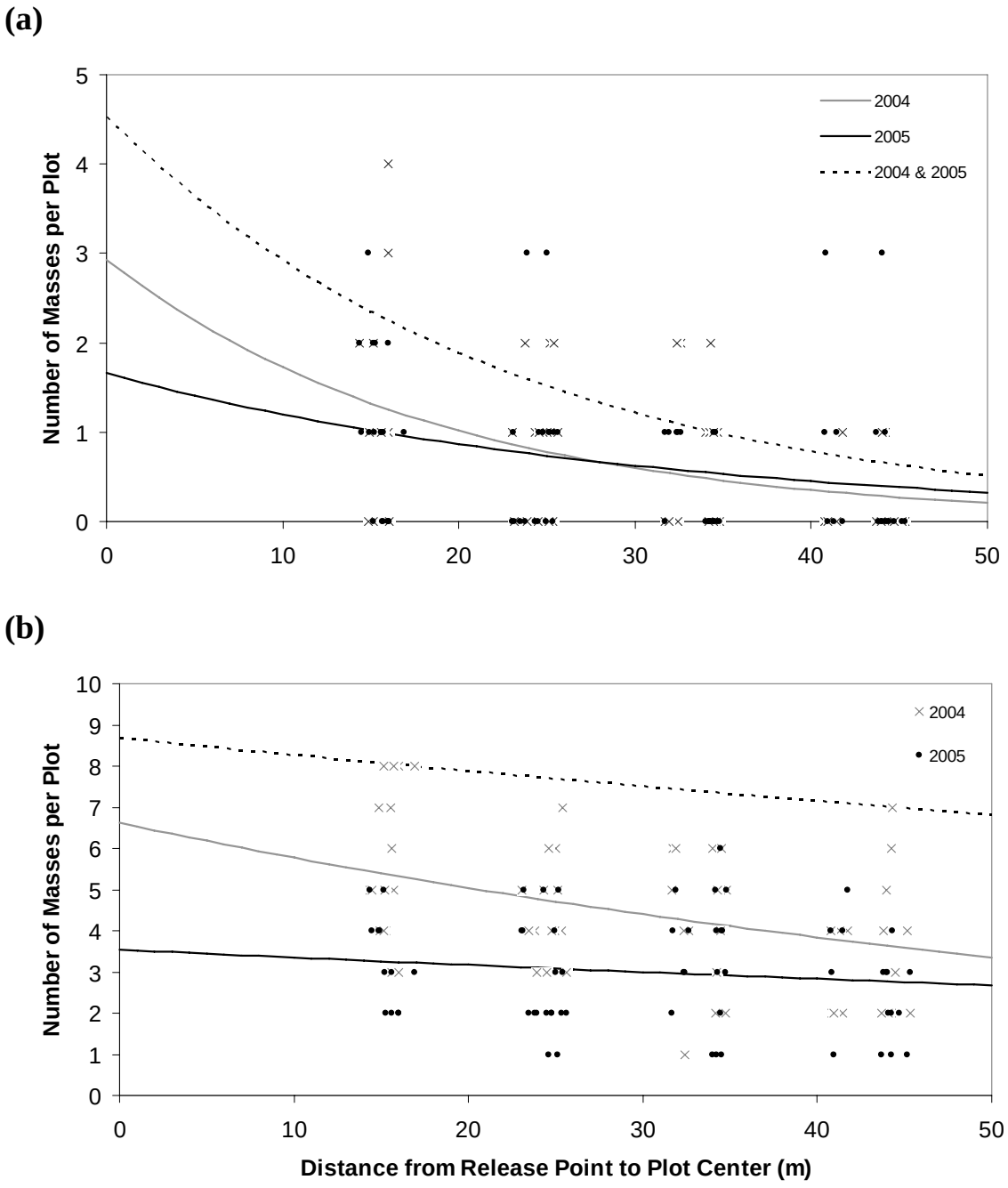


Fig. 2.9. Exponential decay models for (a) colored and (b) uncolored egg mass numbers by plot distance from moth release point. Note that the curve portions <15m are inexact extrapolations, as the area <10 m from the release point was a weedy aggregation site.

Table 2.6. Parameter estimates for exponential decay models fitted to plot distance effects on colored and uncolored egg mass counts.

		Model			Parameter estimates*			
Egg mass group	Year	*F*	df	*P*	*a*	(95% C.I.)	-*b*	(95% C.I.)
Colored	2004	37.36	2	<0.0001	2.932	(0.938,4.927)	-0.053	(-0.084,-0.022)
	2005	25.60	2	<0.0001	1.659	(0.382,2.937)	-0.033	(-0.064,-0.001)
	2004 + 2005	64.11	2	<0.0001	4.524	(2.254,6.793)	-0.044	(-0.065,-0.022)
Uncolored	2004	256.49	2	<0.0001	6.618	(4.999,8.237)	-0.014	(-0.022,-0.005)
	2005	162.58	2	<0.0001	3.545	(2.329,4.760)	-0.006	(-0.017,0.006)
	2004 + 2005	417.93	2	<0.0001	8.685	(6.871,10.498)	-0.005	(-0.012,0.002)

*Exponential decay model with the form $u = ae^{-br}$; parameter confidence intervals are approximate.

CHAPTER 3.

Farm-Scale Cornfield Heterogeneity Effects on European Corn Borer Oviposition

Introduction

The European corn borer, *Ostrinia nubilalis* (Hübner), is a pest that causes significant damage to corn crops throughout the United States and Canada. Plantings of transgenic corn expressing the toxin *Bacillus thuringiensis* kurstaki (*Bt* corn) have greatly increased yields by reducing damage from larval feeding. Extensive use of *Bt* corn could upset its long-term viability, however, as European corn borer resistance may develop quickly. Therefore, researchers have constructed resistance evolution models and more thoroughly examined European corn borer population dynamics since the market introduction of *Bt* corn in 1996 (Onstad and Gould 1998, Guse et al. 2002, Ives and Andow 2002). Of great interest to these models are the interactions between adult dispersal, mating patterns, field spatial arrangements and cornfield attributes such as variety and isolation. Several modelers suggest that these factors may play a major role in the rate of resistance evolution (Caprio 2001, Ives and Andow 2002).

Very few authors have examined corn borer movement and oviposition at a farm or larger scale, which is an important consideration in resistance prediction models. To address movement of European corn borers, Hunt et al. (2001) and Qureshi et al. (2005) released masses of internally-dyed moths and then recaptured them at varying intervals to determine their flight capacity and dispersal propensity. Local (i.e., within-habitat) female European corn borer dispersal and oviposition was also addressed in the previous chapter. Spatial egg mass distributions found in that study implied that a high proportion of moths leave their natal field, suggesting a need for more dispersal studies conducted at larger scales. No published studies have examined corn borer oviposition or egg mass sampling at scales greater than several large (~50

ha) fields. Furthermore, spatially explicit corn borer studies as yet have only concerned trapping, larval damage or homogeneous fields (Lee 1988, Hunt et al. 2001, Wright et al. 2002, Qureshi et al. 2005).

Wiesenborn and Trumble (1988) used a farm-scale complex of heterogeneous cornfields to examine oviposition patterns in *Heliothis zea* (Boddie). The effects of field size, plant age, plastochron index (a linear measure of plant age), and various measures of spatial field configuration on egg density were quantified during several consecutive sampling intervals. The methods outlined in this study are similar; however, a greater number of fields were sampled and more attributes were measured. In addition, we investigated the impact of early-season adult dispersal on oviposition. Previous studies on corn borer choice suggest that various field and plant traits (e.g., humidity, height, maturity) influence corn borer oviposition site choice (Huber 1939, Spangler and Calvin 2000, Hunt et al. 2001, Chapter 2). The relationship between egg-laying and each of these factors was analyzed.

The goal of this study was to examine how female European corn borers interact with a spatial mosaic of fields at a farm scale through oviposition site choices over time (i.e., shifting corn stage availability). The study also aimed to determine how field attributes, such as planting date, plant density, field area, and *Bt* variety, affect egg-laying. Egg densities were examined to see if the spatial distribution of masses among fields can be predicted at a farm or landscape level based on planting date and relative maturity. Finally, the large scale of the study provided a way to glean insight into European corn borer movement over a heterogeneous landscape. Since the initial 2005 farm-scale study was too labor-intensive to complete a second year, a small plot study was substituted in 2006. The smaller study used various planting dates to mimic plant maturity differences observed across the larger landscape in 2005. To evaluate the predictive power of an oviposition preference model based on research by Spangler and Calvin (2000), data from both years were used.

Materials and Methods

2005 Landscape Study. The study area consisted of numerous fields on the Russell E. Larson Agricultural Research Station in Rock Springs, PA. The research farm covers approximately 1800 acres (728 ha) of mostly Hagerstown silt loam soil just north of Tussey Mountain. Most of the research fields range in area from 1 to 5 acres, with the largest covering about 28 acres. This is typical of Pennsylvania fields, which average about 5 acres in size. However, almost all of the privately-owned fields north, west and east of the research farm were significantly larger than the average experimental field. The cornfields sampled in this study covered an aggregate area of 106 acres (42.9 ha). All corn was grown according to recommendations set forth by the Pennsylvania Agronomy Guide (2005), though tillage, fertilization and pesticide schedules differed according to each field's needs.

Fields planted to corn in 2004 were sampled for overwintering European corn borer larvae in April 2005. Thirty-eight fields with old corn stubble were selected in a central portion of the farm and georeferenced. Each field had been planted with 30 in (0.76 m) rows, and approximate per-acre plant populations were estimated by counting the number of stalks in 1/1000 of an acre (17.6 ft (5.36 m) of row). Total plant populations per field were determined by using the median number of stalks from six row samples taken per field. Ten consecutive stalks from ten sites in each field were split open to estimate European corn borer densities. Overwintering larvae from each field sample were used to estimate the percentage of stalks infested and total larval population per field. Larval infestations in each field are shown on an aerial map (Fig. 3.1).

In May 2005, 31 fields planted to field corn were selected and georeferenced. Fig. 3.2 shows an aerial map of the farm with each field identified. All fields were planted with 30 in (38.1 cm) rows; other field attributes are summarized in Table 3.1. Ten sampling sites were randomly selected in each field, with all sample sites at least 25 ft (7.62 m) from the field edge.

The midpoint of each sampling site was georeferenced with a handheld global positioning system unit (eTrex Legend® 010-00256-00, Garmin Ltd., Olathe, KS). Ten consecutive plants at each site were flagged to facilitate location each week. Egg mass sampling commenced the week of 20 June and ended the week of 22 August. Each week all 100 flagged plants per field were searched for European corn borer egg masses. As many fields as possible were sampled each week. If an egg mass was found, the plant number, number of eggs per mass, egg mass location (leaf number from first emerged leaf), plant phenological stage (V1-18; R1-5 as described by Ritchie et al. 1992), and plant height (to whorl opening if whorl stage, else to base of last fully exposed leaf) were recorded. Each egg mass found was circled with a permanent marking pen to prevent recounts. The height and plant stage of the fifth plant at each site was also recorded each week; these data were used to estimate median weekly field-wide plant stages and heights. Field temperature and humidity measurements were taken once weekly at dusk in each field using a digital temperature/humidity meter (Traceable® Model 4096, Control Company, Friendswood, TX). The meter was held at mid-canopy level at least 10 rows from the field edge for 1 minute or until readings stabilized; temperature accuracy was ±1°C and relative humidity range was 25% to 95%, with an accuracy of ±2% at mid-range (±4% elsewhere). All 31 fields were measured within a two hour time period.

Planting Date Field Plot Study. In 2006, a 1 acre (0.40 ha) field was divided into two blocks of a Randomized Complete Block design. The blocks were further divided into seven 0.029-ha plots (treatments), each randomly assigned one of seven planting dates. The treatments were planted on 1 May, 5 May, 11 May, 17 May, 24 May, 1 June, and 9 June with a 101-day, non-*Bt* field corn hybrid (DeKalb DKC51-43). Fertilizer (10-30-10) was applied at 100 lbs/acre (112.1 kg/ha) at planting. The plots also received a 25 gal/acre (233.8 liters/ha) post-planting fertilizer and herbicide applications (s-metolachlor 35.8%/atrazine 28.1% + isoxaflutole + paraquat + nonionic surfactant; urea-ammonium nitrate solution carrier with 83.5 lbs N/acre (93.6 kg/ha)). A

16 gal/acre (149.7 liters/ha) topdress with 53.4 lbs N/acre (59.9 kg/ha) and urea-ammonium nitrate solution carrier was also applied to the entire field.

Five sampling sites consisting of ten consecutive plants were flagged in each plot. Each sample site was located at least 10 ft (3.05 m) from the plot boundary. On 22 June, 28 June, 6 July, 13 July, 21 July, 31 July, 7 August and 23 August, all plants were searched for egg masses. Eggs per mass, leaf location, plant phenological stage and plant height (to collar line of last fully emerged leaf) were recorded for each mass found. All masses were circled and dated with a permanent marking pen. The height and stage of the fifth plant at each site were also used to determine median plot stage and height for each sampling day.

Statistical Analysis: 2005 Landscape Study. Time constraints did not allow sampling of each field every week; therefore, some of the analyses used each field's season-wide egg mass total divided by the number of times it was sampled. This quantity is referred to as the "egg sample estimate" (*ESE*), which represents the approximate number of egg masses were laid on each plant in a given field.

Spearman correlation (PROC CORR, SAS Institute 2004) was used to relate field size with the *ESE*. The distance from two adult dispersal sites was also compared to the number of egg masses found in each field during the first few weeks of sampling (PROC CORR). The adult dispersal sites were chosen due to large expected numbers of emerging moths. One western group of fields contained approximately 50,000 overwintering larvae (Fig. 3.1); egg mass counts from each field were compared with its distance from the emergence field during the first few weeks of the season (20 June, 27 June, 4 July, 11 July). Field distance was hypothesized to be negatively correlated with egg mass deposition during the first-generation European corn borer flight. In addition, field distances from a mass release site in the southeastern part of the study area were compared to their egg mass numbers during the weeks immediately following moth releases (20

June, 27 June, 18 July, 25 July). Field centroid distances from each of these dispersal sites were used in the correlations.

Any effect of *Bt* plants on corn borer oviposition was assessed using a Mann-Whitney-Wilcoxon test (extended Mantel-Haenszel correlation statistic in PROC FREQ, SAS Institute 2004) of a two-way table comparing field hybrid (*Bt* vs. non-*Bt*) and the egg sample estimate. The *ESE* was also compared to the planting period for each field (early: 29 April – 6 May, mid: 7 May – 16 May, late: 17 May – 15 June) with a Mann-Whitney-Wilcoxon test; this was used to determine whether or not planting dates affected the number of egg masses each field received over the entire season.

A multiple regression of eleven possible explanatory variables was generated using stepwise selection in PROC REG (SAS Institute 2004). As in the Wiesenborn and Trumble (1988) study, the regression was used to explore the effects of different measurable field attributes on egg mass deposition. The dependent variable was specified as the *ESE*; explanatory variables included (1) field acreage, (2) acres of older corn (i.e., corn acreage with an earlier planting date, only including those fields in the study), (3) median number of meters to older corn, (4) number of meters to the closest cornfield, (5) number of meters to the closest older cornfield, (6) plant density (i.e., number of plants per acre), (7) plant density in the closest cornfield, (8) plant density in the closest older cornfield, (9) total number of plants in field, (10) total number of plants in the closest cornfield, and (11) total number of plants in the closest older field. Distances were measured from the centroid of each field using ArcMap software (ESRI, Redlands, CA), and only the 31 fields sampled in the study were considered when calculating older corn acreage or distance between cornfields. The independent variables were entered into and remained in the model unless their *p*-values exceeded 0.15. Several methods, including principle component analysis (PROC PRINCOMP, SAS Institute 2004) were used to assess multicollinearity between variables. Fields 11, 12, 16, 19 and 24 were eliminated to improve the model fit; their *ESE* values appeared

as outliers, mostly due to unusually high egg mass numbers or low mass observations resulting from few weekly samples.

Two additional multiple regressions were conducted using approximate first- and second-generation European corn borer subsections of the data. This was done to determine if different generations exhibited differences among field explanatory variables. New *ESE* values were calculated for all fields, based on the number of egg masses found in the first four weeks (20 June, 27 June, 4 July, 11 July) and final six weeks (18 July, 25 July, 1 August, 8 August, 15 August, 22 August).

The Moran's *I* statistic, an indicator of spatial association among lattice data (Banerjee et al. 2004), was used to determine whether fields in close proximity exhibited similar egg mass recruitment. Neighbors were defined as those fields with inter-centroid distances < 300 meters apart. This value was selected to maximize the number of comparisons while excluding relationships between fields that clearly did not lie within the same vicinity. Other Moran's *I* statistics were calculated using neighbor definitions of < 250 m, < 450 m, and < 600 m for comparison. Moran's *I* multiplies a covariance term from neighboring field values by an adjacency matrix **W**. The statistic is generally supported on the interval [-1,1], with positive and negative values indicating positive and negative spatial autocorrelation, respectively (O'Sullivan and Unwin 2003). If plots *i* and *j* were neighbors, $w_{ij} = 1$; if not, $w_{ij} = 0$. Fields 1, 12, 13 and 28 did not have any neighbors within 300 meters.

Finally, a Mantel-Haenszel mean score statistic for special rank scores was calculated using PROC FREQ for a Friedman's chi-square test of the differences in humidity and temperature between fields.

Statistical Analysis: 2005 and 2006. An analysis of variance (PROC ANOVA, SAS Institute 2004) was performed on the seasonwide egg mass totals from each plot in the 2006 study to determine if planting date affected total season egg mass recruitment. Longitudinal analysis of

weekly egg mass counts was also performed on the 2005 and 2006 data; the effect of planting date and time on egg mass deposition was tested using generalized estimating equations (PROC GENMOD, SAS Institute 2004). Only fields with 7 or more sampling events were used to minimize missing values. A negative binomial distribution was specified in both years' analyses to provide a more flexible distribution that could account for overdispersion.

A relative preference model developed by Spangler and Calvin (2000) was used to predict the number of egg masses found in a field based on its relative oviposition preference value compared to that of all other available stages. See Appendix B for details regarding preference calculations and specific values. Predicted distributions of egg masses across all sampled stages were generated for each week; these were compared to observed distributions with chi-square tests of proportions. Approximate total plant leaf areas, based on sampled plants, were also estimated for each plant stage (Table B.1). These were used to estimate the percentage of lifetime peak leaf area existing in each stage. A second set of predicted distributions, based on these leaf area percentages in a modified stage model, were calculated for each year and compared with chi-square tests of proportions.

Finally, PROC CORR was used to determine if corn borers laid larger egg masses on preferred plants relative to all others available. Egg mass size (i.e., number of eggs per mass) was correlated with the predicted percentage of eggs laid in its respective stage (compared to all other available stages that week). To determine if overall plant maturity was related to egg mass size, the number of eggs in each mass was also correlated with the percentage of plant development completed (Table B.1). Both correlations used combined 2005 and 2006 egg mass data.

Results

In 2005, pre-planting stalk infestation percentages ranged from 0.00 to 0.26, with estimated totals from 0 to 38,500 larvae per field. A group of three large fields in the western portion of the field study contained the highest concentration of overwintering larvae (Fig. 3.1). One of these fields, number 22, was chosen to represent the major source of early-season adult emergence. Egg mass deposition during the first two weeks of the study, however, was not correlated with field distance from this source of emerging adults (20 June: $r = -0.036$, $P = 0.850$, $n = 30$; 27 June: $r = 0.207$, $P = 0.272$, $n = 30$; 20 + 27 June: $r = 0.094$, $P = 0.621$, $n = 30$). However, egg mass numbers were negatively correlated with distance from the source field in later weeks (4 July: $r = -0.425$, $P = 0.034$, $n = 25$; 11 July: $r = -0.308$, $P = 0.098$, $n = 30$; 4 + 11 July: $r = -0.423$, $P = 0.040$, $n = 24$).

As part of the study described in Chapter 2, lab-raised European corn borers were released from a southeastern field on 19 June, 13 July, 29 July and 12 August 2005 (Fig. 3.1). Between 2000 and 2500 internally-dyed females were estimated to disperse from each release event. Correlations between field distance from the release point and egg mass deposition in the weeks after the first two releases were not significant (20 June: $r = 0.013$, $P = 0.945$, $n = 30$; 27 June: $r = -0.302$, $P = 0.105$, $n = 30$; 20 + 27 June: $r = -0.264$, $P = 0.158$, $n = 30$; 18 July: $r = 0.034$, $P = 0.880$, $n = 22$; 25 July: $r = -0.044$, $P = 0.842$, $n = 23$; 18 + 25 July: $r = 0.040$, $P = 0.866$, $n = 20$).

A total of 245 egg masses were found on 21,300 plants during sampling in 2005. Out of 5600 plants sampled in 2006, 148 egg masses were found. Week-by-week aerial maps of median plant stage and egg mass site locations for each sampled field are shown in Figs. 3.3 – 3.12. The maps show egg mass deposition in each field relative to its respective weekly plant stage. Most early-season egg masses were laid in the oldest fields. Early reproductive

stages were preferred relative to vegetative stages (Fig. 3.9), though many moths laid eggs in later vegetative stages (Fig. 3.10). Late in the season, earlier reproductive stages (i.e., R1 and R2) appeared to be more attractive than older corn (i.e., R3 and R4) (Figs. 3.11, 3.12).

The fields planted with *Bt* corn did not receive significantly different egg mass numbers than non-*Bt* fields ($Q_{MH} = 0.035$, df = 1, $P = 0.853$). Weekly temperature and relative humidity measurements were not significantly different between fields (Temp: $\chi^2 = 32.22$, df = 30, $P = 0.358$, $n = 248$; Humidity: $\chi^2 = 40.33$, df = 30, $P = 0.099$, $n = 248$).

Field size did not significantly affect the number of egg masses found in each field ($r = -0.167$, $P = 0.368$, $n = 31$). This was also evident in a multiple regression of the *EME* against eleven explanatory variables. The only variables remaining in the model were the plant density in the closest cornfield, total number of plants in the closest older field, and plant density in the closest older cornfield. All other independent variables, including field area, acres of older corn, median number of meters to older corn, number of meters to the closest cornfield, number of meters to the closest older cornfield, plant density in the closest older cornfield, and total number of plants in the field did not improve the model fit. The field plant density variable significantly contributed to the model but was eliminated due to significant correlation with the plant density in the closest field. The final best model, in the form $y = a + bx_1 + cx_2 + dx_3$, explained nearly 60% of the variation in season-wide egg mass deposition ($R^2 = 0.584$, $F = 10.28$, df = 3, 22; $P = 0.0002$); coefficients (± S.E.) were $a = 4.08 \pm 0.915$, $b = 0.0000935 \pm 0.0000243$, $c = -0.000002 \pm 0.0000008$, $d = -0.000197 \pm 0.0000373$, where x_1 = plant density in the closest field ($t = 3.85$, df = 1, $P = 0.0009$, Type 1 SS = 0.1735), x_2 = the number of plants in the closest older field ($t = -2.20$, df = 1, $P = 0.039$, Type 1 SS = 0.0515), and where x_3 = plant density in the closest older field ($t = -5.27$, df = 1, $P < 0.0001$, Type 1 SS = 2.0644).

Temporal partitioning of egg mass deposition resulted in less precise models. The only variable explaining a significant amount of variation in oviposition during the first generation

flight was the plant density in the closest older field; as plant density decreased, egg mass numbers increased (t = -1.93, df = 1, P = 0.0645; R^2 = 0.125, F = 3.73, df = 1, 27). In contrast, later-season egg mass counts were not influenced by any of the older cornfields. The second-generation model included two significant variables: field plant density (t = -4.55, df = 1, P = 0.0002) and plant density in the closest field (t = 2.85, df = 1, P = 0.0096), explaining nearly 50% of the variation in egg masses (R^2 = 0.499, F = 10.47, df = 2, 23; P = 0.0007).

The Moran's I statistic also suggested some moderate spatial autocorrelation in egg masses between neighboring fields within 250 m (I = 0.273, $\Sigma_{i \neq j} w_{ij}$ = 37, n = 27) and 300 m (I = 0.306, $\Sigma_{i \neq j} w_{ij}$ = 40, n = 27). When the definition of neighboring distance was increased to 450 m (I = 0.089, $\Sigma_{i \neq j} w_{ij}$ = 85, n = 31) and 600 m (I = 0.120, $\Sigma_{i \neq j} w_{ij}$ = 119, n = 31), evidence of spatial autocorrelation was diminished. These results, along with the multiple regression analyses, imply that egg mass counts in closer fields are spatially related.

Planting date did not affect season-long egg mass totals in either year (2005: Q_{MH} = 0.796, df = 2, P = 0.672; 2006: F = 0.95, df = 6, P = 0.523). When modeling egg mass deposition over time, neither year exhibited a significant planting date by time interaction (2005: χ^2 = 5.15, df = 2, P = 0.076; 2006: χ^2 = 6.04, df = 6, P = 0.418), indicating that planting date preferences did not change significantly over time. However, the 2005 analysis resulted in a nearly significant interaction. Furthermore, it did not include all fields; any subtle changes among planting dates is difficult to ascertain, particularly later in the season when many fields were not sampled.

Weekly predicted and observed egg mass proportions for each available stage are presented in Tables 3.2 and 3.3. Unfortunately, the oviposition preference models were not successful in predicting stage preferences of ovipositing females. All of the comparisons between predicted and observed proportions in 2005 were significantly different, with the stage model

accurately predicting oviposition preference proportions only for the last week in 2006 (Table 3.2).

Finally, the correlation between mass size and its preference value (the predicted percentage of egg mass deposition in that stage compared to all others available) was not significant (r = -0.044, P = 0.393, n = 385; both variables square-root transformed). The percentage of plant development completed also was not correlated with mass size (r = 0.057, P = 0.266, n = 385; square-root transformed egg mass size).

Discussion

These studies afforded a new look at large-scale heterogeneity in egg mass deposition and corn borer oviposition preferences. Unfortunately, the overreaching extent of the farm-level study rendered consistent sampling nearly impossible. Nonetheless, some valuable conclusions can be made from the results. First, field size apparently does not affect the proportion of plants infested with egg masses. It seems that each acre (or plant) attracted the same number of ovipositing females, contingent on other field attributes such as maturity.

Distance effects of adult dispersal on oviposition were also unapparent. A western field with a high overwintering larval population was expected to contribute a large proportion of the early moth flight. In addition, mass releases of lab-raised, internally-dyed moths were made from a point in the southeastern part of the farm. A pattern of decreasing oviposition over increasing distance was anticipated from females dispersing from the two major sources. Egg mass deposition from the weeks of 4 July and combined 4 + 11 July were negatively correlated with field distance from the natural emergence field. This was expected, but it occurred later than the typical first generation flight in Pennsylvania. No other weekly oviposition counts were significantly associated with field distance.

Marked egg masses from mass releases in the southeastern part of the study area were found in fields 3, 17, 18, 26, and 27. These colored masses did not illustrate any decay by distance from the release point, though this was dependent on the position of the sampled fields relative to the release. Egg mass deposition in the study using the colored moth release (Chapter 2) followed an exponential decay, but this effect was diluted and likely unapparent (with non-exhaustive sampling methods) beyond 50 m of the release point. Therefore, effects of field distance on oviposition are likely exhibited only at a local scale.

The field distance correlation results suggest several possible explanations. First, the fields identified as source sites may not have contributed a significant portion of all local adults. Many of the surrounding fields were privately owned and not sampled for larvae, so adults originating in other areas could have flown into the study region. The displacement of adult females emerging from the field identified in the western portion of the study landscape also depended on their chosen flight pattern (i.e., upwind motor flight or downwind displacement on air currents), which is unknown. Showers et al. (2001) cite evidence that potential female dispersal range is at least 49 km, with many flying more than 800 meters. Flight mill data from Dorhout et al. (2008) showed females tended to fly long distances, frequently at least 1 or 2 kilometers. Qureshi et al. (2005) argued dispersal propensities were much lower. They presented data showing that the majority of released corn borers were trapped within 350 m of their release point; however, low captures in farther traps due to dispersal decay indicate some moths flew well beyond 350 meters. The greatest inter-field distance in our study area was 9340 meters, below the median dispersal distance found for most moths in the Dorhout et al. (2008) study. Therefore, the pre-oviposition female dispersal distances may have been quite great.

Dispersal effects on oviposition also may not have been apparent due to local population reorganization. Females observed in Chapter 2 and the aforementioned dispersal studies exhibited different flight patterns. Qureshi et al. (2005) also noted the extensive distribution of feral moths

within their approximately 50-ha fields. After emerging, male and female European corn borers fly to "aggregation sites", or areas of dense vegetation, to mate (Showers et al. 1976; DeRozari et al. 1977; Sappington 2005). After mating, the moths disperse to different areas and generally do not return (Reardon et al. 2006). Sappington (2005) and Bailey et al. (2007) also found that corn borers' tendency to settle in aggregation sites is spatially related to the presence or absence of nearby corn. In most corn-growing areas, including the Corn Belt, aggregation sites consist of grassy field borders and waterways (Sappington 2005, Bailey et al. 2007). At the Rock Springs farm, however, most field borders and ditches were continually mowed. No traditionally-defined aggregation sites were found within the study area; the only observations of corn borer activity were made within cornfields or fields planted with forage grasses or alfalfa. Thus, emerging adults likely redistributed themselves between fields acting as mating sites.

Moths emerged over a series of days or weeks, and dispersal strategies can differ by age or mating status (Dorhout et al. 2008). Therefore, it seems nearly impossible to characterize the majority of the animals' flight paths from emergence locations to aggregation sites to fields chosen for oviposition, especially when their timing and dispersal strategies are interacting with a highly heterogeneous landscape of undefined aggregation sites. In addition, field sampling was likely too coarse-grained to find definitive oviposition patterns. Large-scale efforts will be required to more clearly assess inter-field population movement within a farm or agricultural landscape.

Plant height and maturity are often cited as major attractive factors in female corn borer choice for oviposition sites (Huber 1939, Beard 1943, Everly 1959, Spangler and Calvin 2000). However, moths also did not lay larger egg masses on plants with more advanced maturities or greater preference values. In addition, planting date did not affect the total number of egg masses found over the entire season. Plant maturity preferences over time were difficult to define; fewer fields were sampled late in the season, leading to considerable data dropout. Neither year

exhibited a significant planting period by time interaction, suggesting plant maturity preferences (if any) did not change over time. However, the weekly egg mass maps exhibit some general preference trends. During the first two weeks of the farm-scale study (Figs. 3.3, 3.4), no egg masses were found in any of the fields with the youngest two stages. After the third week (Fig. 3.5), egg masses were scattered throughout most plant stages and maturity preferences were not as apparent. By the week of 25 July, egg masses were approximately equally distributed between late-vegetative and early-reproductive stages (Fig. 3.8). Interestingly, the youngest field (16) recruited four egg masses with V10 plants. For that week, the Spangler and Calvin (2000) preference model predicted the preference for the youngest field should be about half that of the other fields. Field 16 continued to be very attractive to ovipositing females through the final week, where it had the highest number of egg masses.

The original preference model was modified to include stages V16 through VT, which are commonly completed by field corn. A leaf area preference model was also constructed, based on the leaf area of sampled plants. However, neither preference model could successfully predict the weekly proportion of egg masses laid in each stage. Subtle differences between sampled plants in each field could have contributed to the models' failure, as these probably affected the predicted preference values. Conventional sampling methods also may not accurately detect differences in oviposition choice if natural preference is not consistent or well-defined. It is possible that no model can accurately predict oviposition preference within a landscape, or that model validations are not possible without extensive sampling efforts.

European corn borer females also seem to jointly respond to planting date and plant density factors in neighboring fields. A multiple regression model of corn planting attributes suggested that egg mass deposition was positively associated with field plant density. This result agrees with Lee (1988), who also reported positive correlation between egg masses and corn stand density. However, egg masses were negatively associated with the plant totals and densities from

the closest older fields. This trend suggests that females preferred fields with denser corn stands over nearby fields with sparser stands. In addition, egg mass counts from the first European corn borer generation showed a negative association with the plant density in the closest older field. It is possible that females find denser corn stands more attractive than older corn, particularly in areas with few available aggregation sites like Rock Springs. However, this conflicts with conclusions drawn in Chapter 4, where first-generation females were more attracted to low-density plots. This is especially surprising, considering that a first-generation partition of the landscape study data also showed a nearly significant negative association between egg deposition and plant density in the closest older field.

Second-generation females were not influenced by older cornfields. Late-season trends showed a negative association between egg masses and field plant density, but a positive association with plant density in the closest field. It remains unclear how the interaction of plant totals and stand densities in close-proximity fields contributes to oviposition. None of the individual plant stand variables explained a significant amount of the variation between fields, and the impact of neighboring fields' planting dates on oviposition is also uncertain.

Egg mass deposition in neighboring fields (those located within 300 meters of one another) did exhibit evidence of spatial autocorrelation. When the definition of neighbors increased to 400 meters, however, spatial autocorrelation decreased considerably. These relationships can probably be attributed to the fact that proximate fields were often managed by the same academic unit (Agronomy, Entomology, Horticulture, and Plant Pathology), and many times a department planted the same variety over a short time frame. Therefore, the attributes of nearby fields were generally similar (Table 3.1); this may also explain why egg masses were closely associated with plant stand characteristics in neighboring fields.

These data showed that transgenic *Bt* corn plantings do not affect egg mass recruitment at a landscape or farm level, which confirms conclusions drawn by Hellmich et al. (1999) in small

plot studies. The data suggest that corn borer females cannot detect *Bt* corn while searching for oviposition sites, or at the very least, their behavior is not currently influenced by *Bt* plantings.

Hunt et al. (2001) observed increased corn borer oviposition in irrigated fields in Iowa, suggesting females fly to areas with higher humidity. Andow and Ostlie (1990) noted more egg masses in chisel-plow tillage treatments than no-till corn, and they proposed that higher temperatures in the chisel-plow plots might lead to increased oviposition. Corn at the Rock Springs research farm is not normally irrigated, and field comparisons failed to show a significant difference in weekly humidity or temperature measurements. However, a potato field adjacent to the west side of field 26 was irrigated on 22 June. The egg mass map from the week of 27 June (Fig. 3.4) indicates a much larger concentration of egg masses in that field section compared to all other areas of the farm. It is impossible to conclusively determine that the irrigation led to increased female activity and egg mass deposition in that part of the field, but the irrigation timing and location suggested increased humidity may have attracted ovipositing females.

This study offered a glimpse into farm-level corn borer dispersal and oviposition choice, though it was hampered somewhat by its large scale. The temporal and spatial extent was difficult to sustain over the entire season, especially as our sampling efficiency decreased over time. If landscape-level population dynamics are to be further clarified, finer-scale sampling will be required; this is also true if the oviposition preference of various corn stages can realistically be predicted. Since the study area covered here included small, heterogeneous fields, a comparative survey in a more homogeneous area (such as the Corn Belt) would be valuable.

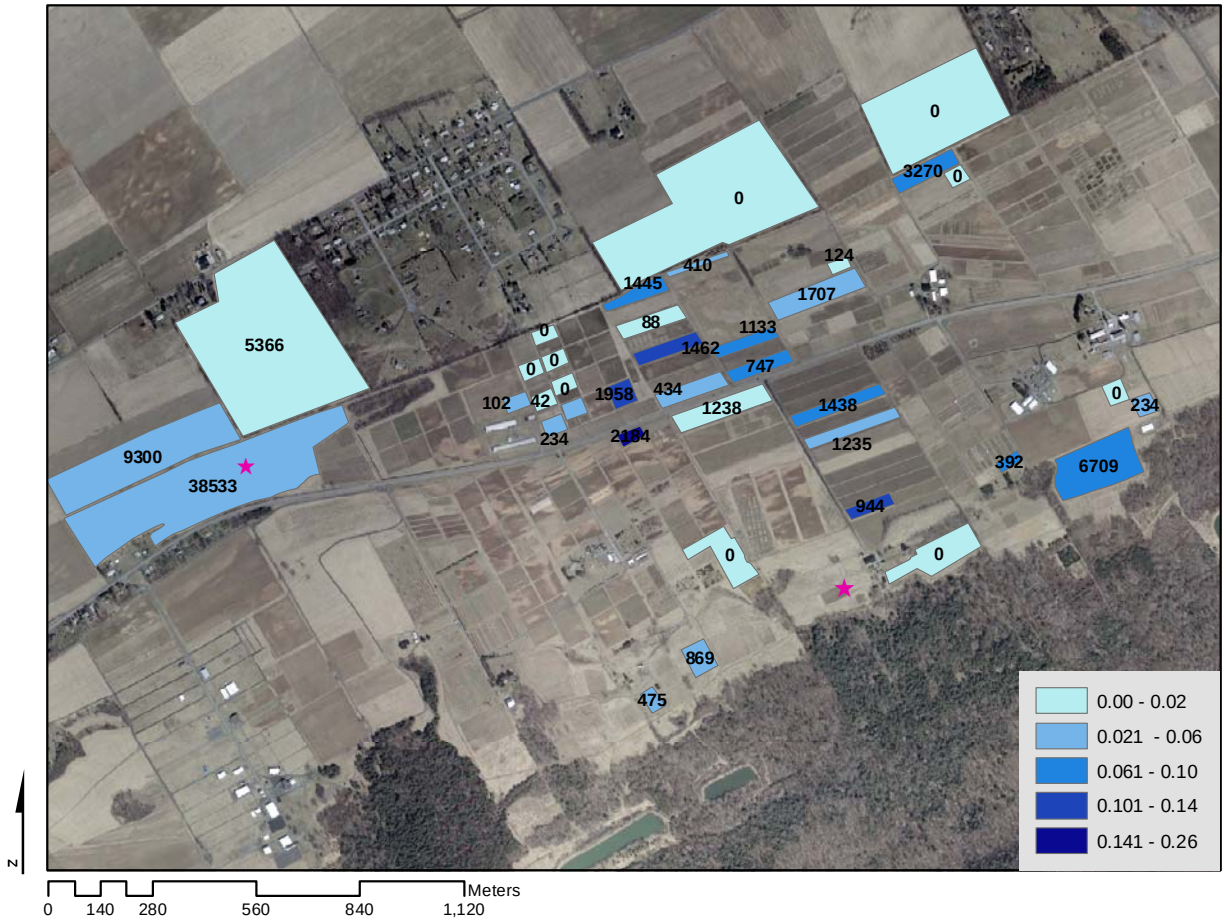


Fig. 3.1. Fields planted with corn in 2004 and stubble sampled in 2005. Numbers within fields signify the estimated total number of fifth instar larvae present, while shades indicate the number of larvae found per 100 stalks. Pink stars represent natural (west) and artificially released (southeast) adult emergence sites.

Table 3.1. Field acreage, hybrid traits, estimated plant populations, planting dates, and number of egg masses found per week in 31 fields.

Field	Area (acres)	Previous crop	Planting date	Corn hybrid	ECB *Bt* trait	Relative maturity	Plant population (x1000/acre)	Number of weeks sampled	Average number of egg masses found (per wk)
1	2.3	Corn	13 May	Pioneer 36B08	N	103	26.5	6	1.17
2	4.1	Wheat	13 May	DKC 51-43	N	106	27.5	7	0.86
3	1.6	Soybeans, Rye	26 May	Pioneer 36B08	N	103	25.5	9	1.11
4	1.1	Clover	9 May	DKC 53-34	Y	103	25.2	8	1.25
5	1.0	Cucumbers	6 May	DKC 53-34	Y	103	24.0	8	1.63
6	1.0	Wheat	6 May	DKC 53-34	Y	103	25.7	9	0.78
7	1.0	Wheat	6 May	DKC 53-34	Y	103	23.0	7	1.29
8	2.0	Corn	1 June	DKC 54-51	Y	104	26.7	8	0.75
9	2.1	Corn	1 June	DKC 54-51	Y	104	25.3	9	0.56
10	3.3	Alfalfa	16 May	DKC53-34	Y	103	28.0	9	1.33
11	2.2	Corn	9 May	FS 5737	Y	107	26.8	3	0
12	1.6	Corn, Soybeans	29 April	Pioneer 34H31	N	109	27.0	5	2.20
13	1.4	Wheat	12 May	NK N65-A1	Y	111	24.3	6	1.67
14	1.8	Soybeans	10 May	DKC 53-34	Y	103	26.0	8	0.75
15	1.2	Wheat	10 May	DKC 53-34	Y	103	25.2	8	0.25
16	2.0	Wheat	15 June	NK N65-A1	Y	111	20.5	7	2.86
17	1.7	Soybeans	5 May	DKC 51-43	N	101	29.2	7	1.29
18	1.7	Soybeans	5 May	DKC 51-43	N	101	29.3	9	0.89
19	1.8	Corn	6 May	Garst 8590 IT	N	101	29.3	7	2.00
20	1.0	Corn, Snap beans	13 May	DKC 60-13	N	110	30.0	7	0.86
21	1.0	Corn, Snap beans	13 May	DKC 60-13	N	110	29.0	6	0.67
22	28.3	Corn	10 May	DKC 58-80	Y	108	29.5	3	0.83
23	13.6	Corn	6 May	DKC 60-14	Y	110	24.8	8	1.75
24	3.5	Corn	13 May	Pioneer 36B08	N	103	24.7	3	0
25	2.3	Soybeans	8 May	DKC 53-34	Y	103	25.0	7	1.43
26	2.1	Clover	8 May	DKC 53-34	Y	103	24.5	10	1.50
27	1.9	Clover	8 May	DKC 53-34	Y	103	26.2	10	1.40
28	6.9	Corn	4 May	Pioneer 34H31	Y	107	27.3	3	0.67
29	3.6	Corn	5 May	DKC 53-34	Y	103	25.3	4	0.75
30	1.0	Corn	12 May	Trial	N	109	30.3	8	1.13
31	5.8	Corn	6 May	DKC 57-84	Y	107	25.8	4	0.25

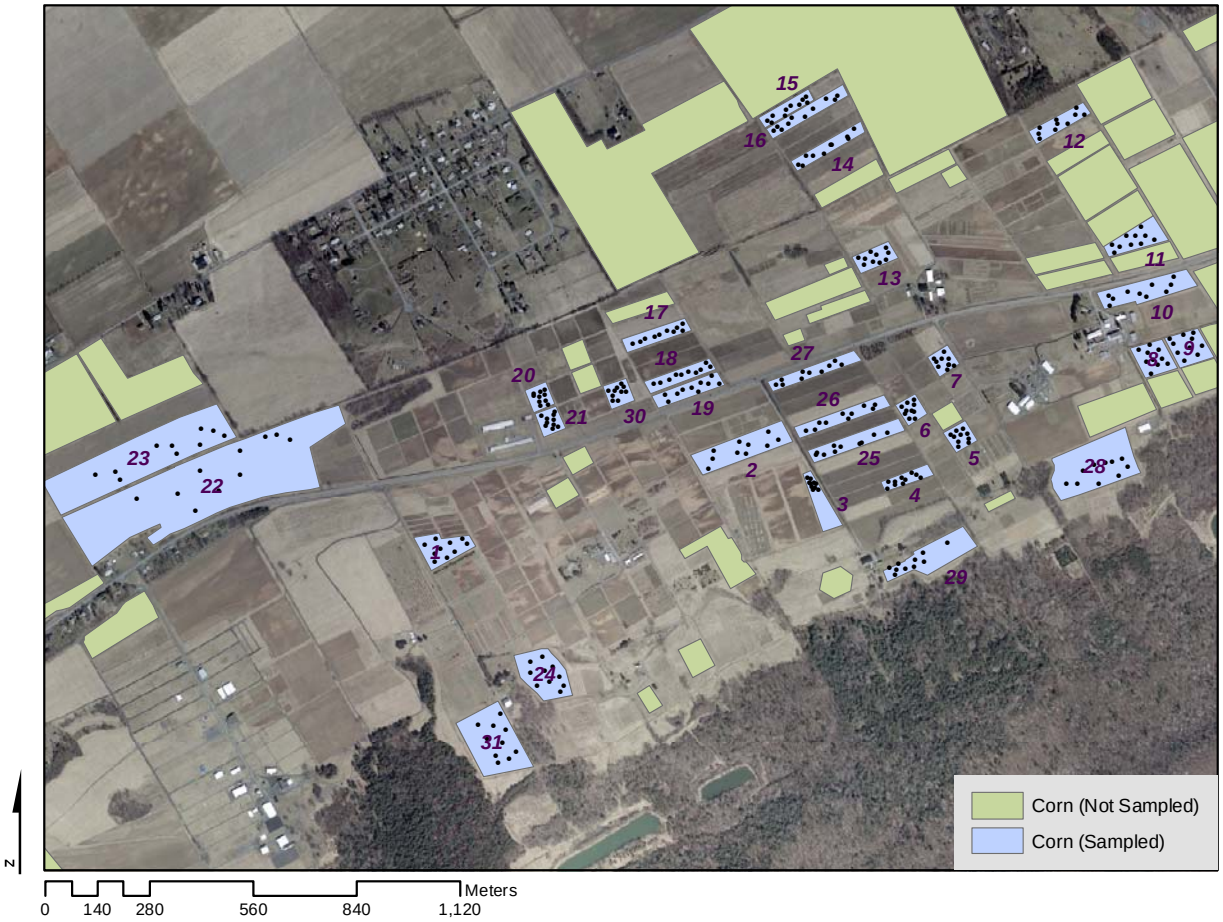


Fig. 3.2. Aerial map of the Rock Springs research farm and surrounding area. Below right is Tussey Mountain; area in upper left is privately owned. All outlined fields were in corn in 2005; blue fields were sampled in the study, while yellow fields were not. Dots represent the ten 10-plant sampling points in each field.

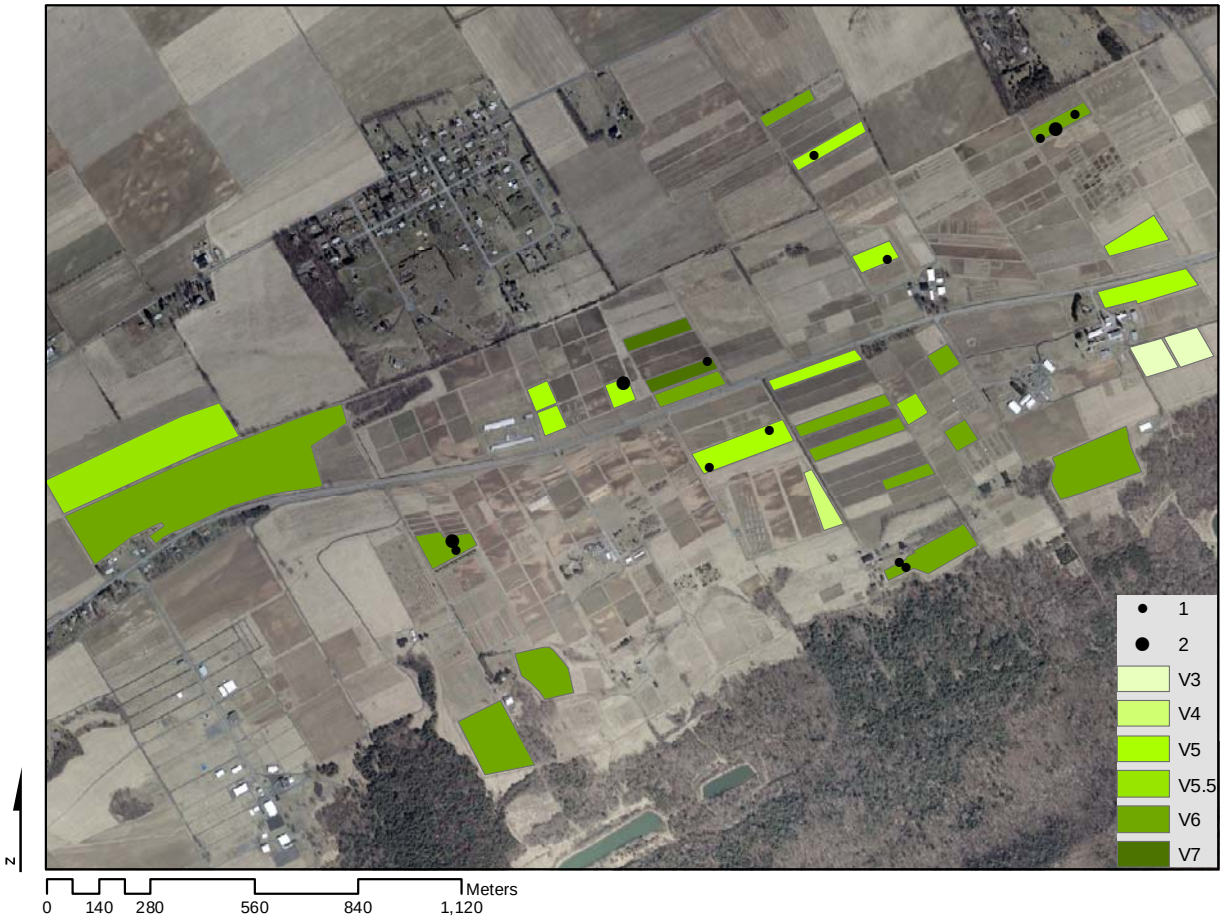


Fig. 3.3. Median plant stage (green shades) and number of egg masses found per site (black circles) in sampled fields the week of June 20, 2005.

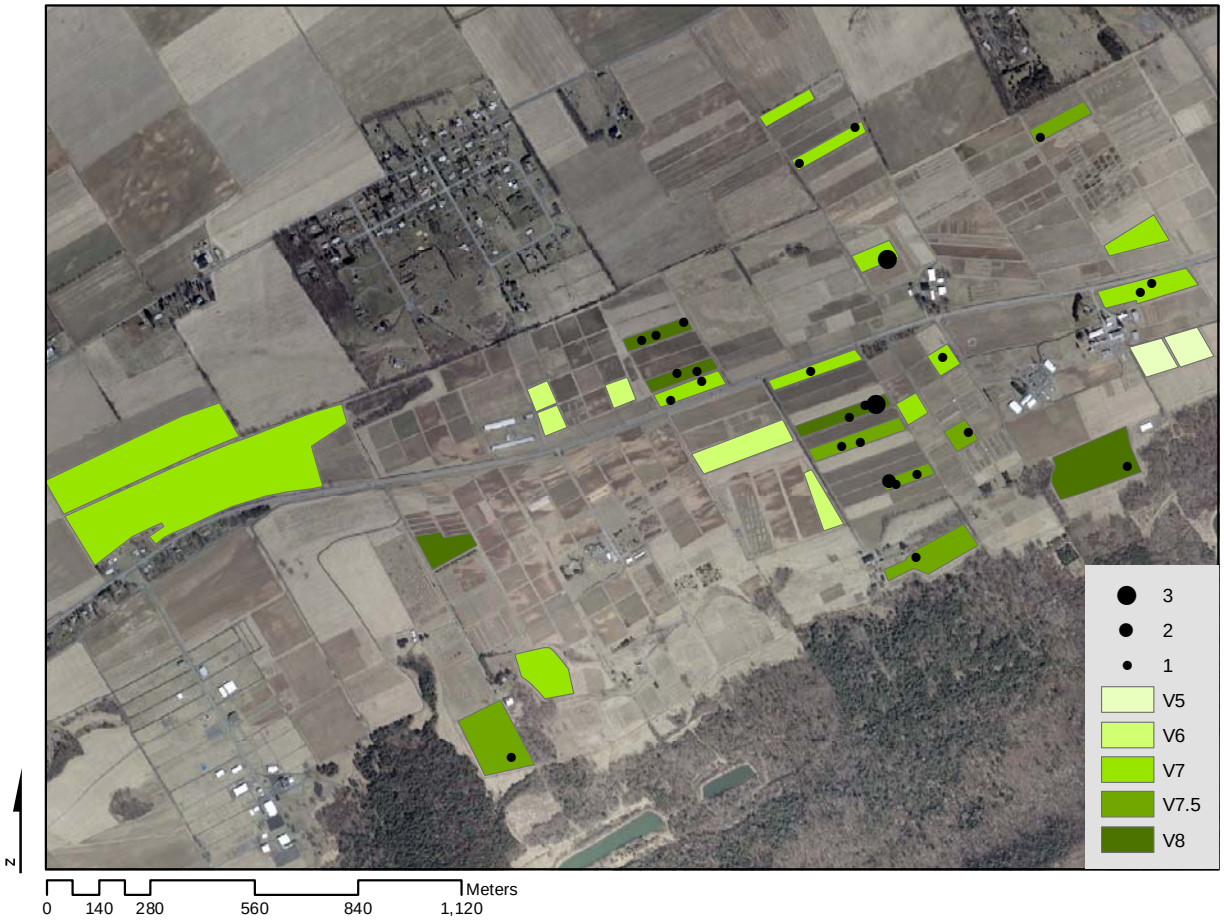


Fig. 3.4. Median plant stage (green shades) and number of egg masses found per site (black circles) in sampled fields the week of June 27, 2005.

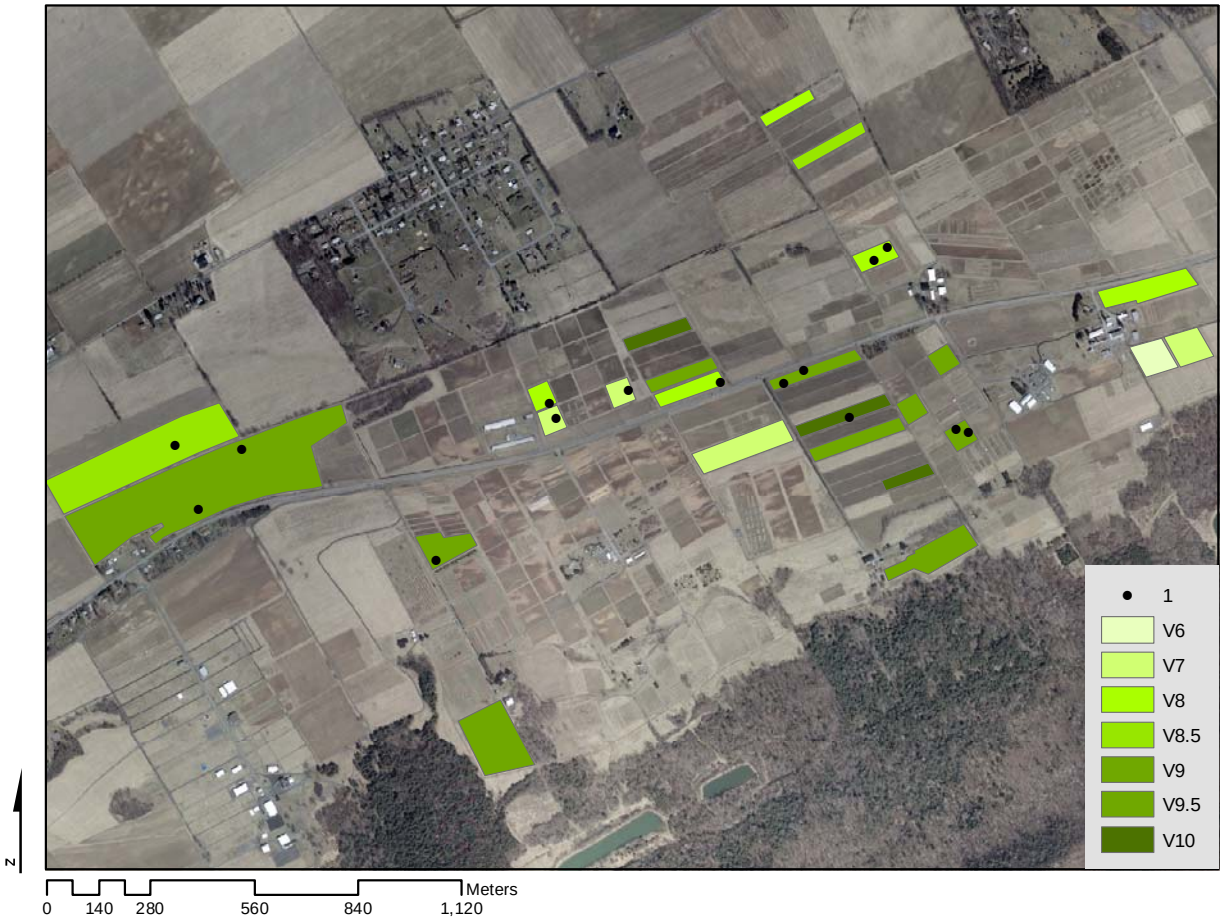


Fig. 3.5. Median plant stages and egg masses found in sampled fields the week of July 4, 2005.

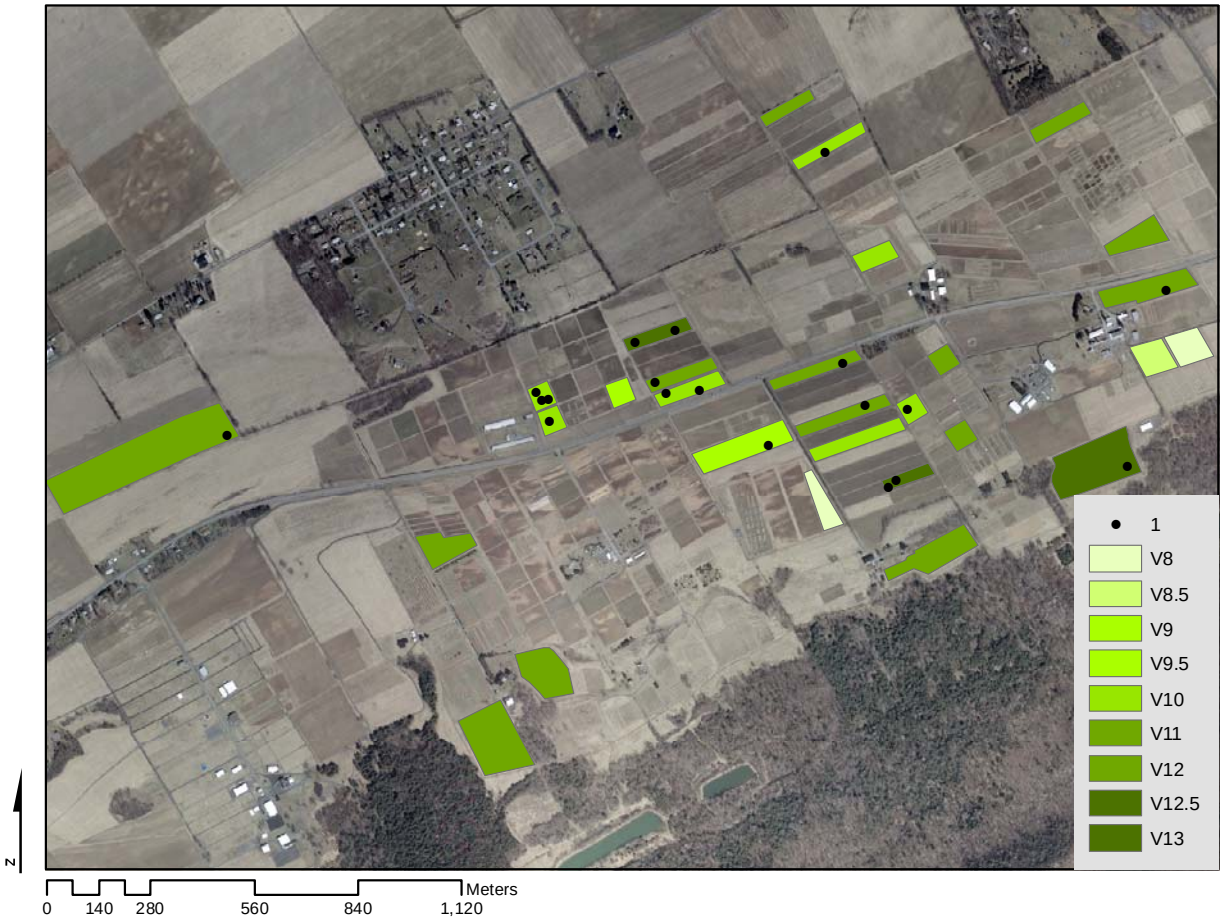


Fig. 3.6. Median plant stages and egg masses found in sampled fields the week of July 11, 2005.

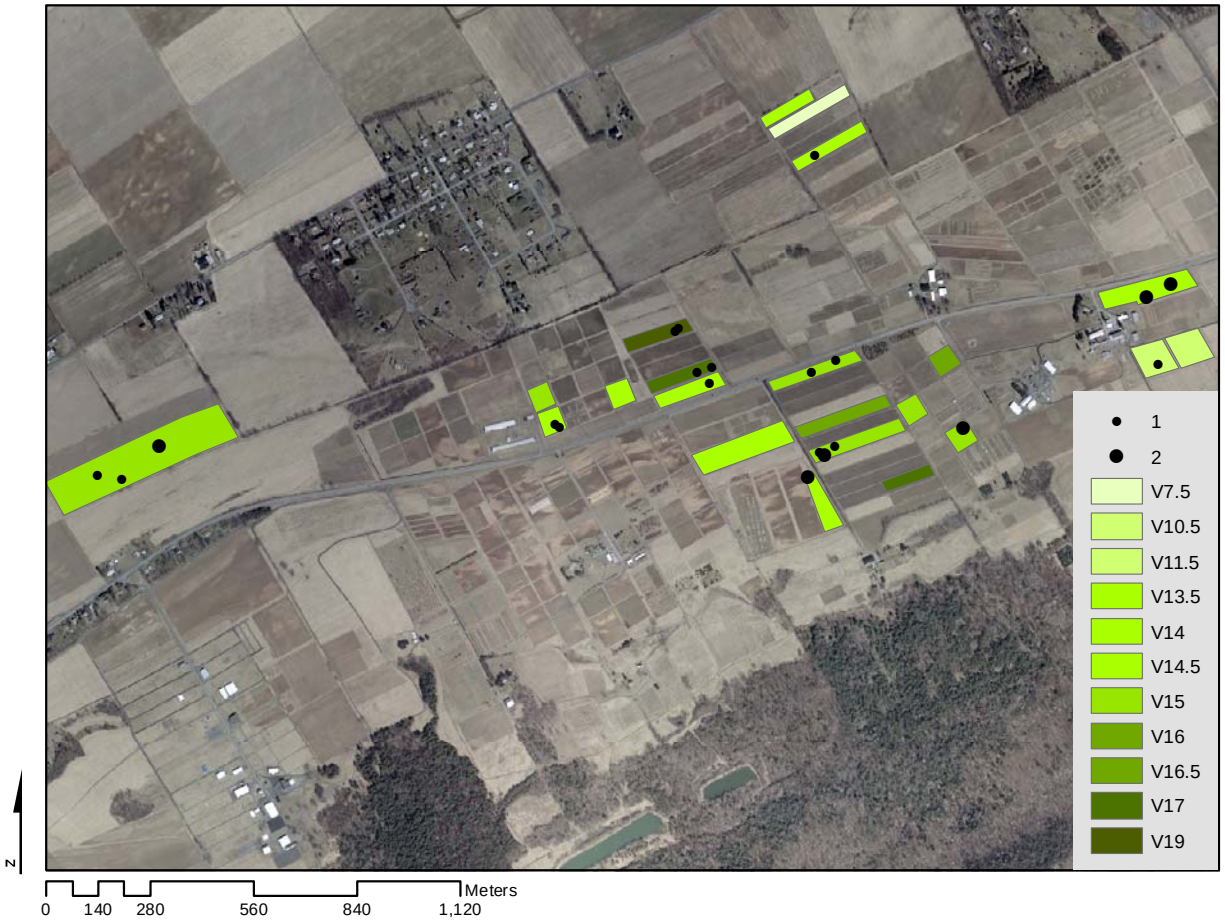


Fig. 3.7. Median plant stages and egg masses found in sampled fields the week of July 18, 2005.

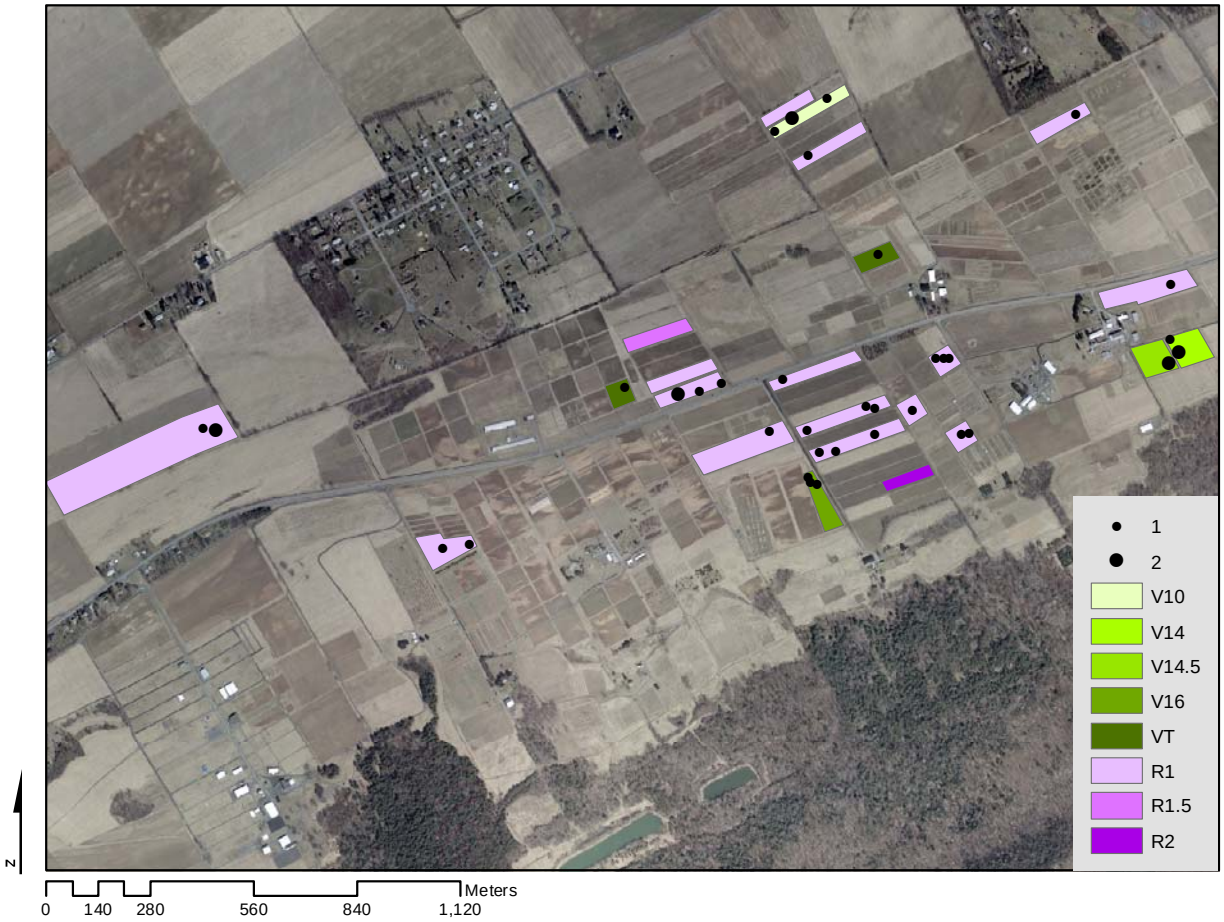


Fig. 3.8. Median plant vegetative (green) or reproductive (purple) stages and egg masses found in sampled fields the week of July 25, 2005.

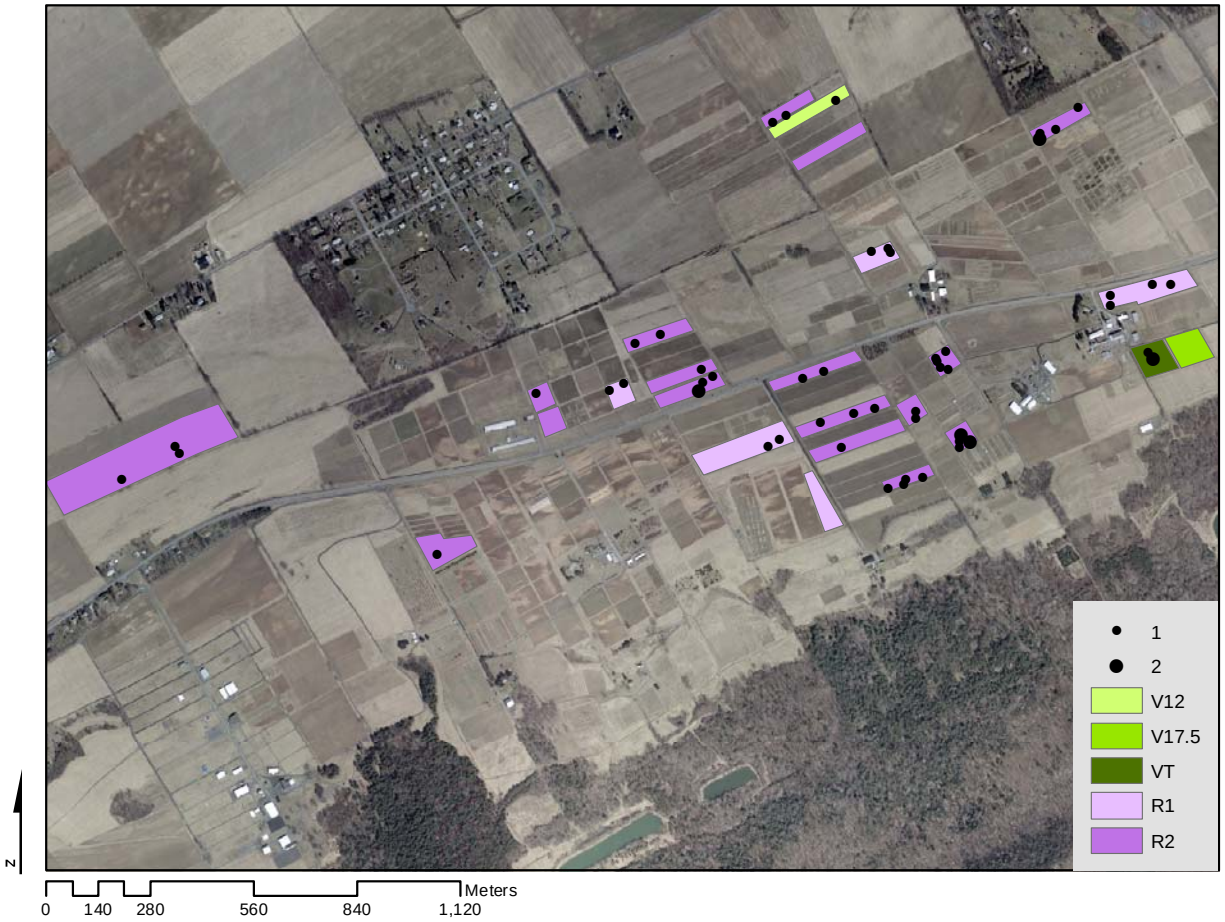


Fig. 3.9. Median plant stages and egg masses found in sampled fields the week of August 1, 2005.

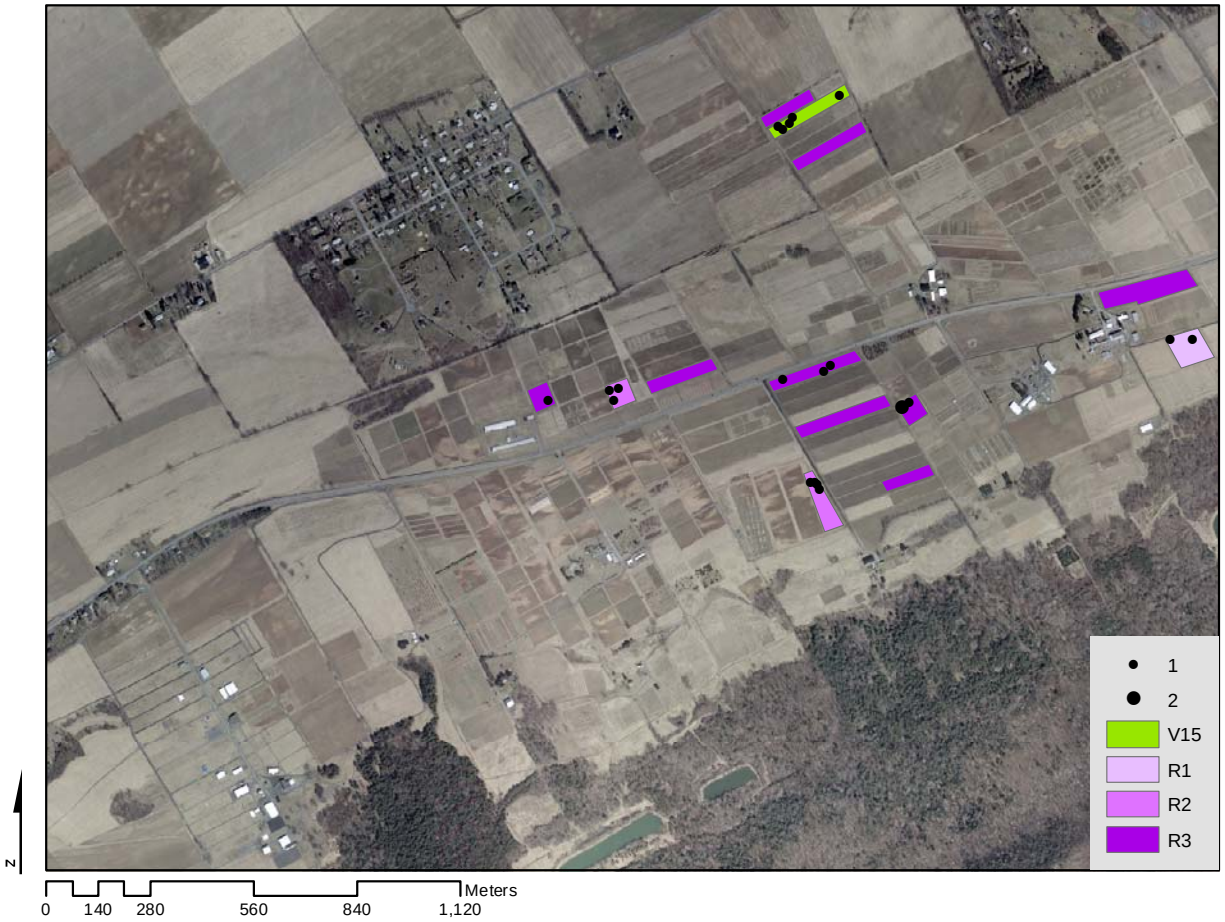


Fig. 3.10. Median plant stages and egg masses found in sampled fields the week of August 8, 2005.

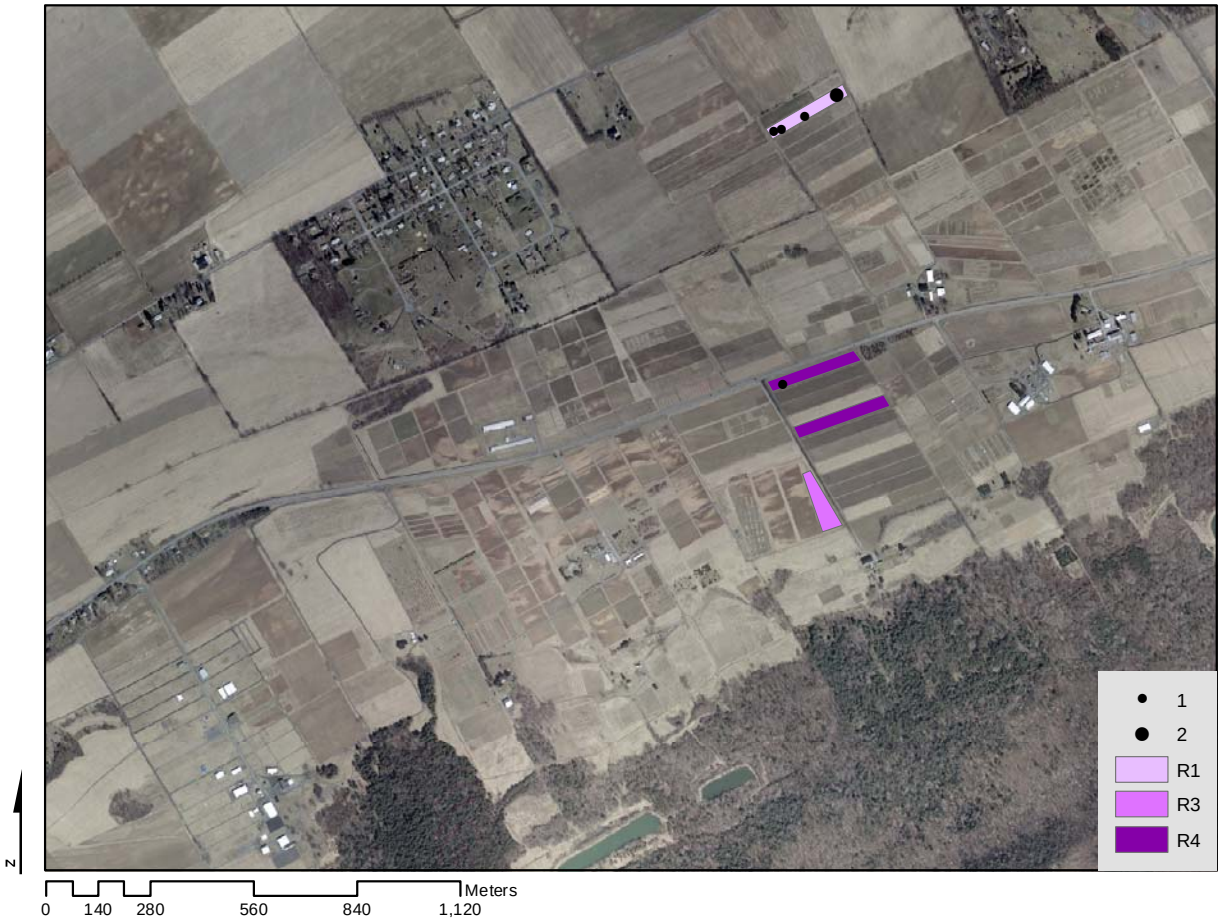


Fig. 3.11. Median plant stages and egg masses found in sampled fields the week of August 15, 2005.

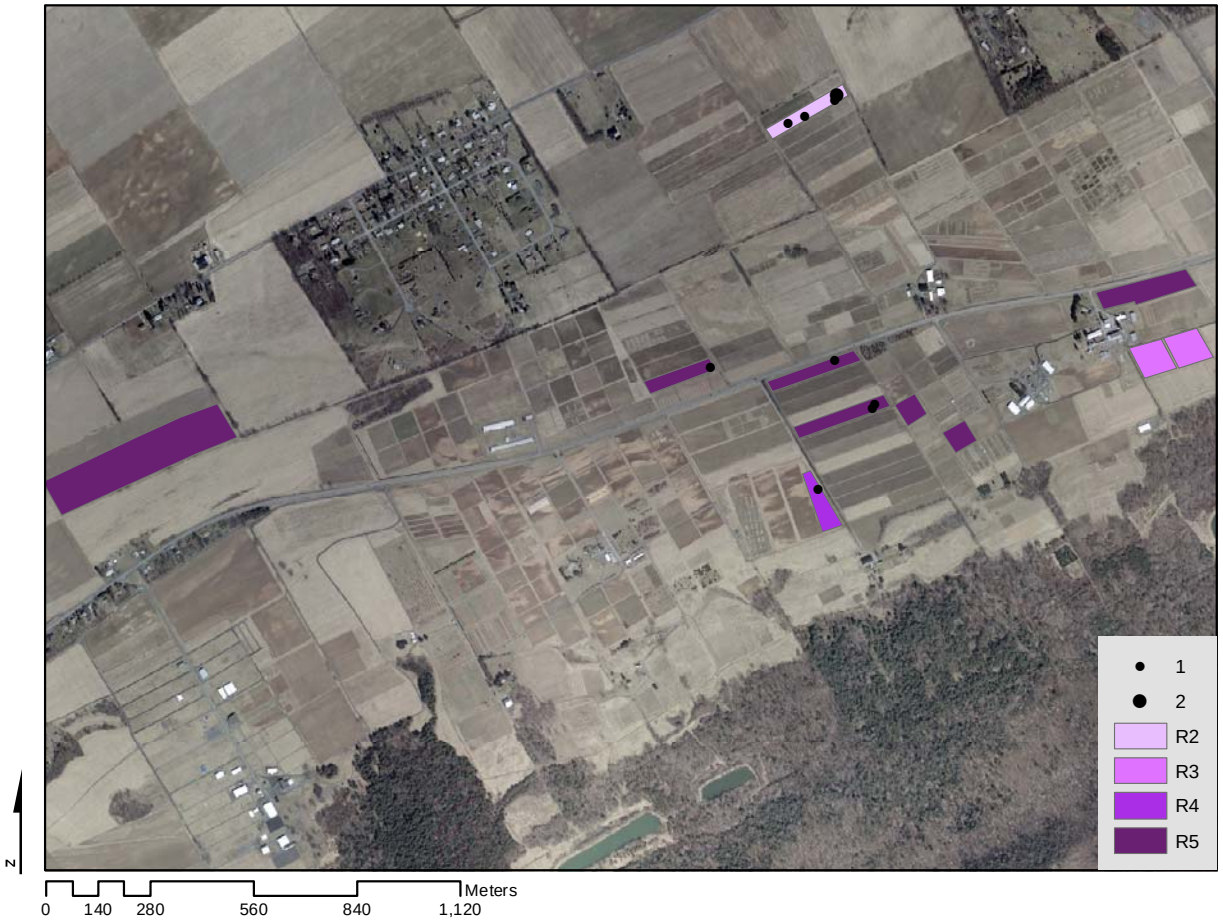


Fig. 3.12. Median plant stages and egg masses found in sampled fields the week of August 22, 2005.

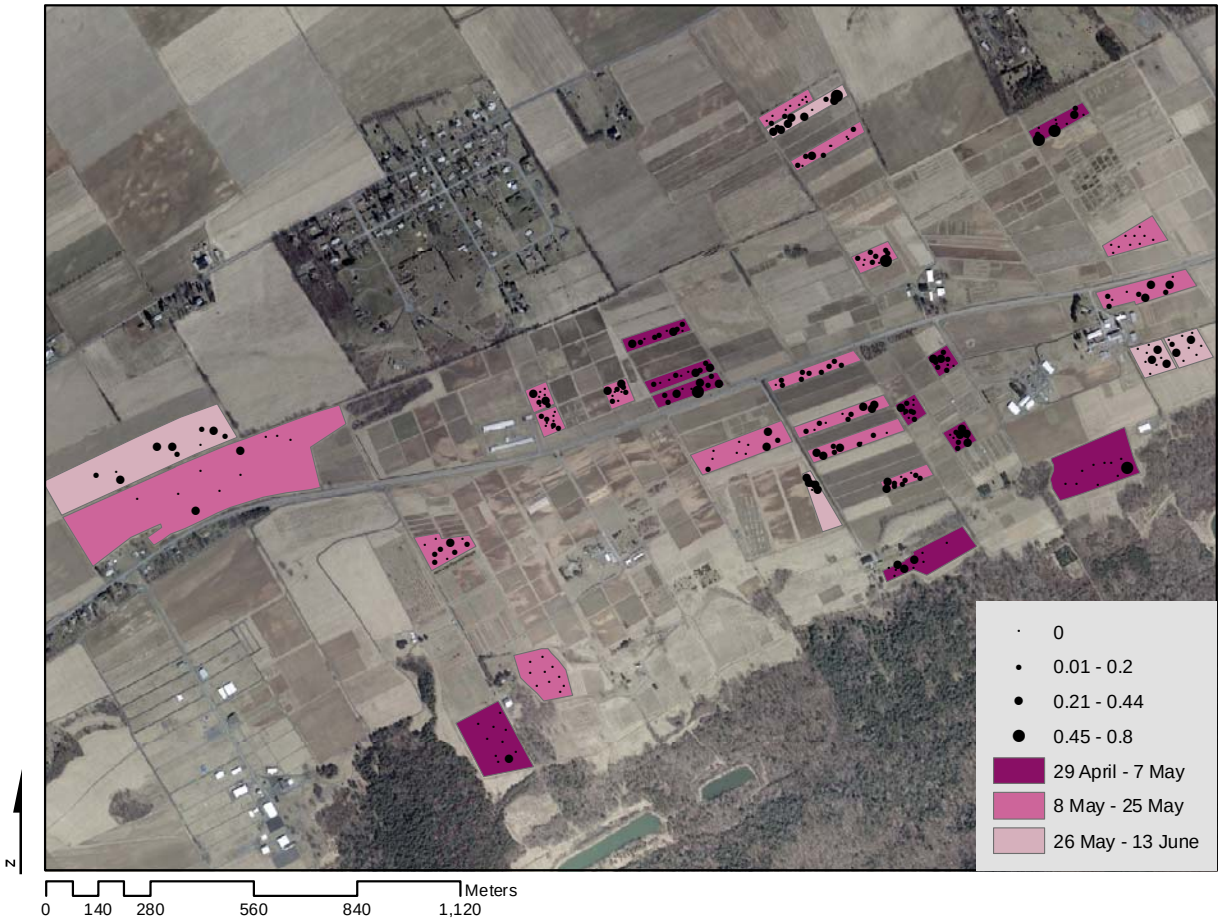


Fig. 3.13. Egg mass deposition per sampling site: dot size indicates the total number of masses found at each site divided by the number of weeks it was sampled. Shading represents field planting date.

Table 3.2. Predicted and observed proportions of egg masses laid in plant stages sampled each week in 2005. Chi-square test of proportion statistics are shown below each week; *n* is the number of fields sampled.

	June 20		***June 27***		***July 14***		***July 11***		***July 18***		***July 25***		***August 1***		***August 8***		***August 15***		***August 22***	
	Pred	Obs	Pred	Obs	Pred	Obs	Pred	Obs	Pred	Obs	Pred	Obs	Pred	Obs	Pred	Obs	Pred	Obs	Pred	Obs
V3	0.04	0																		
V4	0.07	0																		
V5	0.13	0.4	0.07	0																
V5.5	0.17	0																		
V6	0.21	0.34	0.11	0	0.11	0.03	0.01	0												
V7	0.38	0.26	0.2	0.19	0.19	0.06														
V7.5			0.27	0.35					0.01	0										
V8			0.35	0.46	0.33	0.1	0.03	0												
V8.5					0.45	0.13	0.04	0												
V9					0.57	0.17	0.05	0.16												
V9.5					0.78	0.23	0.08	0												
V10					1	0.29	0.1	0.19			0.05	0.27								
V10.5									0.05	0										
V11							0.13	0.07												
V11.5									0.07	0.11										
V12							0.17	0					0.14	0.12						
V12.5							0.19	0.33												
V13							0.2	0.25												
V13.5									0.1	0										
V14									0.1	0.15	0.12	0.2								
V14.5									0.1	0.17	0.12	0.14								
V15									0.11	0.23					0.22	0.44				
V16									0.11	0	0.13	0.2								
V16.5									0.11	0										
V17									0.11	0.11										
V17.5													0.2	0						
V19									0.11	0.23										
VT											0.13	0.07	0.21	0.35						
R1											0.14	0.12	0.22	0.25	0.25	0.18				
R1.5											0.15	0								
R2											0.15	0	0.23	0.29	0.26	0.31			0.25	0.76
R3															0.27	0.07	0.5	0	0.25	0
R4																	0.5	1	0.25	0.15
R5																			0.25	0.09
χ^2	91.98		23.43		100.16		78.23		71.99		134.07		32.04		40.24		99.91		145.00	
df	5		4		6		9		10		7		4		3		1		3	
P	<0.0001		0.0001		<0.0001		<0.0001		<0.0001		<0.0001		<0.0001		<0.0001		<0.00001		<0.0001	
n	30		30		25		29		22		23		25		13		4		11	

Table 3.3. Predicted and observed proportion of egg masses laid in available field plot growth stages on eight days in 2006. Chi-square test of proportion statistics are shown below each week.

	June 22		***June 28***		***July 6***		***July 13***		***July 21***		***July 31***		***August 7***		***August 23***	
	Pred	Obs	Pred	Obs	Pred	Obs	Pred	Obs	Pred	Obs	Pred	Obs	Pred	Obs	Pred	Obs
V2	0.03	0														
V4	0.09	0.25	0.03	0												
V5	0.16	0.13	0.05	0.13												
V6	0.26	0.38	0.08	0.2	0.02	0.06										
V7	0.46	0.25	0.15	0.3			0.02	0.15								
V8			0.26	0.18	0.07	0.13										
V9			0.44	0.2	0.11	0.19	0.06	0.15	0.03	0.05						
V10					0.2	0.32	0.11	0.3	0.05	0.1						
V11					0.26	0.17	0.14	0.11								
V12					0.35	0.13	0.19	0.08								
V13							0.23	0.1	0.1	0.1	0.14	0.26				
V14							0.24	0.11	0.11	0.13	0.15	0				
V15									0.11	0.1						
V16									0.12	0.13			0.23	0		
V17									0.12	0.25	0.17	0				
V18									0.12	0						
VT									0.12	0	0.17	0.39				
R1									0.13	0.15	0.18	0.04	0.25	0.44		
R2											0.19	0.31	0.26	0.34	0.33	0.26
R3													0.26	0.22		
R4															0.33	0.35
R5															0.33	0.39
χ^2	48.56		63.12		45.13		147.79		46.95		90.17		41.23		2.55	
df	4		5		5		6		9		5		3		2	
P	<0.0001		<0.0001		<0.0001		<0.00001		<0.0001		<0.00001		<0.0001		0.2794	

Table 3.4. Chi-square tests of proportions for predicted vs. observed proportions of plants in each available stage, using the original stage model and the modified stage model based on percentage of net lifetime leaf area present.

Year	Week	Model	χ^2	df	*P*
2005	20 June	Original	92.0	5	< 0.0001
		Leaf area	79.8	5	< 0.0001
	27 June	Original	23.43	4	0.0001
		Leaf area	36.3	4	< 0.0001
	4 July	Original	100.2	6	< 0.0001
		Leaf area	56.2	6	< 0.0001
	11 July	Original	78.2	9	< 0.0001
		Leaf area	88.9	9	< 0.0001
	18 July	Original	72.0	10	< 0.0001
		Leaf area	77.5	10	< 0.0001
	25 July	Original	134.1	7	< 0.0001
		Leaf area	95.4	7	< 0.0001
	1 August	Original	32.0	4	< 0.0001
		Leaf area	33.2	4	< 0.0001
	8 August	Original	40.2	3	< 0.0001
		Leaf area	34.4	3	< 0.0001
	15 August	Original	99.9	1	< 0.0001
		Leaf area	109.3	1	< 0.0001
	22 August	Original	145	3	< 0.0001
		Leaf area	119.3	3	< 0.0001
2006	22 June	Original	48.6	4	< 0.0001
		Leaf area	125.9	4	< 0.0001
	28 June	Original	63.1	5	< 0.0001
		Leaf area	22.4	5	0.0004
	6 July	Original	45.1	5	< 0.0001
		Leaf area	16.7	5	0.0051
	13 July	Original	147.8	6	< 0.0001
		Leaf area	50.4	6	< 0.0001
	21 July	Original	47.0	9	< 0.0001
		Leaf area	43.4	9	< 0.0001
	31 July	Original	90.2	5	< 0.0001
		Leaf area	89.6	5	< 0.0001
	7 August	Original	41.2	3	< 0.0001
		Leaf area	39.9	3	< 0.0001
	23 August	Original	2.55	2	0.2794
		Leaf area	7.5	2	0.0235

CHAPTER 4.

Effects of Plant Height, Maturity, Leaf Area, and Density on European Corn Borer Oviposition

Introduction

The European corn borer, *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae), is one of the most severe corn pests in North America. Its influence can be seen in the current widespread use of transgenic corn events expressing the toxin *Bacillus thuringiensis* kurstaki (*Bt* corn). *Bt* plantings offer season-long protection from European corn borer damage, negating the need for insecticide sprays or cultural controls such as planting date manipulation. Early recommendations for controlling larval infestations involved resistant corn strains and late plantings to avoid European corn borer attraction to taller corn (Huber 1939). More recent management recommendations are based on the number of generations in the region, but they highlight the advantage of early plantings (Mason et al. 1996). In areas with two or more generations per year, early-planted corn can avoid some yield loss from second-generation larvae and increase yield potential. However, crops planted too early can be highly attractive to first-generation adults and suffer severe damage.

The earliest European corn borer oviposition preference studies cited taller plants as more attractive, though as time passed the theory was rephrased as a preference for more mature or phenologically advanced corn (Huber 1939, Everly 1959, Spangler and Calvin 2000). The latter is more inclusive of the possible reasons for such preferences, because a variety of plant traits naturally increase with plant maturity: height, leaf area, and ostensible volatile production. Work by Spangler and Calvin (2000) addressed European corn borer oviposition preference in various stages of sweet corn. They observed a positive relationship between egg deposition and plant

maturity during vegetative stages, but reproductive stages were the most preferred. Their conclusions concurred with bivoltine generational preferences for plant maturities present in the field: early plantings are generally chosen by first-generation females, while later plantings are favored by the second flight (Mason et al. 1996).

Differences in egg-laying among plant stages leads to a natural question: Which aspects of plant maturity drive female preference? Other than choice experiments comparing oviposition between planting dates, no experiments have been conducted to directly address possible effects of leaf area or plant height. Evaluating the effect of leaf area and plant height on oviposition preference would be valuable in several respects. First, knowledge about individual plant choice would clarify how the moths perceive plants. Other authors have explored the impact of plant leaf chemicals on European corn borer ovipositional choice; however, most of these studies were conducted under highly artificial conditions (Derridj et al. 1989, Binder et al. 1995, Udayagiri and Mason 1997). Second, information about plant-trait preferences would help pest managers more precisely define which fields are more attractive and susceptible to plant injury throughout the growing season. Finally, refined knowledge about oviposition preference could help insecticide resistance management efforts. In the case of *Bt* corn, crops could be manipulated to attract ovipositing females to refuge plantings. Attractive plant traits could even provide the basis for creating new corn hybrids that lack these qualities.

This study used growth chamber, field cage and field plot experiments to examine the effects of changing plant maturity traits (i.e., leaf area and plant height) on corn borer oviposition. The first set of experiments was designed to characterize any effects that plant height may have on egg-laying. Actual plant height did not differ between treatments; rather, it was visually simulated by placing plants on stands. This was done to eliminate the confounding effect of differing leaf area between plants of varying height. The second set of experiments was designed to determine the impact of leaf area on oviposition; these utilized plots of varying maturity but equal plant leaf

area to analyze differences in egg mass deposition. Finally, a larger field plot experiment was conducted to address the interaction of plant maturity, plant density, and microclimate factors on corn borer oviposition preference.

Materials and Methods

All plants used for the following studies were a conventional (non-*Bt*) 101-day field corn hybrid (DeKalb DKC51-43). Pots used in the growth chamber and field cage experiments were 7.33 liter heavy-duty plastic pots (Nursery Supplies Inc., Chambersburg, PA). Field cages were used for several experiments. These were placed within a one-acre (0.40 ha) cornfield. Part of the area surrounding the cages was planted with fill corn; the remainder of the field was used for a separate study.

Growth chamber experiments were conducted in a walk-in Conviron chamber (CMP4030, Controlled Environments Ltd, Winnipeg, CA) set at 80% RH and 25:21°C on a 16:8 L:D schedule; lights and humidifiers were covered with nylon net fabric to prevent moths from flying into the fixtures. The central fan area was also blocked with a muslin sheet to reduce wind flow within. Plants were grown in the chamber using a 3:1 mix of topsoil:sphagnum peat moss. The topsoil was obtained from Rock Springs Farm and sterilized, then mixed with 19-6-12 Osmocote® granules (Scotts-Sierra Horticultural Products Co., Marysville, OH) according to manufacturer recommendations.

Waxed, corrugated cardboard rings containing between 500 and 1000 lab-reared European corn borer pupae were shipped via overnight courier from the USDA-ARS Corn Insects and Crop Genetics Research Unit in Ames, IA. After the initial setup for each experimental run in a field cage or growth chamber, one-half of one ring was placed on a stool in the center of the chamber and moths were allowed to emerge over two to three days. Each plant was then checked daily for

new egg masses; the number of eggs per mass, leaf level of each mass, and plant stage (V1-18; R1-5 as described by Ritchie et al. 1992) were recorded. All egg masses were removed with a cotton ball soaked in 70:30 ethanol:distilled water after observation to minimize their influence on subsequent egg-laying.

Height treatment experiments: Field cages. All height experiments used a randomized complete block arrangement with subsampling. Potted corn plants were grown using topsoil obtained from the Russell Larson Experimental Field Station at Rock Springs, Pennsylvania. Most of the farm is located on Hagerstown silt loam soil. The plants were watered daily and fertilized with all-purpose 20-20-20 fertilizer according to manufacturer recommendations. Two 13 x 11 x 7.5 ft (3.96 x 3.35 x 2.27 m) Coleman Insta-Clip Screenhouses (C.I. 9392B131, The Coleman Company, Inc. Wichita, KS) were set up as north and south blocks. Each cage had a center height of 7.92 ft (2.41 m) and was constructed with mesh sides and a 50+ UPF-rated polyester fabric ceiling. The screenhouse support poles were slipped over rebars secured in the ground, and soil was heaped on the bottom flaps. Within each cage, three rows of six holes were dug and a pot was placed in each hole. One corn plant was randomly assigned to each hole position. Nine positions in each cage block were randomly assigned to the raised height treatment, while the remaining plants were placed in-ground. The raised plants were placed on stands set over the holes. Each stand was constructed of 4 in (10.24 cm) schedule 40 polyvinyl chloride pipe bracketed to a 12 in (30.5 cm) square piece of plywood. The other end of the pipe was set into a toilet flange screwed to another plywood square. Each stand had a finished height of 32 in (81.3 cm). Cement blocks were placed on the stand bases to prevent tipping, and all plants were watered periodically to ensure similar soil moisture (raised plants every three days, in-ground plants weekly). Raised plants were approximately 3.42 ft (1.04 m) higher than the in-ground treatment. Each experimental run of observations lasted nine days, and two runs with re-

randomized positions were conducted in 2006. Plant stages ranged from V8 to V10 and V6 to V7 for the first and second runs, respectively.

After emergence, most moths tended to rest on the screenhouse ceiling. This was most likely due to moisture collecting on the fabric, but could have been a natural tendency to fly upward for dispersal. Even though most of the raised plants did not touch the ceiling, many moths also rested on the raised plants' upper leaves. Since the microclimate imposed on the field experiment (i.e., free water availability, reduced wind flow and/or daytime temperatures) likely affected the moths' behavior, the experimental design was changed slightly in 2007. Two blocks were set up in the same configuration, but no cages were used. Each potted plant was inserted into an empty pot screwed to the top of each stand for stability. The in-ground treatment was modified as an on-ground treatment whereby pots were screwed onto squares of plywood and set on the ground rather than in-ground (to prevent raised plants from drying out more quickly than unraised plants). All plants were watered daily. In the 2007 design, raised plants were 32 in (81.3 cm) higher than the on-ground treatment.

Local corn borer populations were supplemented with lab-raised pupae set in a release box constructed of plywood and plastic garden mesh; this was mounted on a fence post located 1 m from the blocks. Two rings of pupae were hung in the box every two to three weeks. Due to low population densities in the area, plants were observed for 20 days in each of two experimental runs. The experiment was set up on 24 June with V7 plants and re-randomized on 9 August with V8 plants. Plants were approximately stage V13 at the end of each run.

Height treatment experiments: Growth chamber. The chamber was divided into four blocks, with plants randomly assigned to four positions in each block (16 total). Two positions in each block were randomly chosen as raised treatment plants and placed on the same stands used in the field experiments. All other pots were placed on the floor. Two experimental runs were

conducted, each lasting eight days; stage V5-V8 and V9-V11 plants were used for the first and second runs, respectively.

Equal leaf area experiments: Field cages. Randomized complete block designs were used for all leaf area experiments. In 2006 and 2007, six 14 x 16 ft (4.27 x 4.88 m) blocks were divided into 14 x 8 ft (4.27 x 2.44 m) plots. Plots in each block were randomly assigned to an early or late planting date. Early plantings were seeded with 30 in (76.2 cm) row spacing on 10 May 2006 and 13 May 2007. Late planting plots were planted on 15 June 2006 and 19 June 2007 in 15 in (38.1 cm) rows. All plots were periodically treated with 20-20-20 fertilizer according to manufacturer guidelines.

Before each experiment, the plants around the edge of two adjacent blocks were cut down to accommodate 13 x 11 x 7.5 ft (3.96 x 3.35 x 2.27 m) screenhouse cages. After the cages were secured, any plants touching the interior sides of the cages were cut at ground level. At least three plants cut from each planting date were measured with respect to height and leaf area. All fully-emerged, green leaves from each sample plant were cut at the leaf collar; any leaves not fully emerged were cut at the collar level of the last fully emerged leaf. Leaf length (cm) and widest part of each leaf (cm) were recorded. The area of one side (cm^2) was also measured with a LI-COR™ Portable Leaf Area Meter (Model LI-3000, Lambda Instrument Corp., Lincoln, NE). Each plant's total leaf area and pre-cut height (distance from ground to last fully emerged leaf collar) were used in a regression model to estimate total leaf area of the remaining plants in the screen cages. A new regression model was generated for each experiment.

The heights of the remaining plants in each plot were measured, and the regressions of the sample plant leaf areas were used to determine per-plant total leaf area. The leaf areas of both plots were then equalized by cutting out plants from each planting date. The total leaf area for each experiment was approximately 40,000 to 45,000 cm^2 per plot. Three experimental runs of

six days were conducted each year. Before the second and third runs each year, the cages were moved to the next set of plots.

Before the cages were set up for the third run in both years, 1.5 ft (0.46 m) extensions were made by screwing wood feathering strips to lengths of blue tarpaulin material and the tarp strips at the base of each cage. Washers were also welded to rebars, which were then pounded into the ground. The cage supports were slipped over the rebars down to the washers, which formed a base for the raised cages. Finally, soil was heaped over the tarp strips on all sides of the cage. The raised cages ensured that no plants touched the ceiling, which was found to influence corn borer behavior in the field cage height experiment.

In the 2006 experiments, plant stages for the respective early and late plantings ranged from V14 – R1 and V5 – V8 during the first run, R2 – R4 and V12 – V16 for the second run. The plant growth stages of early and late plantings were R1 and R5 during the final run. In 2007, plant stages were V15 – R1 and V6 – V8 (first run), R1 – R2 and V8 – V11 (second run) and R4 – R5 and V16 – R1 (third run).

Equal leaf area experiments: Growth chamber. In the growth chamber, two sets of potted plants were seeded 20 days apart. Before each experimental run, the heights and leaf areas of four plants from each planting date were measured. Leaf area was regressed against plant height to estimate the remaining plants' leaf areas.

The growth chamber was divided into two blocks corresponding to the area under each light fixture. The blocks were divided into two plots, with each planting date treatment randomly assigned to each plot. The number of plants in each plot were manipulated to ensure equal leaf areas between all plots. Two experimental runs were conducted, each lasting four days. During the first run, eight plants (four per block) from the early planting and 10 (eight per block) late-planting plants were used. Early- and late-planting stages ranged from VT – R1 and V9 – V12

during the experiment. Ten early (five per block) and 20 late (10 per block) plants were set up for the second run, with respective R1 and V12 – V14 stages.

Equal number of plants experiment: Growth chamber. Two sets of plants were grown in the growth chamber, approximately 22 days apart. As in the equal leaf area growth chamber experiments, the chamber was divided into two blocks with the planting date treatments randomly assigned to the two plots within each block. Five plants from each planting were positioned in each plot. Two six-day experimental runs were conducted. Early-planting stages were R2 – R3 and R2 during the first and second runs, respectively; the late-planting treatment stages ranged from V11 – V16 and V6 – V10.

Planting date—density field experiment. A 1.8-acre (0.728 ha) field was planted as a two-factor factorial of two planting dates and two plant densities with four replicates. Each plot covered 10,000 sq. ft (929 sq. meters) and was planted on 9 May and 1 June 2007 using 30 in (0.76 m) rows at 5.5 in (14.0 cm) or 8.88 in (22.5 cm) seed spacing. High and low seed densities resulted in plant populations from 36,000 – 39,000 plants per acre (high density) and 23,000 – 24,000 plants per acre (low density). Small areas of filler corn (~6,000 sq. ft) were planted on two opposite edges of the field on 1 June at the high density. Fertilizer (10-30-10) was applied at 100 lbs/acre (112.1 kg/ha) at planting. Plots also received a 22 gal/acre (205.8 liters/ha) herbicide application (s-metolachlor 35.8%/atrazine 28.1% + isoxaflutole + paraquat + nonionic surfactant; urea-ammonium nitrate solution carrier) the day after planting. A 28 gal/acre (261.9 liters/ha) topdress with 93.52 lbs N/acre (104.9 kg/ha) and urea-ammonium nitrate solution carrier was also applied to the entire field on 28 June.

To increase local corn borer populations, three cages for artificial moth releases were set in the two sections of filler corn and at the field center. The cages were made of plywood and plastic garden mesh and mounted 1 m from the ground on fence posts. Every two to three weeks from 23 June to 15 August, two cardboard rings of pupae were set in each release cage.

Four sampling sites were flagged in each plot, with 25 plants per site. All sites were located at least 25 ft (7.62 m) from plot edges. Each plant was examined once per week for corn borer egg masses. If a mass was found, the plant number, number of eggs per mass, mass location (leaf number from first emerged leaf), plant phenological stage, and plant height (to collar line of last fully exposed leaf) were recorded. Each egg mass found was circled with a permanent marking pen to prevent recounts. The height and stage of every tenth plant was also recorded weekly to estimate median weekly plot-wide plant stages and heights.

Weekly plant leaf samples were taken to estimate per-plant leaf areas in each plot; these were used to create regression models predicting leaf area from plant height. Details regarding the regressions are given in Appendix C. To determine if microclimate factors differed between plots, a HOBO® data logger (Pro Series RH/Temp, Onset Computer Corporation, Bourne, MA) was placed in each plot of the second and third replicates. Each logger was screwed to a piece of wood and protected from rain by a polyvinyl chloride coupler fitting; the logger was then hung at mid-canopy level on a fence post between rows in the center of the plot. Each logger was set to record temperature and relative humidity readings every 30 min from 17 July to 25 August.

Statistical analysis: All studies. Poisson regression models specified the negative binomial or Poisson distribution with a log link (DIST=NEGBIN or POISSON, LINK=LOG in PROC GENMOD, SAS Institute 2004), depending on the nature of the data. A Pearson scaling factor (SCALE=PEARSON) was also used to manage overdispersion when needed. In those cases, the dispersion parameter was estimated by the Pearson's chi-square divided by its degrees of freedom.

Statistical analysis: Height treatment studies. Generalized estimating equations (GEE, PROC GENMOD, SAS Institute 2004) were used to determine if raised and unraised plants showed differences in egg mass recruitment. The number of egg masses on every plant each day were used as the response in GEE models comparing the effects of run, block and height treatment

for the 2006 field cage and growth chamber experiments; day was included as a covariate. A negative binomial distribution and working independent correlation structure were specified to minimize the effects of overdispersion (Stokes et al. 2000). Plant height effects were tested in the 2007 field experiment with a logistic regression main effects model (PROC LOGISTIC, SAS Institute 2004) comparing total run-wide egg mass counts on each plant with the effects of run, block, and height treatment.

The effect of the plant stand treatment on within-plant egg mass location was tested using analysis of variance models for each set of experiments (PROC GLM, SAS Institute 2004). The response variable was the leaf location of each egg mass found, while main effects were block, run, and height treatment; plant stage was also included as a covariate. Using PROC GENMOD, a Poisson regression of egg mass size (number of eggs per mass) against run, block and height treatment was used to determine if moths laid larger masses on raised or unraised plants.

Statistical analysis: Equal leaf area studies. Egg mass totals from each plot were analyzed to determine if oviposition differed between plots of different maturity but equal leaf area. This was accomplished with Poisson regressions of main effects (planting treatment, run, and block) and relevant interactions. Additional analyses were conducted using the total number of egg masses laid on individual plants; these also included leaf area covariates which were estimated from the regressions of sample plant heights and leaf areas used in the experimental setup. A Poisson regression of egg mass size against run, block and leaf area effects was also used to determine if similar mass sizes were laid on different plant maturities.

Statistical analysis: Equal number of plants experiment. As in the equal leaf area experiments, Poisson regressions of total per-plot and per-plant egg masses were conducted. Each plant's leaf area was also used as a covariate to explain variation in egg mass deposition. Egg mass sizes were compared in a Poisson regression of run, block and maturity treatment.

Statistical analysis: Planting date—density field experiment. Season-wide plot egg mass totals were compared between planting date and plant density treatments using a two-way analysis of variance (PROC ANOVA, SAS Institute 2004). The same analysis was also conducted on egg mass data divided into early-season (first four weeks) and late-season (last five weeks) to compare first- and second-generation European corn borer planting date preferences. Treatment effects on oviposition over time were assessed with a GEE model of planting date and density, with week acting as a covariate. The effect of another covariate, per-plant leaf area, was also tested; leaf area estimates were obtained from the regressions of sample plant leaf areas (Appendix C) and estimated using weekly plot height medians.

To reduce cyclical trends in the temperature and relative humidity readings, daily means were calculated for each data logger. Humidity data from one of the units placed in a low-density second planting plot were discarded due to logger malfunction. A mixed-model analysis of repeated measures using a first-order autoregressive covariance model (TYPE=AR(1) in PROC MIXED; Littell et al. 2002, SAS Institute 2004) compared temperature and humidity among planting dates and plant densities. The between-subjects (i.e., logger) effect was assumed to be random. Finally, mass size differences between treatments were evaluated with a two-way analysis of variance (PROC ANOVA).

Results

Table 4.1 summarizes per-plant egg mass means for all height experiments. Type 3 analysis of the 2006 height cage study showed that female moths laid significantly more egg masses on raised plants ($\chi^2 = 41.74$, df = 1, $P < 0.0001$). Run and day effects were significant except for the blocking factor (Run: $\chi^2 = 24.01$, df = 1, $P < 0.0001$; Block: $\chi^2 = 1.39$, df = 1,

$P = 0.2377$; Day: $\chi^2 = 7.10$, df = 1, $P = 0.0077$). The model also exhibited significant run*block and run*raise interactions (Run*block: $\chi^2 = 5.45$, df = 1, $P = 0.0196$; Run*raise: $\chi^2 = 19.22$, df = 1, $P < 0.0001$). A plot of the run*raise interaction suggested that egg mass deposition on both raised and unraised plants was much lower during the second run; however, the decrease was much more marked for the unraised plants. Nevertheless, egg mass deposition was much higher on the plants placed on stands.

Egg mass numbers on plants in the growth chamber experiments showed that females did not prefer raised plants over those placed on the ground ($\chi^2 = 0.06$, df = 1, $P = 0.814$); all other effects were significant except for block (Run: $\chi^2 = 18.30$, df = 1, $P < 0.0001$; Block: $\chi^2 = 6.88$, df = 3, $P = 0.0759$; Day: $\chi^2 = 18.65$, df = 1, $P < 0.0001$). Similarly, the height treatments from the 2007 field experiment exhibited no difference in oviposition (Wald $\chi^2 = 0.0046$, df = 1, $P = 0.9457$); no other main effects were significant (Run: Wald $\chi^2 = 1.2706$, df = 1, $P = 0.2597$; Block: $\chi^2 = 0.2107$, df = 1, $P = 0.6462$).

In the 2006 cages, females laid more masses on higher leaves on raised plants than those in-ground ($F = 5.34$, df = 1, $P = 0.0210$). The effect of run and plant stage were also significant, while block was not (Run: $F = 33.76$, df = 1, $P < 0.0001$; Block: $F = 1.60$, df = 1, $P = 0.2059$; Stage: $F = 165.16$, df = 1, $P < 0.0001$). In contrast, none of the main effects influenced within-plant orientation of egg masses in 2007 (Height: $F = 0.08$, df = 1, $P = 0.7769$; Run: $F = 3.16$, df = 1, $P = 0.0956$; Block: $F = 0.04$, df = 1, $P = 0.8474$); the stage covariate was significant ($F = 11.63$, df = 1, $P = 0.0039$). These results were also reflected in the growth chamber studies (Height: $F = 0.01$, df = 1, $P = 0.9189$; Run: $F = 0.12$, df = 1, $P = 0.7331$; Block: $F = 0.26$, df = 3, $P = 0.8523$). The stage covariate was significant ($F = 14.69$, df = 1, $P = 0.0001$), as was the block*raise interaction ($F = 2.86$, df = 3, $P = 0.0365$). The interaction plot indicated that females

tended to lay egg masses on higher leaves of the unraised plants in the blocks aligned with the light fixtures on the left side of the chamber and vice versa.

Results from the equal leaf area field cage and growth chamber analyses of per-plot and per-plant mass recruitment are summarized in Table 4.2. With the 2006 and 2007 combined field cage data, planting treatment was a highly significant factor in both total plot and individual plant egg deposition. However, planting date was not significant with the 2006 data alone; this also held true in an analysis of the first two runs in each year. In contrast, planting treatment in the combined data from the third and sixth run (final run from each year) was nearly significant. These runs compared egg mass deposition between two reproductive stages. In both years, the second planting, or younger corn, was preferred.

All main effects were significant in the analysis of oviposition on individual plants within field cages from both years. In addition, the leaf area covariate contributed significantly to variation in egg mass deposition. This is also apparent in a graph of per-plant egg mass numbers against plant leaf area (Fig. 4.1). In any case, the results from the individual plant analysis are difficult to interpret due to interactions over six experimental runs. The overwhelming significance of all factors and the interactions suggest that variation between moth releases, stage comparisons, and other unknown factors (e.g., weather and field cage effects) probably affected the outcome.

In the equal leaf area growth chamber experiments, planting treatment did not affect total egg masses laid when comparing plots nor individual plants. The leaf area covariate explained a significant amount of the variation in egg mass deposition among individual plants. Egg mass numbers (per plant per day) are shown with their respective plant leaf areas in Fig. 4.2.

In the growth chamber experiment comparing equal numbers of plants, planting date was highly significant in both plot and individual plant comparisons (Table 4.3). Significant treatment*run and treatment*block interactions were observed in all analyses. Examination of the

interactions revealed that females preferred older plants in all cases, but more so in the second run. This seems sensible because the disparity between plant maturity was greater in the second run. Oviposition within treatments also varied between blocks, but moths chose to lay more eggs on the older plants. These trends were reflected in an examination of egg mass deposition on individual plants (Fig. 4.2). Oviposition on plants of the first planting date was much higher than on the younger plants, but when individual plant leaf area was taken into account, planting date ceased to be significant.

Seasonwide plot egg mass totals in the 2007 planting date—density field study differed between treatments. Females laid significantly more egg masses in the second planting date ($F = 8.36$, df = 1, $P = 0.0126$) and low-density ($F = 5.12$, df = 1, $P = 0.0415$) treatments. GEE analysis of oviposition over time confirmed these differences (Planting date: $\chi^2 = 3.15$, df = 1, $P = 0.0758$; Density: $\chi^2 = 3.76$, df = 1, $P = 0.0526$) and indicated egg mass recruitment varied from week to week ($\chi^2 = 8.13$, df = 1, $P = 0.0044$). The GEE model also suggested planting date preferences changed over time (Planting date*week interaction: $\chi^2 = 8.86$, df = 1, $P = 0.0029$). This trend is apparent when examining weekly egg mass counts from each treatment combination (Fig. 4.3). When the per-plant covariate was included in the model, its effect was significant ($\chi^2 = 12.23$, df = 1, $P = 0.0005$), while density ceased to contribute significantly to egg mass variation ($\chi^2 = 2.51$, df = 1, $P = 0.1129$). Planting date and week effects remained significant (Planting date: $\chi^2 = 11.43$, df = 1, $P = 0.0007$; Week: $\chi^2 = 12.58$, df = 1, $P = 0.0004$). Interestingly, there was no interaction between week and planting date. Instead, a week*density interaction contributed to the model ($\chi^2 = 4.76$, df = 1, $P = 0.0291$).

When season-wide egg deposition was divided into early- and late-season groups, the early-season oviposition showed no difference between planting dates ($F = 0.04$, df = 1, $P = 0.8370$). However, first-generation females were more attracted to low-density plots

($F = 11.28$, df = 1, $P = 0.0050$). Late-season moths showed a marked preference for later-planted corn ($F = 13.18$, df = 1, $P = 0.0030$) but no preference for either density ($F = 0.09$, df = 1, $P = 0.7670$).

Daily mid-canopy temperatures varied over time, but not between planting dates (Table 4.4). Temperatures were generally higher in the lower density plantings, though this varied by day. Plot humidity changed over time, but did not vary by treatment. Humidity also exhibited a mild interaction between plot treatment factors (Table 4.4). High-density corn appeared to have higher humidity in the later plantings, while the opposite trend emerged in the low-density plots; however, these conclusions are uncertain due to missing humidity data from one logger.

Table 4.5 summarizes the influence of run, block, and treatment effects on egg mass size in all growth chamber and field cage experiments. Egg mass size did not differ between height treatments or blocks in the 2006 cages, though size did differ significantly between runs. Mass size also did not differ between plants in the 2007 field or growth chamber studies, though mass size was marginally larger on unraised plants in the 2007 experiment. This difference likely occurred due to the influence of two large egg masses. Leaf area treatments in the field cage and growth chamber experiments had no effect on the number of eggs per mass. Mass size varied significantly between runs in all of the leaf area experiments. In the 2007 field plot experiment, neither planting date ($F = 0.07$, df = 1, $P = 0.8015$) nor density ($F = 0.00$, df = 1, $P = 0.9471$) affected the number of eggs per mass.

Discussion

Authors have cited female European corn borer preferences for plant height and maturity since the early published literature. Huber et al. (1928) observed more moths in early-planted corn, and taller plants were reported to be preferred for oviposition in other studies (Patch 1931,

Huber 1939). Everly (1959) asserted that plant height was the most attractive early-season factor, while late-season borers were influenced by stage. Later studies emphasized the effect of plant maturity on oviposition rather than height (Andrew and Carlson 1976, Spangler and Calvin 2000).

It is difficult to distinguish between plant height and maturity effects because both are correlated during vegetative stages. Furthermore, other plant characteristics may change with maturity and thereby influence European corn borer behavior, provided they are perceptible to adult females. Huber et al. (1928) suggested the emanation of some substance that changed in quality or quantity over the life of the plant. Some factors could include leaf texture or angle, volatile emissions, epicuticular chemicals or leaf carbohydrates (Schurr and Holdaway 1970, Andrew and Carlson 1976, Derridj et al. 1989, Udayagiri and Mason 1997). Laboratory choice experiments have been used to test various plant chemical effects on corn borer oviposition, but no published studies have separated the influence of visibly correlated factors (i.e., plant height, leaf area and maturity) on egg-laying behavior.

This group of studies integrated various field cage, growth chamber and field plot experiments to clarify corn height, leaf area and maturity effects on oviposition choice. The experiments imposed constraints on normal moth dispersal, yet they allowed plant manipulations not generally possible in a typical field. Differences in egg mass deposition between runs, blocks, and days were expected, and indeed inevitable, due to several factors: varying numbers of moths were released in each experiment, adult emergence fluctuated over time, and temperatures in the field cages likely influenced activity. Some females also escaped, died or stopped laying eggs.

One problem not anticipated was the field cage effect on oviposition. The plant height experiments were designed to simulate taller plants while keeping plant maturity equal between treatments. However, it was impossible to predict the effect of a confined, artificial environment on moth behavior. Movement was greatly restricted within the cages, so many of the moths were

observed resting on the screenhouse ceilings or on the undersides of the raised plants' upper leaves. The ceiling fabric tended to retain moisture from rainwater or condensation, so the microclimate near the ceiling likely affected the moths' behavior. This was reflected in the elevated egg mass counts on raised plants versus in-ground plants in 2006. An interaction between the height treatment and experimental run suggested differences in resting preferences between lab-raised cohorts or times in the season. Females in the field cages also laid eggs on higher leaves within raised plants than unraised plants; this was also attributed to their resting tendencies.

Due to cage influence on behavior, the cage experiments were modified as open-air blocks in 2007. Low local European corn borer numbers led to few egg masses, but several observations were made. First, raised plants did not recruit more egg masses than those placed on the ground. Also, within-plant egg deposition did not differ between height treatments. During the second experimental run, reproductive stage corn surrounded the study area, with tassels at approximately the same height as the upper leaves of the raised plants. However, oviposition on raised and unraised plants was roughly equal. Therefore, a plant's height may not make it more or less apparent to ovipositing corn borers. The extent of female plant perception during flight is unknown; if adults orient toward taller plants, this was not reflected in oviposition preferences.

The growth chamber height experiment also represented an unnatural environment. Unlike the field cages, however, moth resting tendencies were unapparent. In addition, oviposition between height treatments did not differ. Variation in within-plant egg mass placement among blocks was likely due to minor differences between light fixtures, humidifier output, or airflow within the chamber.

If the field cages had any effects on the outcome of the equal leaf area experiments, they were impossible to ascertain. The cage ceilings appeared sufficiently far enough away from upper leaves, especially when elevated for the third experimental run each year. These runs were the

only ones in which females showed a definitive preference for one planting date over another when plot leaf areas were equal. Interestingly, these were also the only experiments that compared two different stages of reproductive corn: R1 (silking) and R4/R5 (dough/dent). All other runs compared early vegetative with late vegetative/early reproductive corn or mid/late vegetative with early reproductive stages.

The first two runs from each year, along with the results from the equal leaf area growth chamber experiments, present strong evidence that leaf area is related to oviposition when comparing vegetative stages or vegetative and reproductive stage field corn. When plot leaf area was equalized, plant maturity failed to influence oviposition. This was particularly apparent in the growth chamber studies with an examination of individual plant area; egg mass totals on plants from each treatment did not differ once plant leaf area was accounted for. Similar results appeared in the growth chamber experiments that compared equal numbers of plants. Per-plot and per-plant egg mass totals were much higher in the more mature plantings. When individual plant leaf areas were included as a covariate, however, the planting treatment was not significant.

The results from the 2007 leaf area field cage experiments differed from those of 2006, as seen in the inconsistency between planting date treatment effects on egg-laying. The reason for the disparity is unclear, but it may have reflected differences among plant stage comparisons or moth release cohorts. However, the 2006 data and all growth chamber experiments strongly imply that leaf area is a better predictor of ovipositional preference than maturity during vegetative stage corn.

Spangler and Calvin (2000) suggested that leaf area influences oviposition during vegetative stages, but that the relationship diminishes at the transition to reproductive stages as peak leaf area is reached. This conclusion agrees with the results presented here. However, the authors did not find any difference in egg masses among reproductive stages. Comparisons of oviposition between older (R4-5) and younger (R1) stages indicated a preference for younger

reproductive corn. This preference could indicate increased suitability of silking corn for larval development, possibly due to increased carbohydrate sequestration within the ear. In addition, pollen released during plant silking stages is an excellent protein source for neonate larvae.

After exploring maturity and leaf area at a plant-based scale, the impact of leaf area, maturity and plant density on oviposition was tested in larger field plots. More season-wide egg masses were laid in late-planting and low-density plots than the other treatments. Early-planting plots were preferred during the first two weeks of the study, with approximately equal preference for both planting dates in the second week of July. Analysis of oviposition over time exhibited a preference switch favoring the second planting date beginning with the week of 16 July; this trend continued through the end of the summer. This loosely reflects conclusions drawn by Spangler and Calvin (2000) in sweet corn. The authors observed increased early-season oviposition in early plantings while later plantings were preferred by second generation bivoltine moths. They cited leaf area as a major influence during vegetative stages. During the week of 16 July, however, the second planting was approximately stage V10 while plots of the first planting were V17-V18, or peak leaf area.

One drawback to the planting date—density factorial design was the fact that density and plant leaf area were confounded. Individual plant leaf areas were greater in low-density than high-density plots (Appendix C). A weekly leaf area covariate included in a separate analysis accounted for a significant portion of the variation in egg mass deposition over time. Once leaf area was considered, the plant density effect was no longer significant; planting date preferences remained. These results support the idea that leaf area is an important factor in oviposition, as suggested by Spangler and Calvin (2000).

Analysis of the mid-canopy microclimate indicated higher temperatures in the low density plots, likely caused by increased solar penetration between rows. No inter-plot differences in

humidity were apparent. Temperature variation may influence egg deposition, as Andow and Ostlie (1990) suggest. The team examined larval injury in various tillage regimes, concluding that higher soil temperatures in chisel-plowed fields attracted ovipositing females. In this study, however, plant leaf area seemed to explain most of the variation in oviposition. The density treatment results here contradict those of Lee (1988), who found more egg masses in high-density corn stands. However, his measure of density was based on visual ratings, not a prescribed treatment.

If oviposition preference is an indicator of larval host suitability, does it follow that females lay larger egg masses on preferred plants? None of the plant maturity, height or leaf area treatments affected the number of eggs per mass. In some of the experiments using lab-raised corn borers, egg mass size varied between runs. Therefore, typical egg mass size seems to differ between lab cohorts. Any size variation among feral moths, however, was imperceptible.

These experiments show that plant-scale factors, such as individual plant leaf area, impact European corn borer oviposition behavior. It remains unclear how leaf area acts as an attractant, though it may be an indirect measure of plant volatile emission. Further investigations of variables associated with plant maturity, such as plant volatile production over time and its effect on egg-laying, would be valuable. Both large-scale and plant-based studies are needed to determine how sensory information from plants is processed by ovipositing females, and how it translates to host selection and resulting larval damage.

Table 4.1. Mean ± S.E. number of masses and eggs laid per plant per day on raised and unraised plants in field and growth chamber experiments.

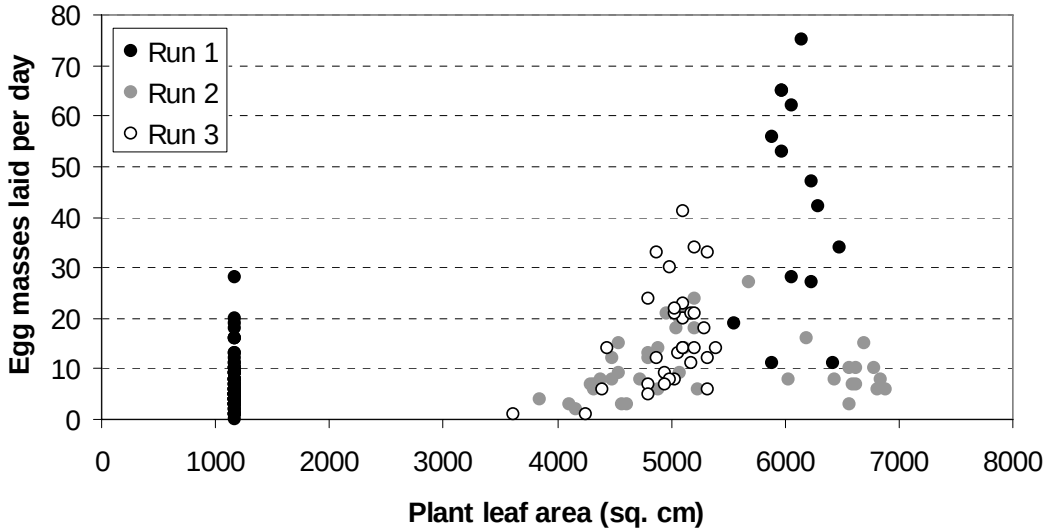
Experiment	Run	Block	Mean number of masses/plant/day (S.E.)*		Mean number of eggs/plant/day (S.E.)*	
			Unraised	Raised	Unraised	Raised
2006						
Field cages	1	North	3.61 (0.48)	5.52 (0.57)	43.15 (5.61)	70.23 (7.34)
		South	2.80 (0.30)	5.77 (0.68)	34.30 (3.79)	72.48 (8.83)
	2	North	0.62 (0.10)	4.12 (0.38)	10.47 (1.84)	63.53 (5.53)
		South	1.27 (0.17)	4.88 (0.44)	19.09 (2.64)	70.31 (6.34)
2007						
Field blocks	1	---	0.22 (0.10)	0.50 (0.17)	8.11 (4.29)	11.17 (4.65)
	2	---	0.28 (0.11)	0.11 (0.08)	8.44 (3.95)	2.06 (1.41)
Growth chamber	1	---	1.95 (0.25)	2.36 (0.30)	38.75 (5.59)	43.48 (6.13)
	2	---	5.00 (0.47)	5.13 (0.56)	100.34 (9.77)	97.44 (9.67)

*2007 Field blocks: Mean masses (or eggs) per plant for duration of run (20 days).

Table 4.2. Likelihood ratio statistics for Type 3 analysis of Poisson regressions comparing egg mass deposition between planting date treatments, experimental runs, and blocks in the equal leaf area field cage and growth chamber experiments.

Analysis	Effect	χ^2	df	*P*
2006 Field cages	Planting	1.28	1	0.2578
(plot totals)	Run	7.48	2	0.0238
	Block	1.20	1	0.2729
Runs 1, 2, 4, 5	Planting	2.72	1	0.0989
(First two runs in field	Run	13.77	3	0.0032
tents each year; plot total)	Block	1.24	1	0.2659
	Block*Run	7.79	3	0.0506
Runs 3 & 6	Planting	3.30	1	0.0694
(Last run in field	Run	6.79	1	0.0092
tents each year; plot total)	Block	1.29	1	0.2554
	Block*Run	4.50	1	0.0339
2006 & 2007 Field cages	Planting	5.85	1	0.0156
(plot totals)	Run	26.38	5	< 0.0001
	Block	2.64	1	0.1040
	Block*Run	13.01	5	0.0233
2006 & 2007 Field cages	Planting	56.67	1	< 0.0001
(individual plant totals)	Run	212.95	5	< 0.0001
	Block	13.78	1	0.0002
	Planting*Run	44.13	5	< 0.0001
	Block*Run	107.23	5	< 0.0001
	Leaf area	93.03	1	< 0.0001
Growth chamber	Planting	1.20	1	0.2738
(plot totals)	Run	7.59	1	0.0059
	Block	4.46	1	0.0347
Growth chamber	Planting	0.04	1	0.8473
(individual plant totals)	Run	0.44	1	0.5067
	Block	18.08	1	< 0.0001
	Leaf area	4.26	1	0.0391

(a)



(b)

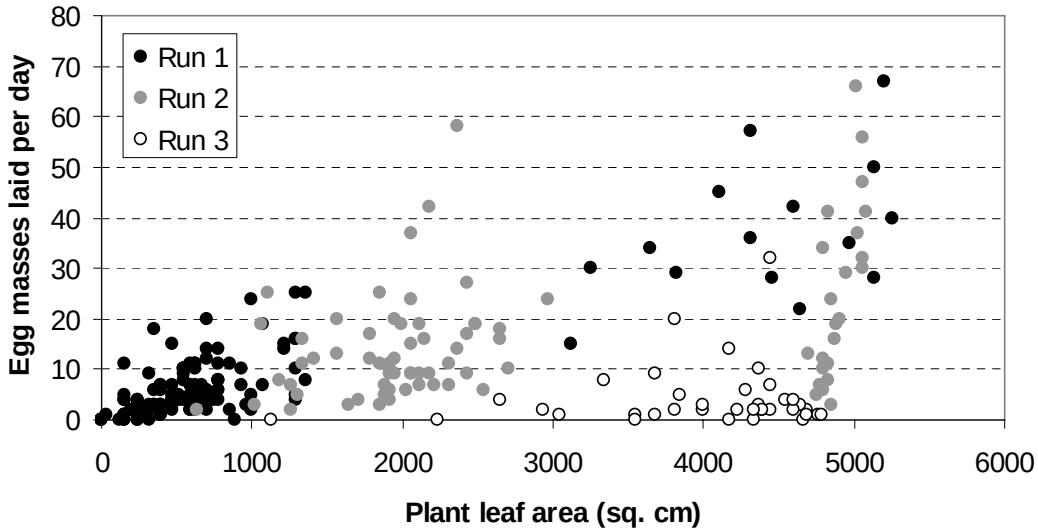
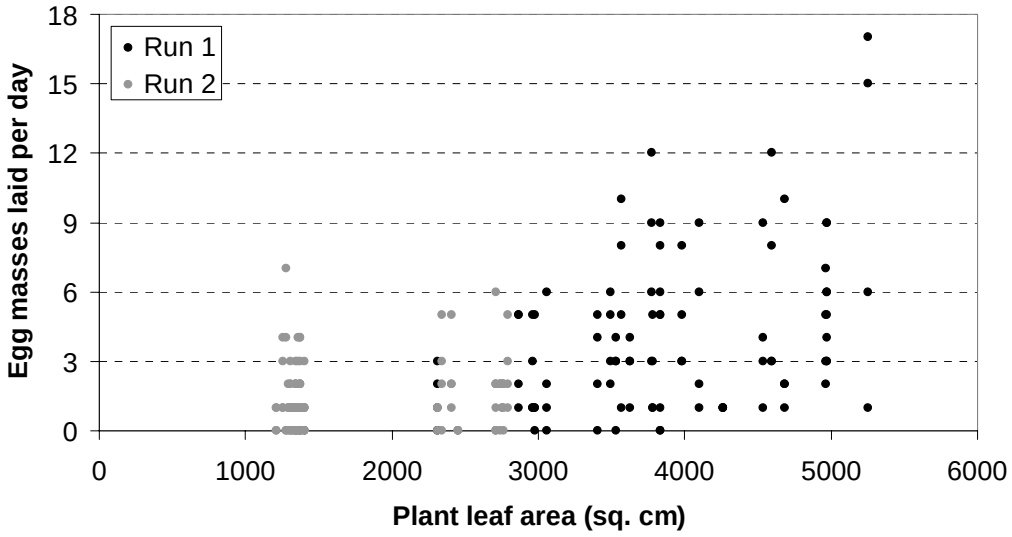


Fig. 4.1. Number of egg masses laid each day vs. leaf area of plants observed in the field cage equal leaf area studies in (a) 2006 and (b) 2007.

(a)



(b)

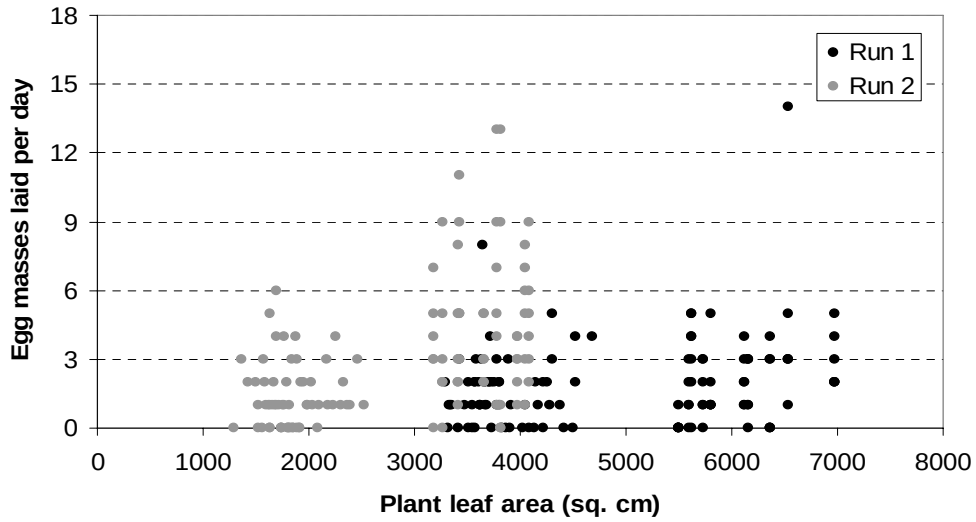


Fig. 4.2. Number of egg masses laid each day vs. leaf area of plants observed in the growth chamber (a) equal leaf area study and (b) equal number of plants study.

Table 4.3. Likelihood ratio statistics for Type 3 analysis of Poisson regression models of oviposition among planting date treatments, experimental runs, and blocks in growth chamber experiments comparing equal numbers of plants per plot.

Analysis	Effect	χ^2	df	*P*
Plot totals	Planting	76.93	1	< 0.0001
	Run	12.53	1	0.0004
	Block	0.01	1	0.9065
	Planting*Run	22.96	1	< 0.0001
	Planting*Block	8.42	1	0.0037
Individual plants (no leaf area covariate)	Planting	27.19	1	< 0.0001
	Run	5.97	1	0.0145
	Block	0.04	1	0.8419
	Planting*Run	10.63	1	0.0011
	Planting*Block	3.79	1	0.0516
Individual plants (leaf area covariate)	Planting	0.07	1	0.7894
	Run	4.06	1	0.0439
	Block	0.55	1	0.4596
	Planting*Run	12.74	1	0.0004
	Planting*Block	3.62	1	0.0570
	Leaf area	2.12	1	0.1458

Table 4.4. GEE analysis of daily differences in mid-canopy temperature and relative humidity in field plots with two planting dates and two plant densities.

	Effect	*F*	Numer. df	Denom. df	*P*
Temperature	Planting date	1.21	1	5	0.3218
	Density	21.21	1	5.04	0.0057
	Day	3536.54	38	197	< 0.0001
	Density*Day	1.77	38	197	0.0067
Humidity	Planting date	3.86	1	3	0.1442
	Density	1.58	1	3	0.2974
	Day	1020.85	38	197	< 0.0001
	Planting date*Density	9.00	1	3	0.0576

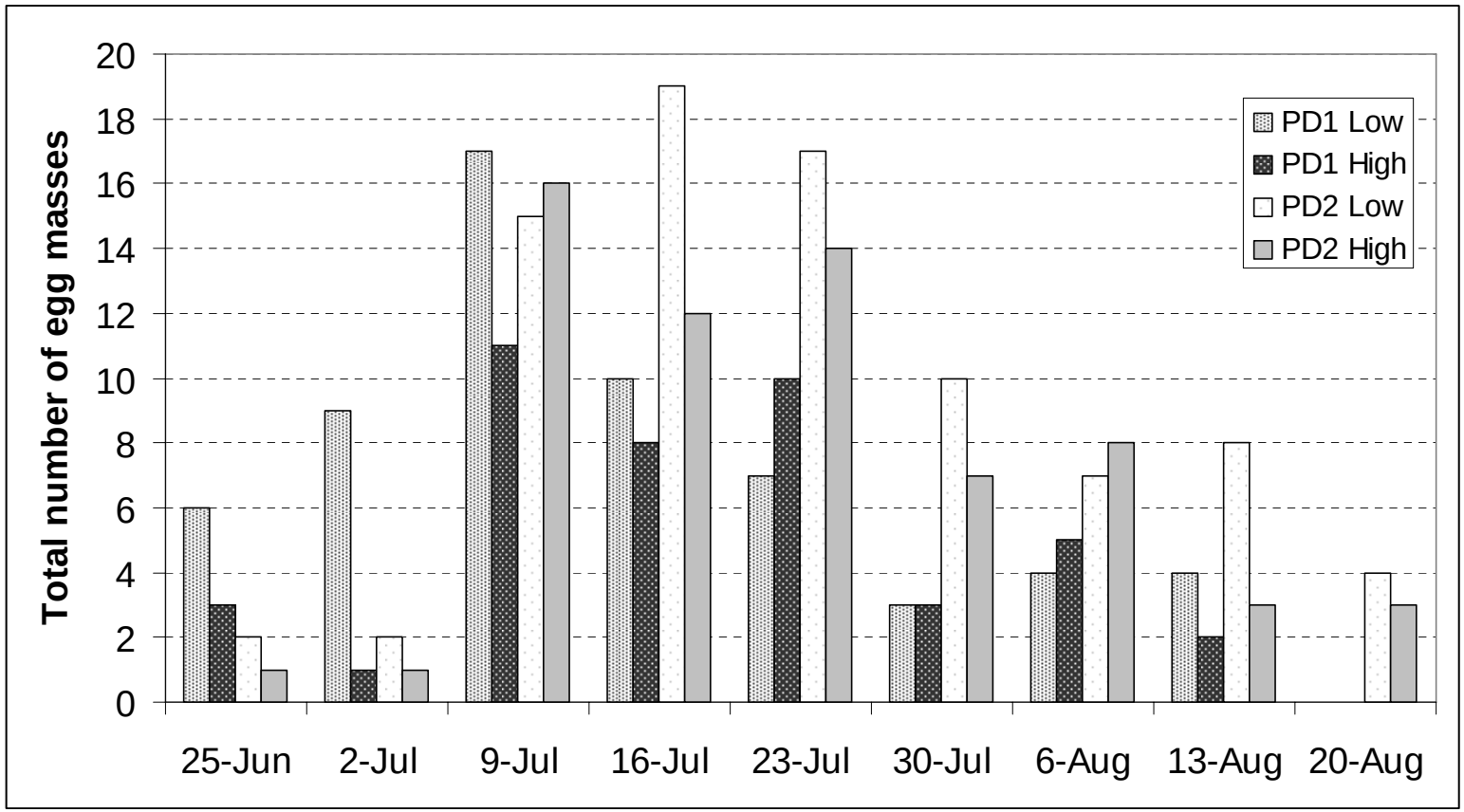


Figure 4.3. Total number of egg masses found in factorial study of two planting dates (PD1, PD2) and two plant densities (Low, High) during nine weeks of 2007.

Table 4.5. Poisson regression analysis of run, block and treatment effects (raised plants, equal plot leaf area, or equal number of plants) on egg mass size in field and growth chamber experiments.

Experiment	*N*	Main treatment effect			Run			Block		
		χ^2	df	*P*	χ^2	df	*P*	χ^2	df	*P*
Plant height										
2006 Field cages	2342	0.07	1	0.7925	27.70	1	< 0.0001	0.21	1	0.6496
2007 Field blocks	20	3.56	1	0.0590	0.70	1	0.4042	0.28	1	0.5985
Growth chamber	915	1.91	1	0.1667	0.00	1	0.9651	6.04	3	0.1096
Equal leaf area										
2006 + 2007 Field cages	4771	1.57	1	0.2102	89.00	5	< 0.0001	1.85	1	0.1733
Growth chamber	545	0.00	1	0.9737	51.00	1	< 0.0001	1.97	1	0.1605
Equal number of plants										
Growth chamber	595	0.15	1	0.6989	4.89	1	0.0270	0.08	1	0.7836

CHAPTER 5.

European Corn Borer Larval Survival on Various Maturities and Leaf Levels Within the Corn Plant

Introduction

Interactions between the European corn borer, *Ostrinia nubilalis* (Hübner), and its primary host plant *Zea mays* L. have been the basis for much of the corn borer research published since its introduction to the United States in the early twentieth century. Many studies have investigated female host selection for oviposition, larval growth and subsequent yield loss. European corn borer oviposition preference has also been examined at a variety of scales. Researchers have conducted choice experiments on foliar extracts and leaf surface components, concluding that females can distinguish leaf qualities through volatiles and contact cues (Schurr and Holdaway 1970, Fiala et al. 1985, Derridj et al. 1989, Binder et al. 1995, Udayagiri and Mason 1995, Udayagiri and Mason 1997). Plant-level and field-level studies have explored the impact of various factors such as maturity, leaf area, and microclimate on female preference (Andrew and Carlson 1976, Andow and Ostlie 1990, Spangler and Calvin 2000, Hunt et al. 2001); these were also addressed in detail in Chapters 2, 3, and 4.

Jaenike (1978) described a preference-performance hypothesis (also termed "optimal oviposition theory") suggesting that in the absence of parental care, female insects can enhance their fitness with the selection of egg-laying sites that maximize the nutrition and habitat requirements of offspring. The theory assumes that plants vary in suitability, and that females can assess host quality through gustatory, olfactory, visual, tactile, or thermo-hygroreceptive cues. Oviposition activity, therefore, should involve female decisions regarding host suitability, age, egg load, and the probability of finding a more suitable host (Leather and Awmack 2002).

Plant maturity has been the predominant factor in plant- and field-level European corn borer oviposition choice studies (Huber 1939, Everly 1959, Andrew and Carlson 1976, Spangler and Calvin 2000). Spangler and Calvin (2000) concluded that European corn borer preference increased with plant maturity during sweet corn vegetative stages and peaked at early reproductive-stage corn. Females rarely lay eggs on corn younger than the 5- or 6-leaf stage (Spangler and Calvin 2000), which may be related to the presence of the plant aglucone 2-4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) (Guthrie et al. 1985). DIMBOA inhibits larval development but its concentration declines with age from very high levels in corn seedlings (Klun et al. 1967, Klun and Robinson 1969). These facts suggest European corn borer oviposition preference is linked to plant suitability.

Within-plant egg mass placement also suggests optimal oviposition activity. European corn borers prefer to lay eggs on leaves at approximately ear level (Windels and Chiang 1975, Calvin et al. 1986, Sorenson et al. 1993, Spangler and Calvin 2001). Egg mass placement ostensibly reduces the distance between the larval emergence site and nutritious reproductive plant organs.

This research represents the most comprehensive work on the relevance of optimal oviposition in the European corn borer. The goal of this study was to use artificial egg mass infestations to test the influence of intra- and inter-plant factors on various measures of offspring success (e.g. survivorship, weight, developmental time). The first experiment was designed to compare larval survival between four planting dates (hereafter referred to as the "plant maturity experiment"), while the second used a two-factor factorial of two planting dates and three vertical infestation positions ("leaf level experiment").

Materials and Methods

In 2006 and 2007, a portion of a 1-acre (0.40-ha) field at the Russell E. Larson Experimental Field Station at Rock Springs, PA was planted in a randomized complete block design with three blocks of four treatments each. One plot in each block was assigned to one of four planting date treatments. Plots were 30 ft (9.1 m) by 69 ft (21.0 m) and planted with 12 30-in (0.76 m) rows of a conventional 101-day field corn hybrid (DeKalb DKC51-43). The remainder of the field was planted with the same corn variety for a separate experiment, and no irrigation was used.

The 2006 no-till plantings were made on 1 May, 12 June, 22 June and 10 July. Fertilizer (10-30-10) was applied at 100 lbs/acre (112.1 kg/ha) at planting. Plots also received 25 gal/acre (233.8 liters/ha) post-planting fertilizer and herbicide applications (s-metolachlor 35.8%/atrazine 28.1% + isoxaflutole + paraquat + nonionic surfactant; urea-ammonium nitrate solution carrier with 83.5 lbs N/acre (93.6 kg/ha)) on 2 May, 15 June, 23 June, or 10 July. A 16 gal/acre (149.7 liters/ha) topdress with 53.4 lbs N/acre (59.9 kg/ha) and urea-ammonium nitrate solution carrier was also applied to the entire field.

Using the same plots, the four plantings were re-randomized the following year. Plots were planted on 9 May, 12 June, 22 June, and 10 July 2007. Fertilizer (10-30-10) was applied at 100 lbs/acre (112.1 kg/ha) at planting along with tefluthrin at 0.68 gal/acre (6.35 liters/ha) to control corn rootworm. All plots received a 22 gal/acre (205.8 liters/ha) herbicide application (s-metolachlor 35.8%/atrazine 28.1% + isoxaflutole + paraquat + nonionic surfactant; urea-ammonium nitrate solution carrier) the day after planting. A 24 gal/acre (224.5 liters/ha) topdress with 80.2 lbs N/acre (89.9 kg/ha) and urea-ammonium nitrate solution carrier was also applied to the entire field on 28 June.

Twenty-two plants in each plot were marked with flagging tape for the plant maturity study; additionally, a second set of 30 plants were flagged in each of the plots planted on the first two dates each year for the leaf level study. All marked plants were located at least 7.5 ft (2.3 m) from the plot edge. Plants were also separated by at least one row and four plants within a row. Plants with obvious European corn borer leaf damage or stalk holes were avoided.

European corn borer egg masses were shipped from the USDA-ARS Corn Insects and Crop Genetics Research Unit in Ames, IA. The masses were produced by laboratory-reared *Z*-strain females according to methods outlined in Guthrie et al. (1985). Each mass was rated according to its approximate diameter and number of eggs. Small masses were about 2 to 3 mm and contained fewer than 25 eggs, medium-sized masses had 25 to 40 eggs with a diameter of 4 to 5 mm, and large eggs were greater than 5 mm and contained more than 40 eggs. On 15 and 16 August 2006, each marked plant was infested by dropping egg masses into the leaf axil using a paintbrush. At the time of infestation, plant stages from each of the four planting dates were R4 (dough), R2 (blister), VT/R1 (tassel/silk), and V10. Most primary ears developed at the 13th leaf node. Each of the 22 plants in the four planting date treatments received one medium egg mass (Blocks 1 & 2) or two small masses (Block 3) placed within the leaf axil of the primary ear (Planting dates 1, 2 & 3) or within the furled leaf sheath at one leaf level above the last emerged leaf (Planting date 4). The 30 remaining plants in each plot were randomly assigned to one of three leaf locations; ten each were infested at high, mid, or low leaf levels. In the high treatment, egg masses were placed in the axil of the third leaf above the primary ear. Low treatment masses were dropped in the third leaf below the ear, and mid treatment masses were placed within the primary ear axil. Large masses were used in Block 1, while Block 2 plants received either one large, two medium, or three small masses; the combinations were chosen randomly. Block 3 plants were infested with three small masses.

The following year, large masses were placed on plants in Blocks 1 and 2 on 16 August as part of the intra-plant leaf level experiment. Corn planted on the first and second dates was approximately R3 (milk) and R1 (silk) at infestation, respectively. The remaining leaf level plants in Block 3 were infested with large masses on 22 August. Plants marked for the comparisons between all four planting dates were also infested with medium-sized masses on 22 August. Corn stages in each planting date were R4, R2, VT/R1, or V9.

After approximately 400 accumulated degree-days, all plants in the leaf level study were destructively sampled. Daily degree-days were calculated by subtracting a base temperature threshold (50°F/10°C) from daily mean temperature (i.e., [½ ($temp_{max}$ + $temp_{min}$) – 50]). Corn from the maturity experiment was sampled after approximately 450 accumulated degree-days. The degree-day intervals were selected to allow most larvae to enter the fourth or fifth instar. The leaf level experiment plants were sampled between 7 and 9 September 2006 when the first and second plantings were at stages R5 (dent) and R4 (dough). Corn infested for the plant maturity experiment was harvested between 11 and 16 September, when the first through fourth planting dates were R5, R4, R3, and R1, respectively. In 2007, the first two blocks of the leaf level study were cut on 7 September 2007, when the first and second plantings of corn were at R5 and R3, respectively. On 16 September 2007, plants in the third block (then at R6 and R5 in planting dates 1 and 2) were harvested. Block 1 of the plant maturity study was cut on 17 September, when the maturities of planting dates 1 through 4 were R6, R5, R3, and R1. Blocks 1 and 2 of the maturity study were sampled two days afterwards.

At harvest, plants were cut at ground level and split lengthwise with a knife. All larvae were collected from within the stalk, ear, and between the leaf sheath and stalk. The instar and vertical location of each larva, based on leaf sheath level, was recorded. Since many of the lower plant leaves were dried up, leaf level was numbered based on the leaf number of the primary ear, which had previously been determined and was generally consistent within planting dates. Larval

cavities located between leaf nodes were associated with their midpoint (e.g., 7.5). In 2007, additional uninfested plants were cut to determine background larval infestation levels; ten plants from each plot (60 total) were harvested for the levels experiment, while eight from each plot (96 total) were sampled for the plant maturity experiment. A portion of larvae from each experiment were accidentally damaged in the sampling process; some of these were included in weight analyses if accurate weights could be obtained. Damaged individuals were discarded from all other analyses.

The larval stage and weight (to one-thousandth of a gram) of each individual was recorded, and each larva was sequestered in a plastic diet cup. A cube of prepared corn borer diet weighing between 8 and 12 g (Southland Products Inc., Lake Village, AR) and a cotton ball moistened with distilled water were placed in each cup. Larvae were placed in an environmental chamber (Percival Scientific, Boone, IA) with photoperiod of 16:8 L:D and temperature 25:21°C. Different scales and chambers were used in the second year. Cotton balls were re-moistened every two weeks, and individuals were re-weighed upon pupation and sexed if development was completed. Larval death and parasitism rates were also noted. Some individuals burrowed into the diet cube and died within, which was unapparent. A number of larvae completed development soon after establishment on diet, while it seemed that others entered diapause. Most viable individuals completed development within 280 days; after this point, any remaining larvae were extracted from the diet cubes to determine final larval death rates.

Statistical Analysis: Both Experiments. Some, but not all, analyses included a blocking factor. Categorical tables and some analysis of variance models collapsed data across blocks to reduce the number of empty cells and increase power. Negative binomial distributions were specified for all Poisson regression models. Response variables (larval/pupal weight, vertical leaf level, or days to adult emergence) in analysis of variance models were square-root transformed. *F*-statistics based on Type III sums of squares are reported here. All analyses of variance were

unbalanced, so the Tukey-Kramer adjustment (ADJUST=TUKEY in LSMEANS statement of PROC GLM, SAS Institute 2004) was used. Some of the analyses presented were not designed for the experiments; rather, they utilized explanatory factors that resulted from each treatment. These are still presented in the discussion due to the near impossibility of designing experiments to test those factors.

Differences in the number of larvae found per treatment (planting date in the plant maturity experiment) or treatment combination (planting date and leaf level) within each block were analyzed using a Poisson regression model in PROC GENMOD (SAS Institute 2004). Poisson regressions also compared the larval numbers from uninfested sample plants from each planting date and block. The effect of treatments and blocks on larval weights was also determined through analyses of variance (PROC GLM) for each experiment.

ANOVA was also used to explain variation in weight between those larvae found within the stalk, ear, and leaf sheath; main effects included year, planting treatment, and leaf level (if applicable). Days to adult emergence were compared between years and treatments in each experiment. Data from both experiments were combined to test if weight differences existed between male and female pupae from each year and experiment. Square-root transformed larval weights from the combined experiments in each year were linearly regressed against their respective vertical in-stalk positions using PROC REG (SAS Institute 2004). Finally, Cochran-Mantel-Haenszel row mean scores were requested from PROC FREQ (Stokes et al. 1991, SAS Institute 2004) to test if parasitism rates varied by year or planting treatment.

Statistical Analysis: Plant Maturity Experiment. The Mantel-Haenszel correlation statistic (PROC FREQ, SAS Institute 2004) was used to determine if larval stages differed between planting dates. The same statistics were used to test differences between the number of individuals completing development in each planting treatment. Additionally, an ANOVA tested differences in pupal weight between years and planting dates.

Statistical Analysis: Leaf Level Experiment. A two-way ANOVA of year and infested leaf level was used to detect differences in larval vertical level within the stalk upon harvest; a separate analysis included larvae within the ear (vertical level 12 or 13). The analysis aimed to ascertain if larval locations were associated with the vertical level at which the plants were infested.

Results

A total of 812 and 458 larvae were found in the combined years of the respective plant maturity and leaf level studies. Table 5.1 summarizes the percentage of individuals that pupated, emerged as adults, and were parasitized. *Macrocentrus cingulum* (Hymenoptera: Braconidae) emerged from the majority of parasitized larvae. *Eriborus terebrans* (Hymenoptera: Ichneumonidae) parasitized five larvae in the combined experiments, while a tachinid fly, *Lydella thompsoni*, parasitized 11 individuals. One other unidentified wasp uncommon to Pennsylvania emerged from a larva in 2007.

The number of larvae found per plant in the plant maturity study differed between planting dates and blocks (PD: $\chi^2 = 209.24$; df = 3; $P < 0.0001$; Block: $\chi^2 = 31.74$; df = 5; $P < 0.0001$); a significant block by planting interaction also appeared ($\chi^2 = 31.18$; df = 15; $P = 0.0083$). The interaction was attributed to differences between years, as blocks within a given year did not exhibit differences in the number of larvae found (2006: $\chi^2 = 3.73$; df = 2; $P = 0.1552$; 2007: $\chi^2 = 3.83$; df = 2; $P = 0.1474$). Planting dates were highly significant within each year (2006: $\chi^2 = 90.83$; df = 3; $P < 0.0001$; 2007: $\chi^2 = 134.07$; df = 3; $P < 0.0001$). The mean number of larvae found per plant in the four planting dates are shown in Table 5.2. In 2006, the highest density of larvae was found in the third planting, while the second planting date supported the most larvae in 2007. Significantly fewer larvae were found in the fourth planting than any

other in both years (Table 5.2, Fig. 5.1). Uninfested plants sampled in 2007 differed between plantings but did not differ between blocks (Planting: $\chi^2 = 9.69$; df = 3; $P = 0.0214$; Block: $\chi^2 = 0.00$; df = 2; $P = 0.9990$); larval numbers in the fourth planting date were significantly lower than all others (Table 5.2).

Larval weights in the same experiment differed between planting dates in an analysis of combined years ($F = 30.71$, df = 3, $P < 0.0001$); differences between blocks were not significant, though a block by planting interaction was present (Block: $F = 0.66$, df =5, $P = 0.6562$; Block*Planting: $F = 2.16$, df = 15, $P = 0.0063$). Mean weights from each year and planting date are shown in Fig. 5.2. In 2006, planting date differences were highly significant, while those between blocks were not (Planting: $F = 16.68$, df = 3, $P < 0.0001$; Block: $F = 1.86$, df = 2, $P = 0.1579$). The heaviest larvae were collected from the second planting (stage R2 at infestation); these were significantly larger than those from all other maturities (PD2 vs. 1: $P = 0.0339$; 2 vs. 3: $P < 0.0001$; 2 vs. 4: $P < 0.0001$). Larvae from the first planting (R4) were also significantly heavier than those from the third and fourth plantings (R1 and V10; 1 vs. 3: $P = 0.0067$; 1 vs. 4: $P = 0.0049$). The third and fourth plantings did not differ ($P = 0.1983$), but this was likely due to the conservative comparison test used. Larval weights from 2007 varied by planting date and block (Planting: $F = 20.73$, df = 3, $P < 0.0001$; Block: $F = 4.70$, df = 2, $P = 0.0095$). The block difference is largely attributed to the sampling of one block several days before the other two. As in the previous year, the weights from second planting plots were higher than all other maturities ($P < 0.0001$). Weights in plantings 1 and 3 did not differ ($P = 0.9382$), but they were larger than those in planting 4 ($P < 0.0001$).

Leaf level and block effects were significant factors in the number of larvae found in the leaf level experiment, but planting date was not (Level: $\chi^2 = 47.69$; df = 2; $P < 0.0001$; Planting: $\chi^2 = 0.01$; df = 1; $P = 0.9322$; Block: $\chi^2 = 55.80$; df = 5; $P < 0.0001$). Table 5.3 summarizes plant means according to year and treatment. Block differences were mainly between years;

interestingly, differences in egg mass inoculations in 2006 (i.e., 3 small, 2 medium, or 1 large) did not seem to affect the number of larvae found per plant. Larvae were 2.29 and 2.79 times more likely to be found on plants inoculated at middle (ear) leaf level than the low and high levels, respectively. No differences were found between blocks or plantings in the 2007 uninfested plants (Planting: $\chi^2 = 0.01$; df = 1; $P = 0.9056$; Block: $\chi^2 = 2.41$; df = 2; $P = 0.2998$). A significant interaction was found between the factors (Planting*Block: $\chi^2 = 7.48$; df = 2; $P = 0.0238$), though the reason for this is unknown. The number of larvae found on uninfested plants (Tables 5.2 and 5.3) suggests that natural infestation levels on each planting date did not affect the conclusions drawn from these analyses.

Fig. 5.3 shows larval weights from the leaf level experiment. Since the combined data exhibited several interactions (due to variation from blocks between years), it was more informative to examine larval weights from each year separately. In 2006, none of the main effects affected larval weight (Planting: $\chi^2 = 2.50$; df = 1; $P = 0.1168$; Level: $\chi^2 = 0.83$; df = 2; $P = 0.4390$; Block: $\chi^2 = 0.79$; df = 1; $P = 0.4545$). However, block by planting and block by level interactions were significant (Block*Planting: $\chi^2 = 3.09$; df = 2; $P = 0.0494$; Block*Level: $\chi^2 = 2.81$; df = 4; $P = 0.0290$). These may have resulted from differences in harvest time, but their cause is not entirely clear. Weights in 2007 did not vary by treatment effect, though there were differences between blocks (Planting: $\chi^2 = 0.02$; df = 1; $P = 0.8748$; Level: $\chi^2 = 0.09$; df = 2; $P = 0.9166$; Block: $\chi^2 = 15.68$; df = 1; $P < 0.0001$). Blocks also varied by level ($\chi^2 = 2.98$; df = 4; $P = 0.0195$).

Frequency distributions of larval vertical stalk position from each experiment showed that tunnels formed a bell curve with a peak just below primary ear level (Fig. 5.4). Most within-stalk positions were associated with a leaf node (integers), though some larvae were identified with an intermodal area. Larval stalk levels were found to vary by leaf level treatment ($F = 6.98$, df = 2, $P = 0.0012$) but not by year ($F = 0.00$, df = 1, $P = 0.9878$). Larvae from plants infested at the high

leaf level tunneled at higher stalk levels than those infested at ear level or lower (H vs. M: P = 0.0024; H vs. L: P = 0.0047). Larval stalk level comparisons of mid and low level infestation treatments were not significant (P = 0.9951). Mean stalk levels for each treatment and year are shown in Fig. 5.5. When larvae found within the ear were included at their respective ear leaf levels, infestation levels failed to show any effect on position (Level: F = 0.30, df = 2, P = 0.7409; Year: F = 0.21, df = 1, P = 0.6497).

Larval weight decreased with increasing vertical stalk position in both years (Fig. 5.6). In addition, the weights of larvae found within the stalk, ear, and leaf sheath differed. In the plant maturity experiment, larval weights differed by year and organ location (Year: F = 7.31, df = 1, P = 0.0070; Location: F = 19.70, df = 2, P < 0.0001). Weights also showed an interaction between year and location factors (F = 20.63, df = 2, P < 0.0001). The smallest larvae were found between the leaf sheath and the stalk each year, while larvae within the ear and stalk were heavier. In 2006, individuals that tunneled into the stalk were heavier than those in the ear, while the reverse was true in 2007. In the leaf level experiment, the same factors were also significant (Year: F = 8.00, df = 1, P = 0.0049; Location: F = 26.82, df = 2, P < 0.0001; Year*Location: F = 6.87, df = 2, P = 0.0012). The smallest larvae collected in 2007 were located within the leaf sheath, while those found in the ear and stalk were heavier. In 2006, larvae found within the stalk were heaviest, followed by those in the leaf sheath and then those in the ear.

Larval instars varied significantly by year (Q = 13.65, df = 1, P = 0.0002) and planting date (Q = 17.58, df = 1, P < 0.0001). Separate analyses of instars (3, 4 or 5) by year yielded similar results (2006: Q = 14.17, df = 1, P = 0.0002; 2007: Q = 10.42, df = 1, P = 0.0012). The second planting was 23 times more likely than the fourth planting to have fourth- or fifth-instar larvae than third instars in 2006. Larvae from the first and third plantings also were more phenologically advanced than those collected from the fourth planting. In 2007, the first planting

had the highest percentage of older larvae (i.e., fourth and fifth instars); this percentage decreased with later planting dates.

Planting date treatments also affected the number of individuals completing development (i.e., emerging as viable adults). Individuals were categorized by the last stage attained (larva, pupa or adult) and compared between planting dates and years. Planting date was highly significant ($Q = 31.48$, df = 1, $P < 0.0001$), as was year ($Q = 12.17$, df = 1, $P = 0.0005$). In 2006, the likelihood of adult emergence decreased with plant maturity; larvae from the first planting date were 1.9, 3.1, and 8.7 times more likely to complete development than the second, third, and fourth plantings, respectively. The trend was not exhibited the second year, however, as the ratio of larvae completing development from the fourth planting was higher than any of the other treatments. Unfortunately, the analysis suffered from much lower sample sizes in the younger plants, so this may have been an artifact of the data.

Planting date was a significant factor in the number of days required to complete development (Table 5.4; $F = 4.33$, df = 3, $P = 0.0052$); time to adult emergence was also significantly higher in 2007 ($F = 14.22$, df = 1, $P = 0.0002$). Interestingly, the time required to complete development increased with plant maturity. Larvae collected from the last planting took significantly less time to complete development than the first two planting dates (PD4 vs. 1: $P = 0.0203$; 4 vs. 2: $P = 0.0354$). The only factor that significantly affected development completion time in the leaf level study was year ($F = 27.92$, df = 1, $P < 0.0001$); again, larvae required more days in 2007 (Table 5.5). Times did not vary by leaf level or planting treatment (Level: $F = 0.91$, df = 2, $P = 0.4028$; Planting: $F = 0.54$, df = 1, $P = 0.4650$). This did not contradict the results from the other experiment, as the times from the first and second plantings in the plant maturity experiment were not different.

Pupal weights were higher in 2006 ($F = 4.88$, df = 1, $P = 0.0285$) and those collected from different planting dates were significantly different ($F = 3.09$, df = 3, $P = 0.0285$). Pupal weight

tended to decrease with increasing plant maturity (Table 5.6). The only significant pupal weight comparison was between planting one and three, though this was only barely significant ($P = 0.0450$).

Another analysis of pupal weight revealed that females weighed significantly more than males ($F = 229.67$, df = 1, $P < 0.0001$). Again, pupae from 2006 weighed more ($F = 5.46$, df = 1, $P = 0.0202$). Pupal weight did not vary between experiments ($F = 0.54$, df = 1, $P = 0.4628$). Table 5.7 summarizes mean weights from the combined studies.

Finally, an analysis of parasitism between planting dates yielded some interesting results. Parasitism rates were different between years in the plant maturity study ($Q = 34.42$, df = 1, $P < 0.0001$). In addition, parasitism increased with decreasing plant maturity ($Q = 38.22$, df = 3, $P < 0.0001$). In 2006, larvae from the fourth planting were 3, 5, and 70 times more likely to be parasitized than the third, second, and first plantings, respectively. Larvae developing on the youngest plants were 1.3, 5.8, and 11 times more likely to be parasitized than those in the third, second and first plantings in 2007. Parasitism in the leaf level study also varied by year ($Q = 6.26$, df = 1, $P = 0.0123$) and planting date ($Q = 4.56$, df = 1, $P = 0.0327$). Larvae in the second planting were 10.6 and 1.2 times more likely to be parasitized than the oldest plants in 2006 and 2007, respectively.

Discussion

While some insects actively care for their young, parental care appears as an anomaly when looking at the life histories of the hundreds of thousands of described species. Most female insects lay eggs and depart from the oviposition site, leaving their offspring to fend for themselves. In this case, female choice of an adequate, nutritious food supply acts as a surrogate for parental care. Optimal oviposition theory, colloquially known as “mother knows best”, posits

that females maximize their fitness by choosing the most suitable plants for larval development, survival, and the resulting fecundity of their offspring (Jaenike 1978). Therefore, oviposition preference should correspond with host suitability. Preference-performance theory assumes that host plants vary in suitability for larval development, and that females are able to discriminate between plants or places on a plant.

Other factors that may act on oviposition searches include the mother's age, egg load, and the probability of finding a better host (Roitberg et al. 1999). In a review of research on optimal oviposition, Leather and Awmack (2002) presented evidence that some insects adjust egg size according to the relative suitability of a host plant. No efforts as yet have compared European corn borer egg size among plants; however, an analysis of 8,525 egg masses from six different experiments (Chapter 4) suggested that the number of eggs laid per mass does not vary across a wide variety of plant maturities. This implies that if European corn borer females make choices following optimal oviposition theory, preferences should be apparent as differential egg-laying among plants or leaves, not mass size.

It is clear that ovipositing European corn borers have plant preferences. Many researchers have asserted that oviposition is positively associated with corn height or maturity (Huber 1939, Beard 1943, Andrew and Carlson 1976). A study of both vegetative and reproductive sweet corn stages by Spangler and Calvin (2000) showed that oviposition increases with vegetative stage; they suggested increasing preference was associated with expanding leaf area. Oviposition was greatest on reproductive-stage corn, but no reproductive stage was necessarily preferred over others. Chapters 2, 3, and 4 also examined the influence of field corn stages on egg-laying choices. Choice experiments designed to compare plant maturity and leaf area (Ch. 4) indicated that leaf area is probably related to oviposition.

The results from this study suggest that European corn borer females make oviposition choices consistent with optimal oviposition theory. Greater numbers of European corn borer

larvae survived on reproductive corn than vegetative stages. Less than 5% of the total larvae found on all plants were collected from mid-whorl stage (V9-10) plants, which was consistent across both years. Larval survival was approximately the same on all reproductive stages (i.e., R1, R2, R4 at inoculation) in 2007, while larvae were more successful on early reproductive (R1) corn than later stages (R2, R4) in 2006. Variation in larval survival could be attributed to a number of plant factors: nutrient availability, microclimate, protection from natural enemies, or the ability to support larvae. Some of the individuals developing on whorl-stage plants may have drowned after entering the whorl.

Larval weights were highest on blister stage (R2) plants in the second planting both years. Considering the numbers of surviving larvae and their weights, reproductive-stage plants were superior to mid-vegetative stages. Early larval development, based on the instars found in each planting date, also progressed faster in older plants. Early reproductive stages (i.e., R1 or R2) seem to be most suitable stages overall for larval development, though it is difficult to identify the best stage due to variation between years.

These assessments follow trends noted in earlier research. Huber (1939) found higher survival rates on taller corn. Beck and Lilly (1949) cited evidence of lower larval mortality and higher weight on taller corn. European corn borer survival tests conducted by Luckmann and Decker (1952) showed base survival began when plants had approximately six fully-emerged leaves and increased steadily until at least pre-tassel. Decreasing mortality levels with increasing host age is consistent with DIMBOA levels expressed by the corn plant.

While young larvae cannot select the plant on which they emerge, they can choose plant parts on which to feed. Newly hatched European corn borer caterpillars are positively thigmotropic and saccharotropic (Beck 1956a). Experimental results indicate that European corn borer larvae tend to select food sources with high sugar concentrations, likely because a glucose-deficient diet leads to retarded growth and poor development in later instars (Beck 1950, Beck

1956a, Beck 1956c). Protein is also essential for early growth, though protein requirements decline as development progresses (Beck 1950, 1956c). The movement of larvae on the plant reflects nutritional value of each plant part. Leaf tissue and pollen, which have significant amounts of protein (Beck 1956c), are primary food sources for young larvae as they make their way toward the center of the corn plant. Older individuals requiring more sugars for proper development begin feeding on developing tassels in the first generation, or silks and pollen later in the season (Beck 1956b, Beck 1956c). Beck (1956b) attributed shifts in feeding behavior to changing sugar levels within the plant.

The second experiment in this study examined larval success resulting from vertical leaf placement of egg masses. Plants infested at ear leaf level had higher numbers of larvae than those infested above or below the ear; this was consistent across both planting dates and reflected conclusions from a study by Barry and Mends-Cole (1991). The results also reveal a similarity between larval survival and leaf preference of egg-laying females. Multiple studies have shown that females prefer to lay eggs on the leaves near the primary ear (Windels and Chiang 1975). Coll and Bottrell (1991) reported that 77% of all European corn borer egg masses were found on the middle third of the plant. Sorenson et al. (1993) found 85% of the egg total within a five-leaf zone surrounding the primary ear. Partial plant sampling was suggested by Calvin et al. (1986) to increase efficiency, as the probability of finding an egg mass formed a bell curve that peaked just around ear level. The placement of most egg masses on sweet corn plants was also greatest at ear level or just above (Spangler and Calvin 2001).

Egg mass placement within the plant did affect final larval vertical position. Larvae hatching from egg masses placed three leaves above the ear were ultimately found at a higher stalk level than those placed at ear level or three leaves below. Larval position on the low leaf level treatment plants was lower than that of the mid leaf plants, but the difference was not significant.

If larval success is indeed greater at ear level, within-plant oviposition preference trends reflect the suitability of corn plant tissues for offspring survival.

Although European corn borer larvae can move between plants, Ross and Ostlie (1990) found that more than 50% of recovered larvae stayed on the infested plant. It seems that many larvae tend to stay within three to four leaves of egg mass placement. Analysis of within-plant corn borer movement by Labatte and Got (1993) revealed reduced organ-to-organ movement after the fourth larval instar. The authors also found that larvae moved downward four to five internodes in the period from silking to harvest. Labatte et al. (1997) suggested that changing larval nutritional requirements, along with temporal shifts in plant nutrient availability and organ development, may drive within-plant larval movement.

Although tunnel height was not a reliable predictor of larval weight, there was evidence that weight decreased with increasing vertical stalk level. This may suggest that parts of the stalk are nutritionally variable. However, individuals that bore into the corn stalk are generally fourth or fifth instars; much of their earlier development may have been spent feeding in other places on the plant. Larvae developing on upper leaves may have had reduced access to carbohydrates within the plant. Lower weights may have also been related to smaller stalk diameters near the top of the plant. Losey et al. (2002) presented evidence that European corn borer survival is better in thicker-stalked hosts, but their experiments only tested stalk diameters between plant species, not diameters within the corn plant. Differences in larval weight between those collected from stalk tunnels, ears, and leaf sheaths revealed that the largest larvae were generally found within the stalk. Larvae feeding between the leaf sheath and the stalk did not fare as well, likely because this area tended to be very wet.

The likelihood of completing larval development seems to decrease with plant maturity, but this is not entirely clear. Larval weight was generally higher on reproductive corn stages than vegetative stages, so larvae on older plants may have developed more quickly with less temporal

risk of death from a predator, parasite or pathogen. Within-plant egg mass placement did not affect larval development time, but plant maturity did. Larvae collected from the final planting completed development significantly faster than those from the reproductive stages. This seems counterintuitive, since larval weights were lowest in the youngest corn. However, several explanations are possible. First, the number of larvae from the last planting surviving to adulthood was much lower than all other planting dates; this could have affected the results. It is also possible that larger larvae from the earlier plantings were on the verge of entering diapause. Infestations were made in mid-August and collected in mid-September when the second generation is preparing to overwinter. A large proportion of larvae were also inactive for several months within the growth chamber, suggesting that many entered diapause. Alternatively, small larvae collected from younger plants spent a greater percentage of their development between egg hatch and final weight in the fifth instar within the growth chamber. The chamber setup simulated a midsummer light and temperature cycle, which may have caused young larvae to complete development rather than enter diapause. Pupal weights also seemed to increase with later planting dates, but this was not necessarily significant. Smaller larvae from the last planting date spent a greater proportion of their active feeding time (and gained more of their total body weight) on prepared diet, which may have been a better food source.

European corn borer larval development and mortality is affected by additional factors, including temperature, moisture stress, and natural enemies (Showers et al. 1978, Ellsworth et al. 1989). Infection by the naturally-occurring pathogen *Nosema pyrausta* slows larval development and often inhibits successful pupation or adult emergence (Solter et al. 1990, Sajap and Lewis 1992). Vertical transmission of *Nosema pyrausta* was avoided by treating the colony used to produce egg masses used for infestations. No effort was made to quantify *Nosema* infection, but infection *per os* could have explained some cases of developmental delay or adult inviability. Siegel et al. (1986) estimated the base mortality of larvae uninfected with *Nosema* as

approximately 18%. Larvae collected from this study may have suffered from stress incurred from the sampling process, transport, and handling. However, between 20 and 65% of larvae from each experiment never completed development; therefore, *Nosema* infection might have been a factor in developmental inhibition of some individuals.

Natural parasitization by all parasitoids ranged from 3.6 to 23.3% and varied between experiments and years. Annual parasitism rates from *Macrocentrus cingulum* reported by Phoofolo et al. (2001) exhibited similar variation to those found in this study. The timing of *M. cingulum* emergence has been found to coincide with that of preferred European corn borer larval stages (Sked and Calvin 2005). Another interesting trend was found here when comparing parasitism rates between larvae developing on different plant stages: parasitism increased with decreasing plant maturity. This association was exhibited in larvae from both experiments and both years. Parasitoid foraging efficiency between plantings probably did not affect parasitism, since rates on each reproductive stage were different even though the plants had the same basic physical structure. One possible explanation for differential parasitism may have been variation in volatiles given off by each plant maturity.

Only European corn borers responding to the *Z* pheromone isomer (*Z*-strain) were studied in these experiments, with the ostensible exception of some larvae collected from uninfested plants. In areas of *E*- and *Z*-strain sympatry, such as Pennsylvania, ovposition preference and larval performance relationships may differ between populations. *E*-strain females tend to oviposit on a wider range of hosts and also seem to survive better on noncorn hosts than the *Z* strain (Mason et al. 1996, Bethenod et al. 2005). *E*-strain moths also tend to emerge earlier in the spring. Therefore, the interaction of individual strain type, oviposition preferences on available corn hosts, and resulting larval survival warrants investigation.

Further research would be required to identify more specific differences in larval survival on a wider range of planting dates, and a variety of measurements assessing larval success should

be used. As demonstrated by this study, "success" as measured by the number of surviving larvae as opposed to larval weight could lead to conflicting conclusions. Also, precise estimates of survival on more stages would be helpful in determining whether or not European corn borer preference-performance is affected by different reproductive maturities, as differences were found between years. Oviposition rates between a variety of reproductive stages were found to be approximately equal by Spangler and Calvin (2000), but research presented in Chapter 4 suggested that females prefer younger (R1) to older (R4/R5) reproductive plants. Additional studies on other aspects of optimal oviposition theory, including the impact of moth age, egg load, and risks versus the chance of finding better host plants, would also add considerable information.

Table 5.1. Total number of larvae collected from plants in each experiment, with sex ratios and parasitism, pupation, and development completion rates for those individuals unharmed in the sampling process.

Experiment	Year	Total number of larvae collected	Number of undamaged larvae	Parasitized %	Pupated %	Viable adults %	Sex ratio M:F
Plant maturity	2006	246	236	23.3	43.6	30.9	36:64
	2007	566	507	4.9	59.2	50.1	51:49
Uninfested	2007	45	39	0.0	48.7	46.2	50:50
Leaf levels	2006	125	115	9.6	45.2	36.5	54:46
	2007	333	274	3.6	69.0	59.9	48:52
Uninfested	2007	32	30	0.0	66.7	46.7	57:43

Table 5.2. Mean ± S.E. number of larvae found per plant in corn from three blocks of four planting dates. Different letters denote significant differences between model parameters in Poisson regressions of each year's infested or uninfested counts ($\alpha = 0.05$).

Year	Plant Stage	*N*	Mean	S.E.	
2006	R4	66	0.924	0.149	b
	R2	66	0.712	0.120	b
	R1	66	1.955	0.194	a
	V9-10	66	0.136	0.043	c
2007	R4	66	2.621	0.344	a
	R2	66	2.909	0.310	a
	R1	66	2.894	0.217	a
	V9-10	66	0.152	0.045	b
2007	R4	24	0.500	0.120	a
(uninfested)	R2	24	0.625	0.168	a
	R1	24	0.625	0.198	a
	V9-10	24	0.125	0.192	b

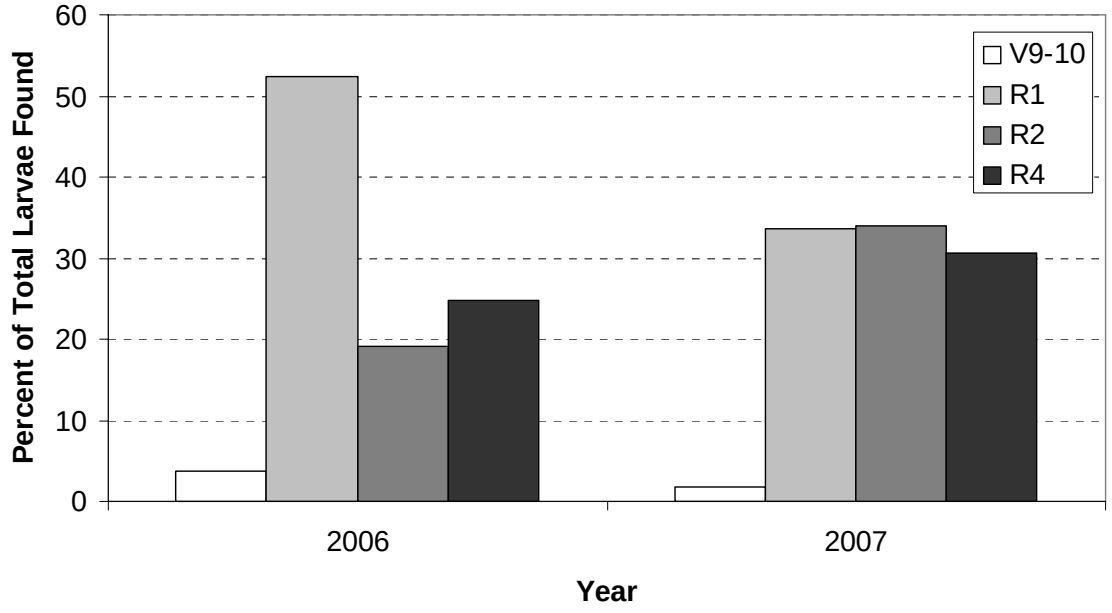


Fig. 5.1. Percentage of all larvae collected in each planting of the maturity experiment in 2006 and 2007. Plant stages listed are those at the time of egg mass inoculation.

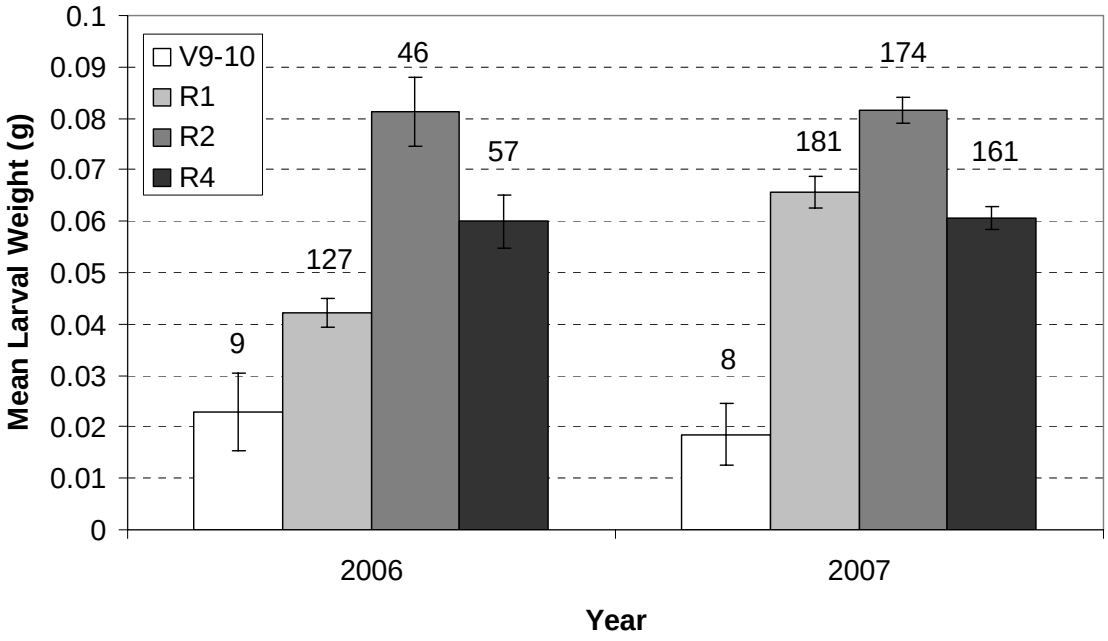


Fig. 5.2. Mean larval weight of larvae found in each planting of the maturity experiment. Plant stages indicate those at the time of inoculation, and sample sizes are listed above standard error bars.

Table 5.3. Mean ± S.E. number of larvae found per plant from three leaf levels and two planting date treatment combinations (plant stage at infestation).

Year	Plant Stage	Level	*N*	Mean	S.E.
2006	R4	L	30	0.733	0.287
		M	30	0.867	0.178
		H	30	0.233	0.079
	R2	L	30	0.667	0.216
		M	30	1.067	0.253
		H	30	0.600	0.103
2007	R4	L	30	1.133	0.202
		M	30	3.900	0.647
		H	30	1.000	0.192
	R2	L	30	1.000	0.240
		M	30	2.900	0.499
		H	30	1.167	0.245
2007 (uninfested)	R4	---	30	0.433	0.124
	R2	---	30	0.633	0.182

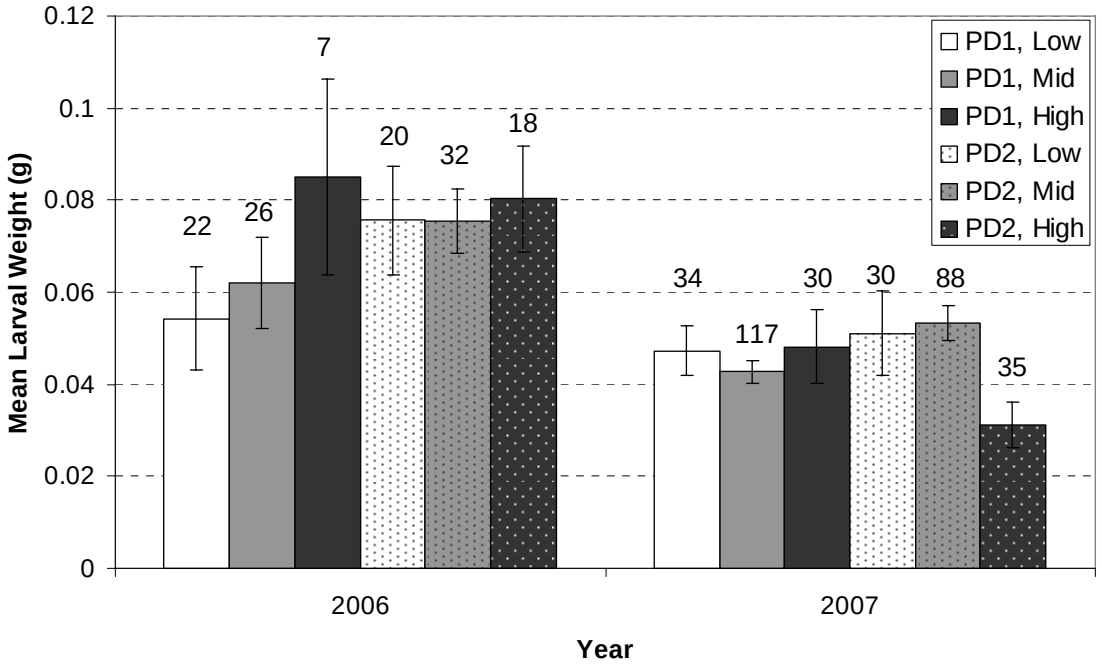
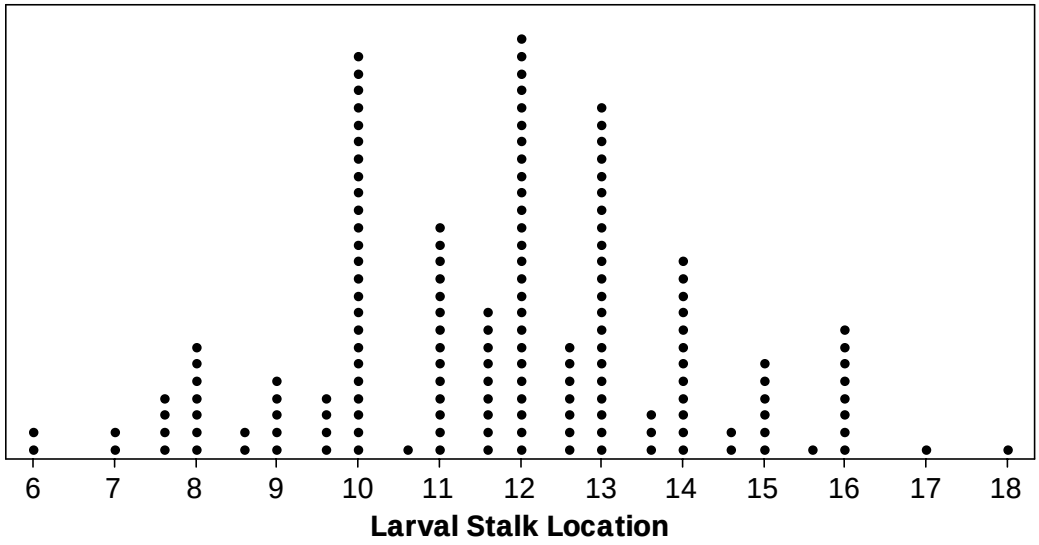


Fig. 5.3. Mean weight of larvae cut from three leaf inoculation levels (Low, Mid, High) and two planting dates (PD1, PD2). Sample sizes are indicated above standard error bars.

(a)



(b)

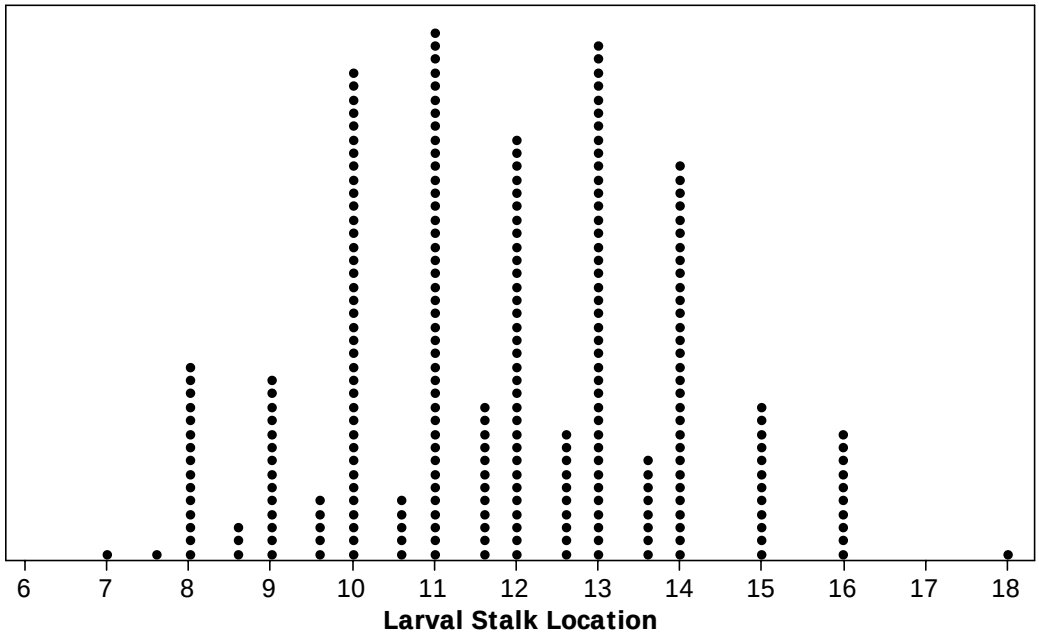


Fig. 5.4. Vertical levels of larvae found within the stalk across years and treatments in (a) leaf level experiment and (b) four planting date experiment. Each dot represents one larva.

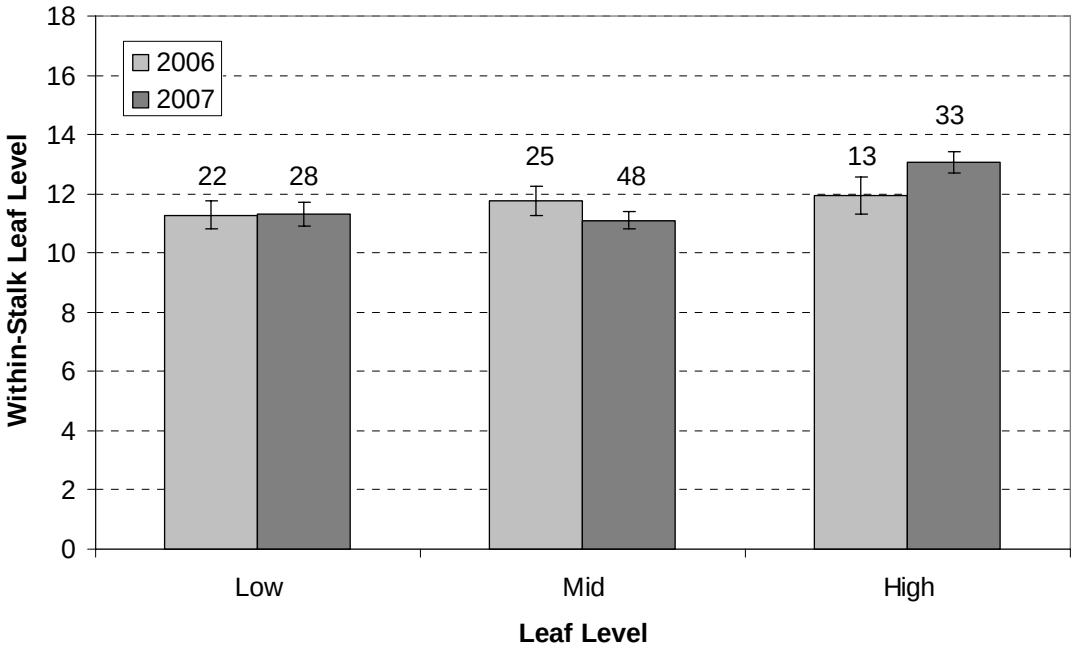


Fig. 5.5. Mean leaf level of tunnels from larvae hatching at three leaf levels. Sample sizes are indicated above standard error bars.

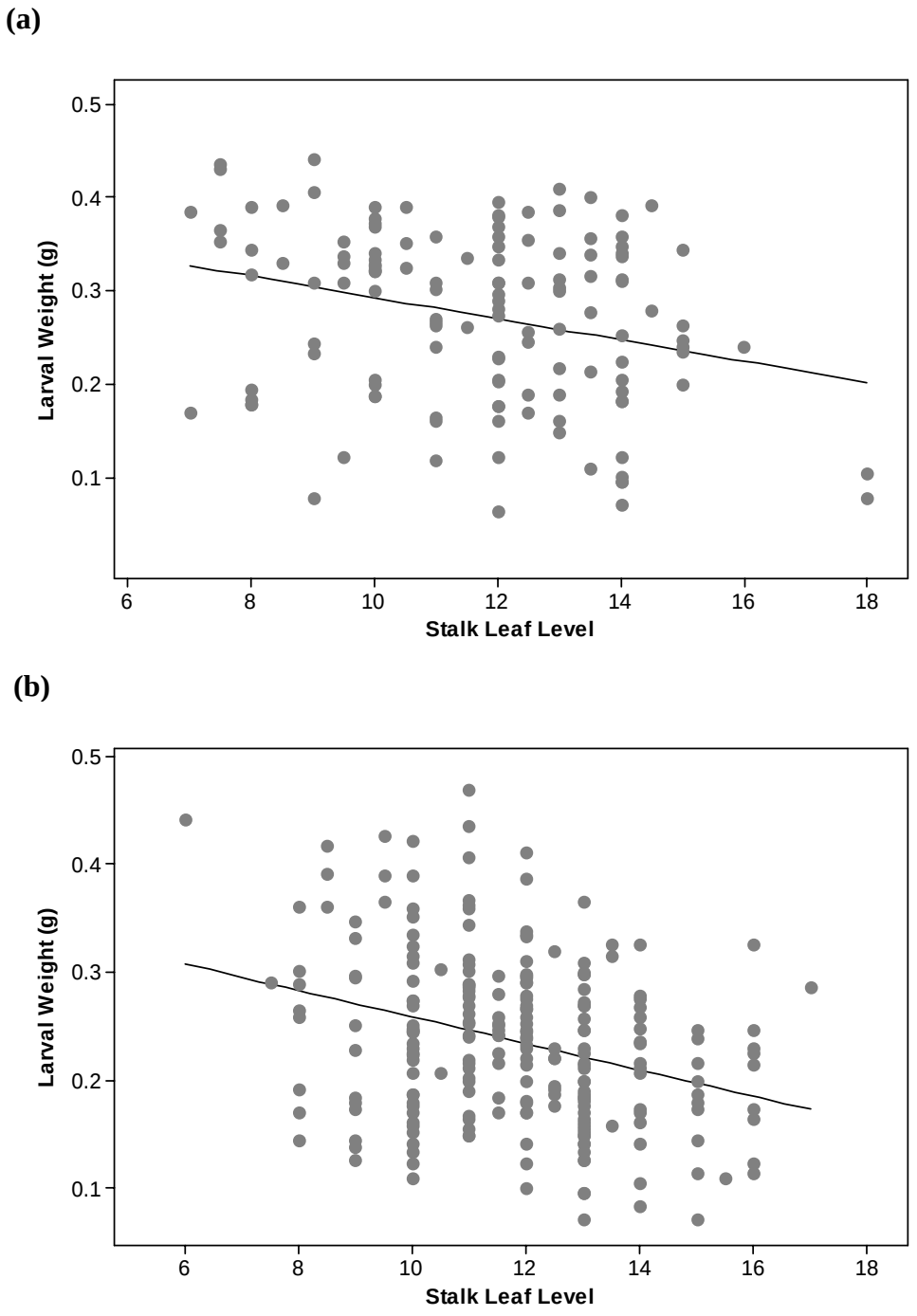


Fig. 5.6. Larval weight (square-root transformed) against vertical stalk level in (a) 2006 and (b) 2007. Weight decreased significantly with stalk level in linear regressions ($y = a + bx$) from each year (2006: $R^2 = 0.077$, $F = 10.99$, df = 1, 132, $P = 0.001$, $a = 0.407$, $b = -0.011$; 2007: $R^2 = 0.096$, $F = 24.60$, df = 1, 234, $P < 0.0001$, $a = 0.382$, $b = -0.012$). Note: No larvae were found below leaf level 6. Leaf levels 1-5 are not included in the figure due to shorter intervals between leaf nodes.

Table 5.4. Mean ± S.E. number of days required for development of viable adults after larval stalk harvest of the plant maturity experiment. Plant stage represents the stage at infestation. Letters indicate significant differences within main effects (year and/or planting date treatment within a year) using the Tukey-Kramer adjustment of least squares means.

Year	Plant Stage	*N*	Mean	S.E.	Larvae Completing Development (%)
2006 b	R4	28	87.5	11.0 a	49.1
	R2	15	83.8	12.2 a	34.1
	R1	29	67.3	10.3 a	23.8
	V9-10	---	---	---	0.0
2007 a	R4	93	105.0	5.1 ab	59.6
	R2	96	101.5	5.5 ab	57.8
	R1	59	91.2	4.7 bc	33.3
	V9-10	6	53.0	12.1 c	75.0
Combined	R4	121	100.9	4.7 a	56.8
	R2	111	99.1	5.0 a	52.8
	R1	88	83.3	4.8 ab	29.0
	V9-10	6	53.0	12.1 bc	35.3

Table 5.5. Mean ± S.E. number of days required for development of viable adults after larval stalk harvest of the leaf level experiment. Plant stage indicates the stage at infestation.

Year	Plant Stage	Level	*N*	Mean	S.E.
2006	R4	Low	13	57.8	15.0
		Mid	13	62.7	10.9
		High	1	159.0	---
	R2	Low	3	66.7	35.3
		Mid	8	73.4	16.9
		High	4	99.8	43.2
2007	R4	Low	9	121.6	23.7
		Mid	65	118.5	6.5
		High	21	119.3	9.0
	R2	Low	11	95.4	13.9
		Mid	44	116.2	9.1
		High	13	99.8	14.9

Table 5.6. Mean ± S.E. pupal weight (g) of individuals collected from the plant maturity experiment. Plant stage represents the stage at infestation. Letters indicate significant differences within main effects using the Tukey-Kramer adjustment of least squares means.

Year		Plant Stage	*N*	Mean	S.E.	
2006	a	R4	12	0.089	0.0075	
		R2	3	0.077	0.0110	
		R1	12	0.110	0.0051	
		V9-10	---	---	---	
2007	b	R4	71	0.081	0.0028	
		R2	58	0.085	0.0030	
		R1	32	0.089	0.0037	
		V9-10	4	0.102	0.0088	
Combined		R4	83	0.082	0.0027	bc
		R2	61	0.085	0.0029	bc
		R1	44	0.095	0.0033	a
		V9-10	4	0.102	0.0088	ab

Table 5.7. Mean ± S.E. pupal weight (g) of males and females collected from both experiments.

Year	Sex	*N*	Mean	S.E.
2006	Female	20	0.107	0.0049
	Male	14	0.082	0.0039
2007	Female	116	0.102	0.0018
	Male	113	0.072	0.0010

CHAPTER 6.

Synthesis of European Corn Borer Female Egg Distribution Process: Landscape Movement, Field Selection, and Within-Plant Oviposition

Crop damage from the European corn borer can have a considerable impact on crop yields, grain quality, and growers' livelihoods. Applied research on the interactions of the corn borer with its primary host plant can provide insight about where and why females lay their eggs. This has become more important with the advent of transgenic *Bt* corn and its widespread use. Insecticide resistance management (IRM) models are predicated on some assumptions that have not been tested in the field. In some cases, IRM models are sensitive to parameters used for insect dispersal (Guse et al. 2002, Onstad et al. 2002).

European corn borer dispersal studies have generally been limited to mark-release-recapture studies using traps or flush bar sampling (Showers et al. 2001, Qureshi et al. 2005, Reardon et al. 2006). Additional studies are required to determine how dispersal affects mating patterns, oviposition between fields and subsequent population gene flow.

Several key objectives were identified in the course of this research to improve knowledge about female dispersal and their host preferences for oviposition:

1. Use egg mass placement (within a field or farm) as a function of European corn borer dispersal to estimate typical female range of oviposition
2. Determine if plant maturity preferences are predictable at different times throughout the growing season
3. Compare plant traits that change with maturity and clarify their influence on female host choice

4. Evaluate optimal oviposition theory (i.e., preference-performance) as it relates to corn borer intra- and inter-plant egg mass placement

A summary of this research follows:

Chapter 2: Local Dispersal and Oviposition Choice of Marked European Corn Borer Females. Research presented in Chapter 2 suggests that many females fly at least 500 meters, though some do mate and lay a portion of their egg load within 50 meters of their emergence site. The majority of females move beyond the immediate vicinity (ca. 1 acre) of their emergence point. However, dispersal distance effects were evident in oviposition patterns at the same scale. Egg deposition decreased with increasing distance from the release point, though this would likely be unapparent if the scale of the experiment had been larger. No directionality was observed in egg mass placement throughout the field, even though wind direction was significantly oriented. Therefore, females moved from the release site equally in all directions at a small plot level.

Planting date (i.e., crop maturity) was the major factor driving oviposition preference between plots at a local scale. The earliest plantings recruited the greatest number of egg masses during the first-generation corn borer activity, while the latest planting was most preferred during the second generation. Both early- and late-planted plots received similar numbers of egg masses over the entire season.

Chapter 3: Farm-Scale Cornfield Heterogeneity Effects on European Corn Borer Oviposition. As suggested in the previous chapter, flight distance effects were not apparent beyond a single field. Egg mass densities were not correlated with field distances from populations emerging at two different sources within the farm. In addition, field size was not a significant factor in egg deposition across a landscape of varying field types. Plant numbers and densities in the closest fields, particularly those planted on an earlier date, influenced oviposition over the entire season. However, proximate older fields did not affect egg mass numbers found during the second generation flight; only plant density during that period seemed to predict mass

density. Moderate spatial autocorrelation in egg mass densities was present between neighboring fields. Similarities in crop management and planting protocols within academic units (and therefore sections within the farm) likely led to these spatial patterns.

Regrettably, comparisons between weekly observed egg mass counts and predicted numbers from an oviposition preference model showed significant differences throughout the season. This suggests that egg infestations within a landscape may not be predictable based solely on field plant maturity. Increased sampling could improve the predictive power of preference models, but it is unlikely based on current sampling efficiency.

Chapter 4: Effects of Plant Height, Maturity, Leaf Area, and Density on European Corn Borer Oviposition. When adjusted for leaf area, plant height (or plant apparency due to height) did not appear to influence egg-laying. During vegetative growth stages, leaf area was an important factor in oviposition. Leaf area did not affect oviposition preference between reproductive growth stages, as females preferred younger reproductive-stage corn. Plant density was also a factor in oviposition preference; however, this was mainly due to confounding with leaf area. Differences in per-plant leaf area accounted for variation among egg mass recruitment in plant density treatment plots.

Plant leaf area seems to be the most important factor in oviposition preferences among vegetative stages. It is not yet clear how leaf area is determined by ovipositing females (e.g., volatile emission, plant examination). In addition, mechanisms for preference between reproductive stages have not been studied.

Chapter 5: European Corn Borer Larval Survival on Various Maturities and Leaf Levels Within the Corn Plant. Offspring success generally corresponded to oviposition preferences, which is consistent with optimal oviposition theory. Corn borer survival was highest in the early reproductive stages (R1 and R2) and lowest in the vegetative stages (V9-V10). Larval weights were also highest in the second planting (R2), followed by R1 and R4 plants. Weights

were lowest in vegetative-stage corn. Survival was highest on plants infested at ear level or lower, with the greatest number of surviving larvae found on ear-infested plants across years and planting dates. Larval weights from ear-level and lower-leaf infestations were slightly higher than those from the higher-leaf infestations in the second planting.

Larvae tended to burrow into the ear, or stalk-bore at or below ear level. The time required to complete adult development was highest for larvae collected from the first and second planting dates. Late-instar larvae that developed more quickly on these plants may have entered diapause, leading to delayed development. Pupal weights also increased with later planting dates. Finally, parasitism rates increased with decreasing plant maturity.

Proposed Description of the European Corn Borer Female Egg Distribution Process

Relevant factors influencing behavior at each step are listed in italics.

Step 1. Female corn borer moth emerges from a source field.

Population genetics (pheromone race, voltinism biotype), weather cues (temperature, day length)

Step 2. Female moves to an action site and mates.

Wind speed/direction, action site availability (distance, plant species composition), site humidity, number of moths within action site

Step 3. Female moves to field and "accepts" a host plant.

Wind speed/direction, field traits (distance, humidity, plant density), plant traits (leaf area, maturity (if reproductive stage), hybrid, insect damage, volatile emission), neighboring field plant density and/or maturity

Wind speeds probably affect local vs. long-range movement. Based on observations in the field, females likely use very light wind flow or their own flight power to move among plants within a field. Stronger winds above the corn canopy would be used for longer

flight distances. After emergence and mating, a female may choose to oviposit in the immediate vicinity (if suitable host plants are present), move to another action site, or fly to a farther field. At least ninety percent of females are expected to move beyond a 1-acre area around their emergence point/initial action site to lay their first egg mass. This may be a type of bet-hedging strategy in which some individuals from the population stay in the immediate area, while others search for more suitable host plants elsewhere.

Step 4. Female chooses a position within the accepted plant.

Plant stage, number of leaves, leaf constituents (surface carbohydrates, physical structure)

Females deposit their egg masses in a bell-shaped curve peaking at or just below the primary ear leaf. This occurs even during vegetative growth. By positioning egg masses within the ear zone, females maximize the fitness of their offspring. Neonates require a high protein/lower carbohydrate diet which is easily found in the pollen and silks of early reproductive stages. Later instars need a higher carbohydrate/protein diet ratio. They acquire carbohydrates by boring into the stalk, where sugars are transported from the leaves (points of photosynthesis) to the ear for storage. Higher larval weights and survivorship found in R1 and R2 stages support this. Lower parasitism rates in these stages also suggest that females depositing eggs at approximately ear level in early reproductive stages maximize the chances of offspring success.

Step 5. Female moves to another plant or field to oviposit.

Factors from Step 3, also: risk of death associated with moving to alternative field, relative "attractiveness" of alternative field, moth age, egg load

Relative preference increases with leaf area during times when only vegetative-stage plants are available. When some or all fields are in reproductive growth stages, females will tend to select early reproductive-stage corn over vegetative or late reproductive

stages. As the majority of fields enter late reproductive stages, preference switches to any available vegetative-stage fields.

Step 6+. Female continues Steps 4 and 5 until death or ovary depletion.

APPENDIX A.

Variation in Egg Mass Coloration of Laboratory-Dyed European Corn Borer Moths

Introduction

Numerous studies have shown the value of tracking insect dispersal to answer questions involving population and spatial dynamics, gene flow, disease transmission, and plant-insect interactions (Caprio and Grafius 1993, Schneider 1999, Blackmer et al. 2006). However, researchers often find dispersal studies challenging due to the high vagility of many species. Various techniques have been used to mark insects for mark-release-recapture studies, including paint, wing punctures, fluorescent powders, stable isotopes, elemental markers, and genetic methods; all have met with varying success (Southwood 1978, Turchin 1998, Qureshi et al. 2004b, Hood-Nowotny and Knols 2007). An ideal marking agent should be cost-effective, persistent, distinguishable from wild unmarked individuals, and not affect the survival, fecundity or dispersal of the insect. A marker is most helpful if evidence of marked insects is easily observable in the field (i.e., from observations of individual insects, trap captures or eggs found).

Ostlie et al. (1984) describe a practical technique for marking European corn borer moths with oil-soluble dyes. Sudan Blue and Red dyes are mixed into a standard meridic diet, which is fed to corn borer larvae; the dyes then accumulate in the insect fat body. This marking method is valuable in several respects. First, researchers can raise large numbers of dyed larvae in the laboratory without investing much in time or money, as the treatment is relatively inexpensive and essentially follows the same procedure for raising undyed larvae (Lewis and Lynch 1969, Hunt et al. 2000). Moreover, the "marker" (i.e. blue or red dye) remains inside both sexes throughout

their lives. Males are easily caught in pheromone traps, and light traps attract both males and females. Females also sequester the dye in their eggs, which makes the technique even more useful; not only can researchers track insect dispersal with individual captures, but females deposit evidence of their movement and oviposition preferences with their eggs. Finally, Hunt et al. (2000) showed that the dye has no discernible negative effects on European corn borer larval survival or adult life span.

Dyed European corn borers have previously been used in studies investigating dispersal capability and movement patterns among mating aggregation sites (Showers et al. 2001, Qureshi et al. 2005, Reardon et al. 2006). The authors also used results from their mark-recapture studies as a basis for predictions involving insecticide resistance management (IRM). All of the aforementioned experiments quantified dispersal with counts of marked adults from trap captures or sweep net samples. Currently, no published corn borer studies have utilized the additional value of dyed moths: colored egg masses. Future research may use dyed female corn borers for work on dispersal and oviposition choice, especially as IRM models are refined. Female spatial range during mating and oviposition, as well as plant preferences, all may contribute to the development of resistance. Therefore, the utility of marked corn borer egg masses in field dispersal studies should be addressed.

The goal of this study was to evaluate the success of red and blue color treatments on egg masses that must be differentiated from white wild-type masses. More specifically, the experiment aimed to determine the effects of each marker dye on egg mass color intensity. The second objective was to ascertain the extent of differences between individual females' egg mass coloration, as well as model within-subject egg color over time. The final goal was to find expected proportions of females laying eggs of each color intensity in lab-raised populations.

Materials and Methods

Insect Culture. For details on rearing methods for the dyed insects, see Chapter 2 ("Released Insect Colony"). 0.4 g Sudan Blue II (C.I. 306436; Aldrich, Milwaukee, WI) or Sudan Red 7B (C.I. 201618) dye was used per liter of diet.

Laboratory Egg Mass Observations. Upon delivery from the Iowa USDA-ARS laboratory, one ring of dyed pupae was placed in a 25cm x 25cm x 25cm wood-frame, wire mesh cage. A cotton wick was placed in a plastic vial filled with a honey-water mixture and hung in the cage as a moisture and energy source for the adult moths. The cage was sprayed daily with distilled water, wrapped in clear plastic sheeting and placed in a growth chamber (Percival Scientific, Boone, IA) set at 25°C with a photoperiod of 16:8 (L:D).

Once approximately 50 moths emerged, the ring was removed from the cage and females and males were allowed to mate. After mating, two-day-old females were removed from the communal cage; each was isolated in a cylindrical 8 cm x 8.5 cm diameter stainless steel mesh cage. The top of each small cage consisted of a standard quart canning ring lid fitted with a circular partition of 6 mm grid galvanized hardware cloth. A circle of waxed paper was placed between the partition and the lid. The openings in the partition were large enough for a female moth to oviposit on the waxed paper. A cotton ball moistened with honey-water was placed in each cage. The individual cages were sprayed with distilled water, placed in a plastic crate, and covered with clear plastic sheeting. The crate was placed in the growth chamber (25°C; 16:8 L:D; approx. 80% RH).

Every day, the waxed paper was replaced in each cage and the number of masses and eggs per mass from the previous night were counted. The color of each mass was rated on the following color scale: 1 (no color), 2 (very light color), 3 (light color), 4 (definite color), and 5 (intense color). Each egg mass was compared to a series of pre-selected Valspar (Minneapolis,

MN) paint chips; this achieved standardization amongst color ratings (Blue masses: Color 1: Lime Wash 258A-1, Color 2: Palm Frond 258A-2, Color 3: Island Sky 252B-3 or Ivy Laurel 255-3, Color 4: Sea of Love 252B-4 or Secret Haven 255-4, Color 5: Patina Tint 222-5 or Teal Green 252B-5; Red masses: Color 1: Ivory Peach 205A-1, Color 2: Sweetheart Rose 209A-2, Color 3: Calamine 209-3, Color 4: Azalea 209-4 or Watermelon 209A-4, Color 5: Oriental Fuchsia 210A-5 or Raspberry Sherbet 210A-6). Daily egg records continued until death, upon which the females were dissected. Fat body coloration, internal egg coloration, and ovarian development stage (Xingquan et al. 2004) were noted for each female. A total of 129 females from seven rearing cohorts (4 blue, 3 red) were caged and observed, with numbers ranging from 15 to 24 individuals per cohort.

Statistical Analysis. Egg mass numbers laid per female were not compared between treatments because each shipment was reared separately; no replication existed because each shipment comprised its own block. PROC FREQ (Stokes et al. 1991, SAS Institute 2004) was used to obtain the extended Mantel-Haenszel mean score statistic for a comparison of average lifetime color (i.e., median color rating, 1-5, for all masses produced by each laying female) between red- and blue-dyed moths. Integer scores were specified, since the ordinal color rating levels were approximately equally spaced. The influence of average lifetime color on the total number of eggs oviposited by each laying female was analyzed with a Kruskal-Wallis test (PROC FREQ; Stokes et al. 1991). The Cochran-Armitage trend option in the TABLES statement of PROC FREQ was also used to determine if the proportion of laying females from each dye treatment in each average lifetime color category (1-5) exhibited a decreasing linear trend with increasing color intensity.

Frequency predictions for each lifetime color intensity were modified based on a cubic regression approximation of the proportion of females from each lifetime color rating in both dye treatments. These frequency predictions were compared to the actual frequency of red and blue

moths exhibiting each average lifetime color intensity using individual chi-squares for individuals from each dye, as well as a heterogeneity chi-square analysis.

Due to the ordinal egg mass color response variable, longitudinal analysis of within-subject egg mass color changes over time was achieved by fitting a cumulative logit model with random subject effects using PROC NLMIXED (SAS Institute 2004) using methods described by Yang (2006)*. The NLMIXED procedure estimates marginal log likelihoods by using Gaussian quadrature (i.e., numerical integration) over the random effect distribution (Fitzmaurice et al. 2004). A general log likelihood specification was used in the MODEL statement. Two different models were used, each using different time interval specifications. The first measured the median egg mass color rating on the first, third and fifth day of egg-laying for each female producing eggs. The second method used color ratings of the first and last egg masses laid by each moth. Parameters from each model were used to determine the log odds of egg mass color change over the life of each individual.

Results and Discussion

Table A.1 summarizes median egg deposition, life span and proportion of females exhibiting each lifetime color rating from each dye treatment. Almost one-third of all females observed did not lay any eggs. Blue individuals tended to live longer and lay more eggs than red moths. Fig. A.1 shows daily oviposition by blue females was greatest about 3 days after emergence, while red moths' oviposition peaked approximately four days after emergence. Daily egg-laying decreased steadily over the next 20 days, as moths died or stopped ovipositing. Red and blue females did not differ in their distribution between average lifetime color ratings (Q_{MH} = 0.14, df = 1, P = 0.711). This suggests both dye treatments are equally suitable for distinguishing colored egg masses in the field. The average lifetime color of a female also did not seem to

influence the number of eggs she laid, as total lifetime egg deposition was not significantly different among females of different egg color intensities ($H_{KW} = 1.67$, df = 4, $P = 0.323$).

Although both blue and red moth lifetime color proportions tended to decrease with increasing color intensity, this did not show a linear trend (Cochran-Armitage $Z = -0.37$, $n = 87$, $P = 0.709$). The decrease more closely approximated a cubic regression model. Populations of dyed, lab-raised females were hypothesized to exhibit median lifetime color ratings with a frequency of 31:23:22:19:5 (1:2:3:4:5). Proportions of females observed (summed across shipments from each dye treatment) in each color category supported this frequency distribution (Blue: $\chi^2 = 0.44$, df = 4, $P = 0.979$; Red: $\chi^2 = 0.62$, df = 4, $P = 0.961$). Both red and blue moths exhibited the frequency (Heterogeneity $\chi^2 = 0.83$, df = 4, $P = 0.935$), so it could be argued that all dyed moths show the same average lifetime egg color frequency distribution. Egg masses (≥ 5 eggs) from each color category were also totaled and compared to the same frequency distribution, but both blue and red egg mass totals differed significantly from it (Blue: $\chi^2 = 40.40$, df = 4, $P < 0.0001$; Red: $\chi^2 = 103.10$, df = 4, $P < 0.0001$). No color frequency distribution was suitable for egg masses from both dye treatments.

Within-subject model parameters of egg color on the first, third and fifth day of egg-laying showed no significant color change over time ($t = -0.49$, df = 208, $P = 0.623$). This conclusion also held in the model of initiation versus conclusion of egg laying ($t = -1.19$, df = 79, $P = 0.239$). However, the second model suggested treatment groups differ at baseline; that is, red individuals' first egg masses are more likely to be lighter than those from blue moths ($t = -2.07$, df = 79, $P = 0.042$). However, this result does not contradict our prior conclusion of no difference in egg mass color between dye treatments, as a significant time by dye treatment interaction in the model was an indication that red moths were more likely to increase their egg mass color intensity over time than blue moths (($t = 2.33$, df = 79, $P = 0.023$).

Egg color intensity within the ovary generally resembled each moth's respective egg mass color rating. All eggs within a given ovary showed uniform coloration, with the exception of one female with an ovariole containing three bright pink eggs and nine very light pink eggs. We originally speculated that dye sequestered in the fat body possibly depletes over the course of egg development and oviposition. However, the evidence suggests inter-female coloration tends to vary much more than that of a given female over time. Females may consume or sequester dye to different degrees. Alternatively, some individuals may incorporate marker dyes more readily into developing eggs than others. In any case, dispersal studies may suffer somewhat from the lack of data from unidentifiable egg masses produced by released corn borers. Researchers using marked moths should assume some percentage of observed, uncolored egg masses are from released females; however, this would be hard to quantify owing to variation among uncolored mass deposition from wild corn borer populations, released moth dispersal patterns and dispersal study scales.

Studies using moth releases may also consider daily oviposition relative to female age (Fig. A.1) when characterizing trends. Field oviposition models can be complicated due to variation in moth emergence over time. However, temporal emergence patterns could be important considerations in the timing of egg mass deposition in relation to plant sampling frequency. In the laboratory, egg-laying peaked soon after mating and decreased steadily over time.

Hunt et al. (2000) addressed the effects of red and blue marker dyes on female longevity and egg mass coloration. The group found no differences between dyes in adult life span, fecundity, or oviposition rate; they also described color variation among individual insects. However, the group reported higher percentages of dyed egg masses: 84% of blue masses and 99% of red masses were sufficiently dyed. Since egg mass coloration in both studies was evaluated on waxed paper, future work on oviposition with marker dyes may benefit from color

assessment on actual corn leaves. The dark green background color of a leaf may impact marked egg mass recognition in the field; therefore, a blind study using egg masses from both marked and unmarked individuals is warranted.

*Note on longitudinal model and data selection:

Two methods are generally utilized for the longitudinal data analysis involving an ordinal response: the Generalized Estimating Equation (GEE) approach for marginal models developed by Liang and Zeger (1986) and Generalized Linear Mixed Models. Parameters resulting from the GEE approach are generally used for conclusions about population averages. In contrast, the random effects included in mixed models allow inference about subject-specific changes over time. Due to the high variability in inter-individual egg mass coloration of dyed moths, the mixed model approach was favored in this case. Furthermore, a model of within-subject changes in egg mass color intensity was more appropriate for our objectives in the longitudinal analysis.

Egg mass color intensity for each individual moth was used as the response variable, rated on an ordinal scale of 1=no color to 5=intense color. Two cumulative logit models with random subject effects were fit using different time intervals. Unfortunately, both time interval specifications presented problems. Most longitudinal studies set an equal time length between observations for all individuals. In this study, daily oviposition was highly variable; therefore, a set time interval between observation days would result in a large number of missing data. We chose to only use those days that each female laid eggs.

The first time interval specification involved taking the median egg mass color from all masses ($\geq$ 3 eggs) laid on the first, third, and fifth days each female laid eggs. This resulted in a variable number of days between measurements for each individual, but the difference was usually no more than two to four days. The first- through fifth-day time spread was chosen to maximize the time over which color depletion may be observed while minimizing the number of individuals

that died or stopped laying eggs before the fifth day. Seventh- and eleventh-day egg colors were also modeled, but the large numbers of missing data due to early death overtaxed the statistical program.

Initiation of egg-laying (first egg mass of three or more eggs) and conclusion of oviposition (final egg mass of three or more eggs) were also used as time interval endpoints. Again, the life spans, numbers of eggs laid, and temporal span of egg-laying activity was variable between individuals. One of our study goals was to model within-subject egg color changes from the first egg mass to the last, however, so we chose to specify the time interval this way.

"Dropouts", or those individuals laying only one egg mass, were included in both models, even though this resulted in missing data for one or more time points. As we previously found no relationship between average lifetime color intensity and total number of eggs laid, dropout was determined to be missing completely at random (MCAR, Fitzmaurice et al. 2004), and the probability of dropout was independent of egg color intensity. Available data analysis was chosen for the cumulative logit model because complete case analysis (i.e., elimination of individuals with dropouts) is generally not recommended due to biased conclusions for treatment effects (Fitzmaurice et al. 2004).

The mixed-effects cumulative logit model was specified as

$$\log\left[\frac{P_{ijk}}{1-P_{ijk}}\right] = \gamma_k - Z_{ij} \qquad (k = 1, 2, 3)$$

where $Z_{ij} = [\beta_0 + \beta_1 \text{Dye}_i + \beta_2 \text{Time}_j + \beta_3 \text{Dye}_i \text{Time}_j + \mu_{0i}]$

μ_{0i} = random subject effects ($N \sim 0, \sigma_u^2$)
γ_k = model thresholds ($k = 3$)

Table A.1. Median (min,max) number of masses and eggs laid and days lived per female from each dye treatment. Median numbers for only those females laying eggs, as well as the proportion of individuals laying eggs of each color intensity, are summarized below.

Dye	Cohorts *n*	Moths *n*	Egg masses laid (≥ 5 eggs)	Eggs laid	Life span (days)	Mean ± SE eggs per mass	Non-ovipositing (%)
Blue	4	72	4 (0,48)	111 (0,661)	16.5 (3,30)	15.4 ± 0.44	29
Red	3	57	1 (0,32)	29 (0,687)	12 (6,24)	14.2 ± 0.61	37

Only females laying eggs:						Proportional average lifetime color				
						1	*2*	*3*	*4*	*5*
Blue	4	49	11 (1,48)	190 (6,661)	18 (5,30)	29	22	23	22	4
Red	3	29	6 (1,32)	101 (24,687)	15 (7,24)	33	25	17	19	6

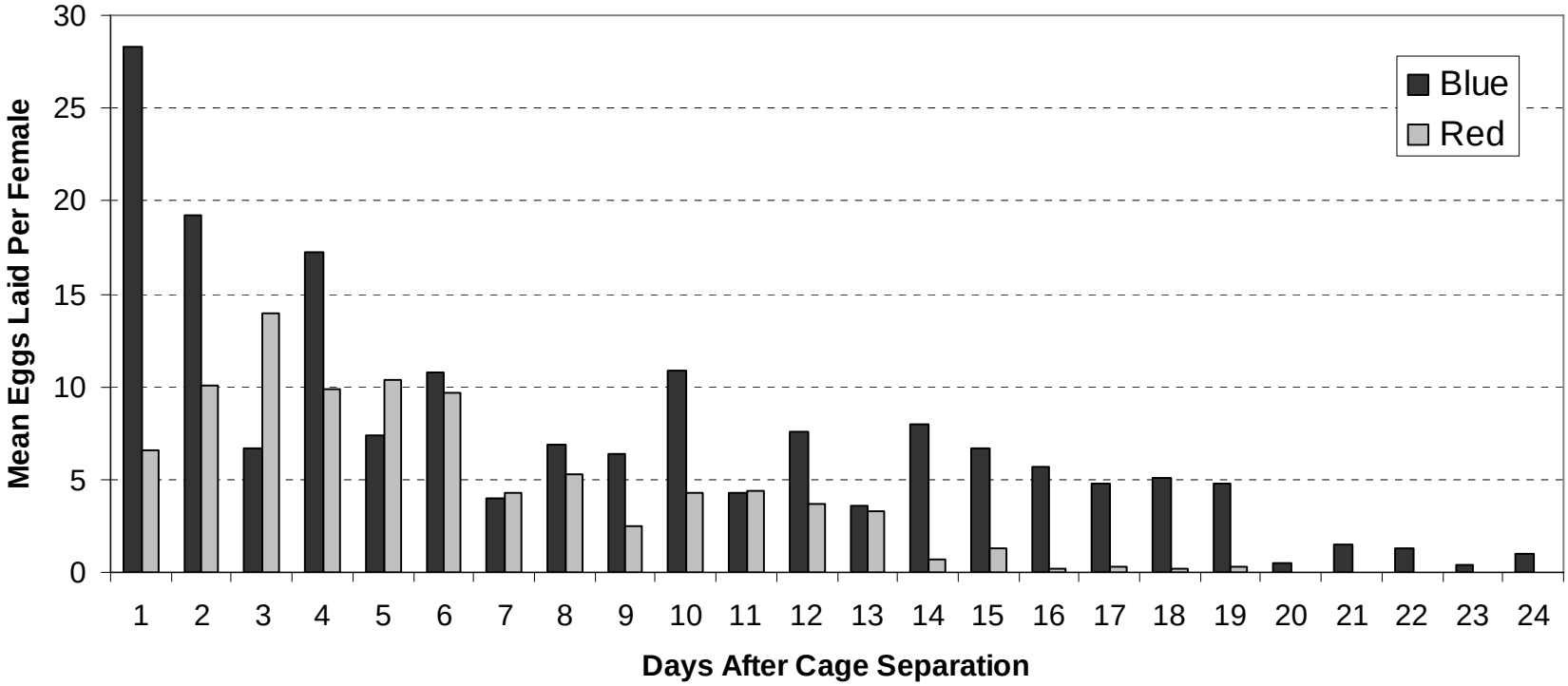


Fig. A.1. Mean number of eggs laid per blue- or red-dyed female on each day after separation amongst individual cages (2 days post-emergence).

APPENDIX B.

European Corn Borer Oviposition Preference Model

A relative preference model for European corn borer oviposition was generated by Steve Spangler and Dennis Calvin (2000) from observed egg mass deposition on sweet corn plant stages V1-16 and R1-5. The preference function is

$$RP = \frac{0.58}{1 + e^{(6.047 - 15.52 * PCDC)}}$$

where RP = relative preference

$PCDC$ = proportion of development completed

Fig. B.1 is a graph of the data used as the basis for the model. Table B.1 shows the predicted preference of each stage. Stages V17-19 and VT were added to include stages commonly completed by field corn.

Predictions of egg mass deposition for a given week were calculated considering the fields of each stage available to ovipositing females. The relative preference of each stage that week was calculated by dividing its predicted preference from Table B.1 by the preference of the most attractive available stage. To find the predicted percentage of eggs deposited in a particular plant stage, the relative preference for the stage was divided by the sum of the relative preferences for all available stages. The predicted percentages were then compared to the observed percentages of egg masses found in fields of each stage that week.

The approximate total leaf area for each stage was added as well; these values were estimated from a regression of total leaf area against plant stage recorded from sample plants in fields from the Chapter 3 landscape study.

Periodically throughout the season, two to five plants were selected at random from several fields and cut at ground level; the heights and leaf areas from these sampled plants were used to estimate the total leaf area of plants sampled for egg masses. All fully-emerged, green leaves from each sample plant were cut at the leaf collar. Any leaves not fully emerged were cut at the collar level of the last fully emerged leaf. Leaf length (cm) and the widest part of each leaf (cm) were recorded. The area of one side (cm^2) of a leaf was measured with a LI-COR™ Portable Leaf Area Meter (Model LI-3000, Lambda Instrument Corp., Lincoln, NE).

A quadratic regression of plant leaf area against plant height was also estimated from sample plant leaf areas using PROG REG; the model was used to estimate per-plant and per-field leaf areas from plant height measurements taken each week. Mean leaf area and median field height from each field plant sample were used instead of individual plant measurements to maximize model fit.

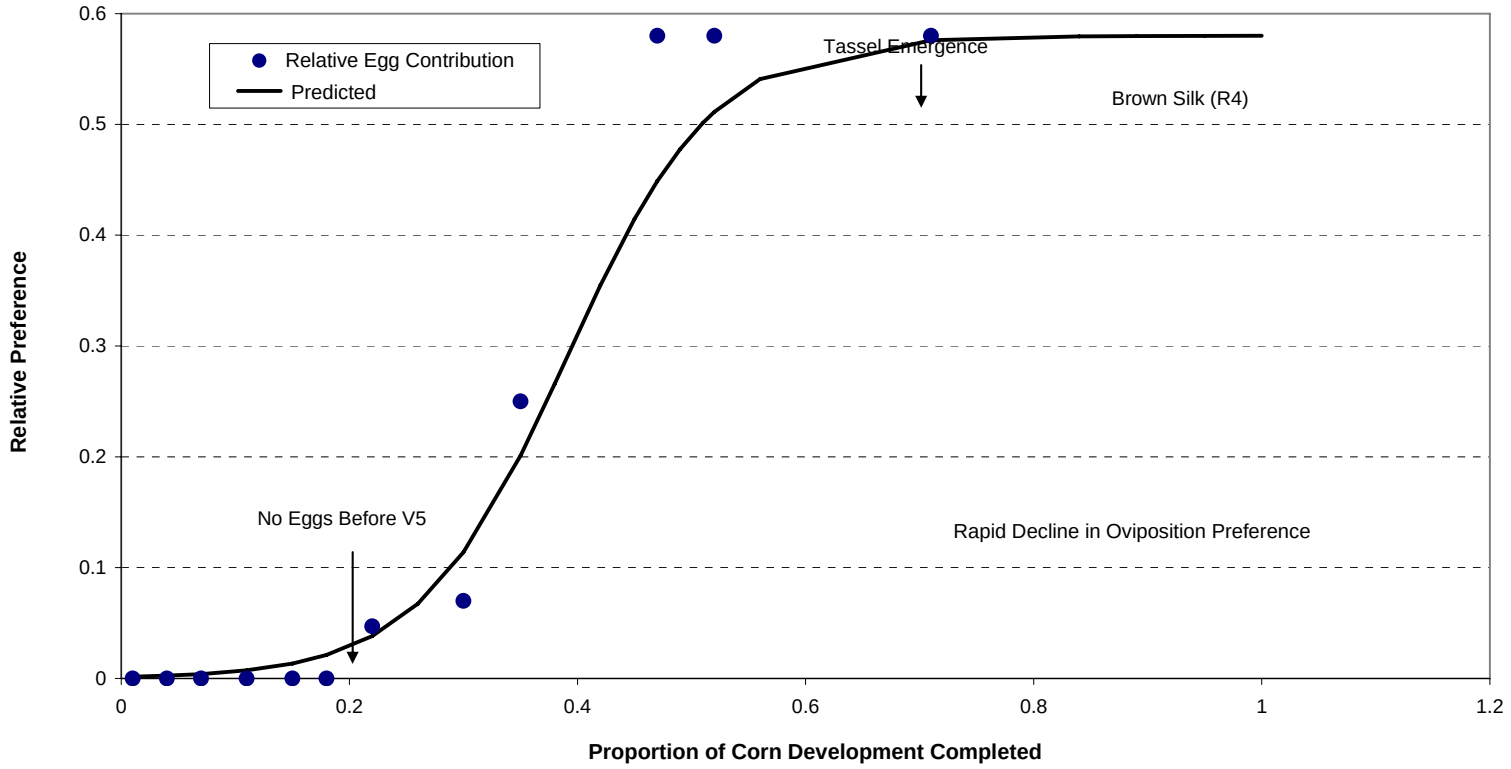


Fig. B.1. Relative oviposition preference of European corn borer females by the proportion of corn development completed (generated from Spangler and Calvin 2000).

Table B.1. Approximate leaf area, developmental completion, and predicted oviposition preference for corn stages V1-VT and R1-R5.

Corn Stage	Leaf Area (cm^2)	Percent of Total Leaf Area Present	Percent of Development Completed	Predicted Relative Preference
V1	40	0.00853	0.01	0.00157
V2	100	0.02133	0.04	0.00249
V3	150	0.03200	0.07	0.00397
V4	230	0.04906	0.11	0.00734
V5	531	0.11316	0.15	0.01350
V6	1041	0.22195	0.18	0.02121
V7	1517	0.32363	0.22	0.03826
V8	1960	0.41819	0.26	0.06736
V9	2370	0.50564	0.30	0.11393
V10	2747	0.58596	0.35	0.20120
V11	3090	0.65918	0.38	0.26583
V12	3400	0.72527	0.42	0.35468
V13	3677	0.78425	0.45	0.41464
V14	3920	0.83612	0.47	0.44878
V15	4130	0.88087	0.49	0.47762
V16	4306	0.91850	0.51	0.50123
V17	4449	0.94901	0.513	0.50372
V18	4557	0.97241	0.515	0.50622
V19	4635	0.98870	0.518	0.50871
VT	4678	0.99787	0.518	0.51121
R1	4688	0.99992	0.52	0.54088
R2	4664	0.99485	0.56	0.57594
R3	4607	0.98267	0.71	0.57946
R4	4516	0.96338	0.84	0.57945
R5	4393	0.93697	0.89	0.57975

APPENDIX C.

Plant Density Treatment Study: Sample Leaf Area Regressions

Weekly leaf area samples were collected from plants in high- and low-density plantings from the two-factor factorial study described in Chapter 4. Three plants were selected at random and cut from each planting date and density treatment combination (PD1-Low, PD1-High, PD2-Low, PD2-High) each week for 7 weeks. Each replicate was sampled at least once.

Each plant's pre-cut height (distance from ground to leaf collar on last fully emerged leaf in cm) and stage (V5-VT, R1-R5) were recorded. All fully-emerged, green leaves from each plant were cut at the leaf collar; any leaves not fully emerged were cut at the collar level of the last fully emerged leaf. Leaf length (cm) and widest part of each leaf (cm) were recorded. The area of one side (cm^2) was also measured with a LI-COR™ Portable Leaf Area Meter (Model LI-3000, Lambda Instrument Corp., Lincoln, NE).

The leaf area data were combined across weeks and planting date. Density treatment differences in leaf length and width were analyzed with a Mann-Whitney-Wilcoxon test (PROC FREQ, SAS Institute 2004). PROC REG (SAS Institute 2004) was used to estimate regression models of total plant and individual leaf areas against plant heights, leaf lengths, and leaf widths. The CLM and CLI options were used to obtain confidence and prediction intervals.

Leaf area regressions of individual leaves. Density treatment did not significantly affect leaf length ($\chi^2 = 1.99$, df = 1, $P = 0.158$, $N = 913$). Leaf areas from both density treatments were regressed against leaf lengths (Fig. C.1). Fig. C.2 shows a scatterplot of leaf area and leaf width for both densities. Leaf width in the low density plots was significantly greater than high density plots ($\chi^2 = 28.16$, df = 1, $P < 0.0001$, $N = 913$). Separate quadratic regressions were estimated for the two treatments; these are presented in Figs. C.3 and C.4.

A multiple regression using leaf length and width provided the best estimation of leaf area, regardless of density ($R^2 = 0.987$; $F = 35324$; df = 2, 910; $P < 0.001$). For the model

$$y = a + bX_1 + cX_2$$

y = leaf area (cm^2, square-root transformed)
X_1 = leaf length (cm)
X_2 = leaf width (cm)

$a = -0.869 \pm 0.075$
$b = 0.185 \pm 0.002$
$c = 1.020 \pm 0.019$

An analysis of covariance using leaf number as a covariate (PROC GLM, SAS Institute 2004) showed that high density sample plants had significantly lower leaf areas than leaves from low density plots (Type III analysis; $F = 7.61$; df = 1; $P = 0.006$; leaf number covariate: $F = 53.93$; df = 1; $P < 0.0001$; $N = 913$).

Whole-plant leaf area regressions. Plant leaf area totals were also analyzed by density treatment. An analysis of covariance (PROC GLM, SAS Institute 2004) using a plant height covariate showed significantly higher total plant leaf areas in the low density sample plants (Type III analysis of square-transformed leaf area; $F = 4.68$; df = 1; $P = 0.034$; $N = 82$; leaf number covariate: $F = 131.62$; df = 1; $P < 0.0001$).

Plant height was a fairly good predictor of total plant leaf area; quadratic regressions of leaf areas against plant heights for each density treatment are shown in Fig. C.5. Total plant leaf area peaked at a height of about 150 cm in each density; this corresponded to a stage of about V16. Total leaf area decreased through the reproductive stages as lower leaves started to yellow and dry out.

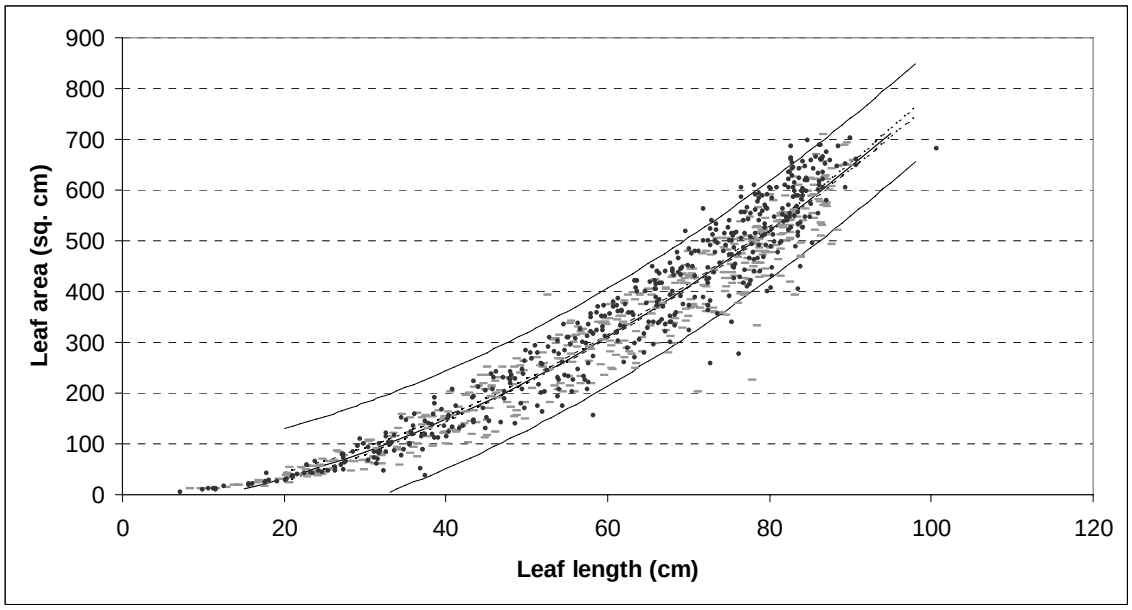


Fig. C.1. Quadratic regression of individual leaf area against leaf length where $y = a + bx + cx^2$. Dots and dashes represent low and high density sample leaves, respectively. The regression line is flanked by 95% confidence interval (dashed) and 95% prediction interval lines. Coefficient values (± S.E.) are a = -32.29 ± 11.28, b = 2.09 ± 0.44, c = 0.06 ± 0.004; R^2 = 0.931, mean square error = 2374, F = 6168; df = 2, 910; $P < 0.001$.

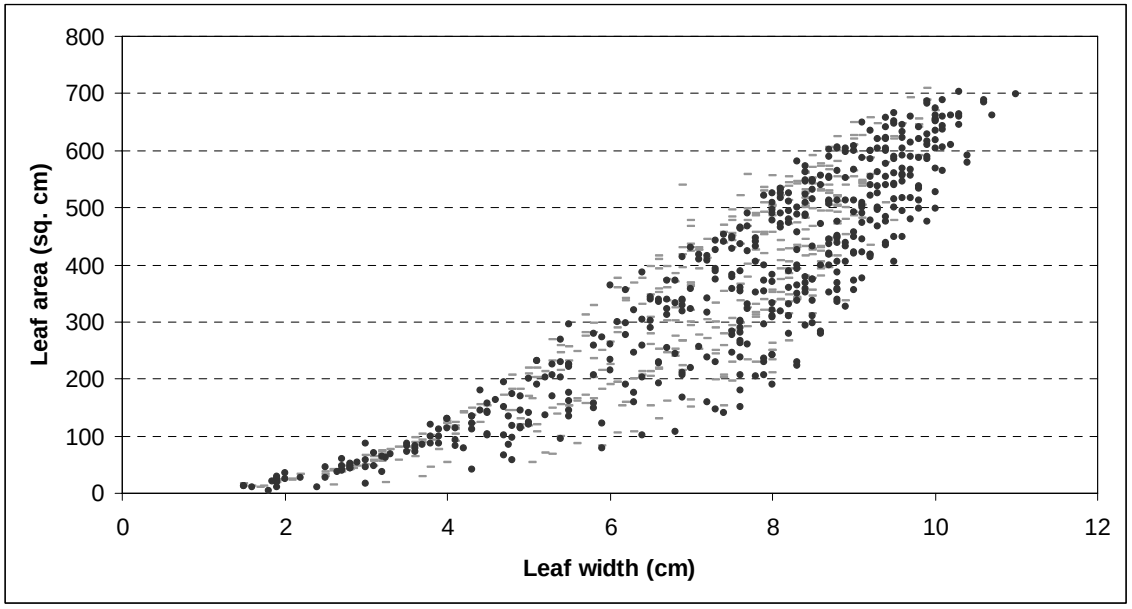


Fig. C.2. Leaf width and area of individual leaves sampled from low- (dots) and high-density (dashes) plant treatment plots.

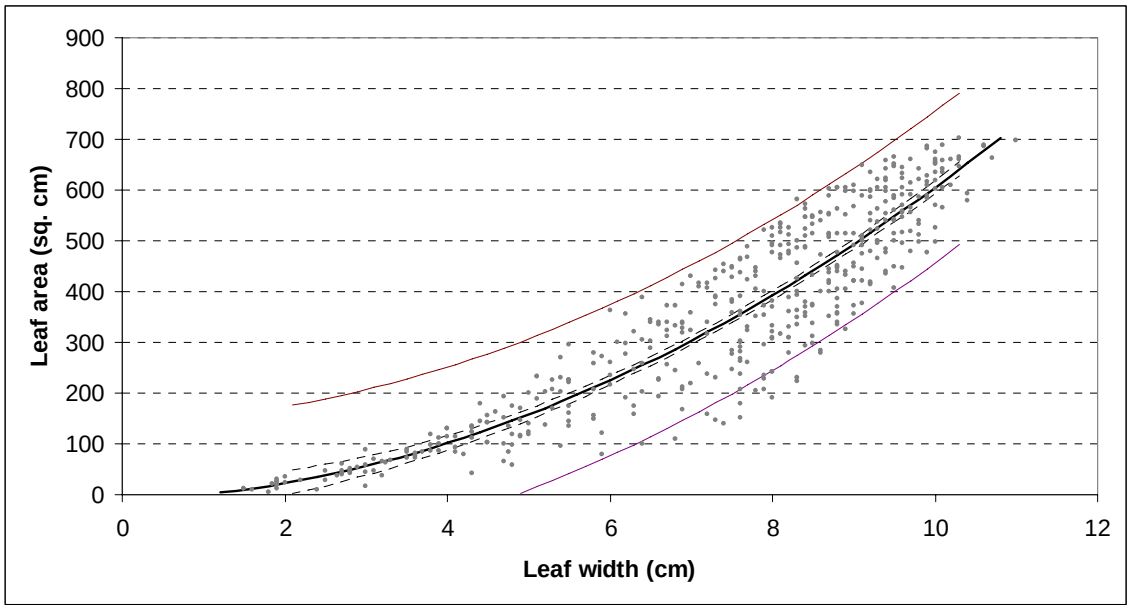


Fig. C.3. Quadratic regression of individual leaf area against leaf width in low density sample plants. The regression line ($y = a + bx + cx^2$) is shown with 95% confidence interval (dashed) and 95% prediction interval lines. Coefficient values are a = -10.85 ± 26.79, b = 5.82 ± 8.87, c = 5.58 ± 0.68; R^2 = 0.844, mean square error = 5709, F = 1259; df = 2, 467; P < 0.001.

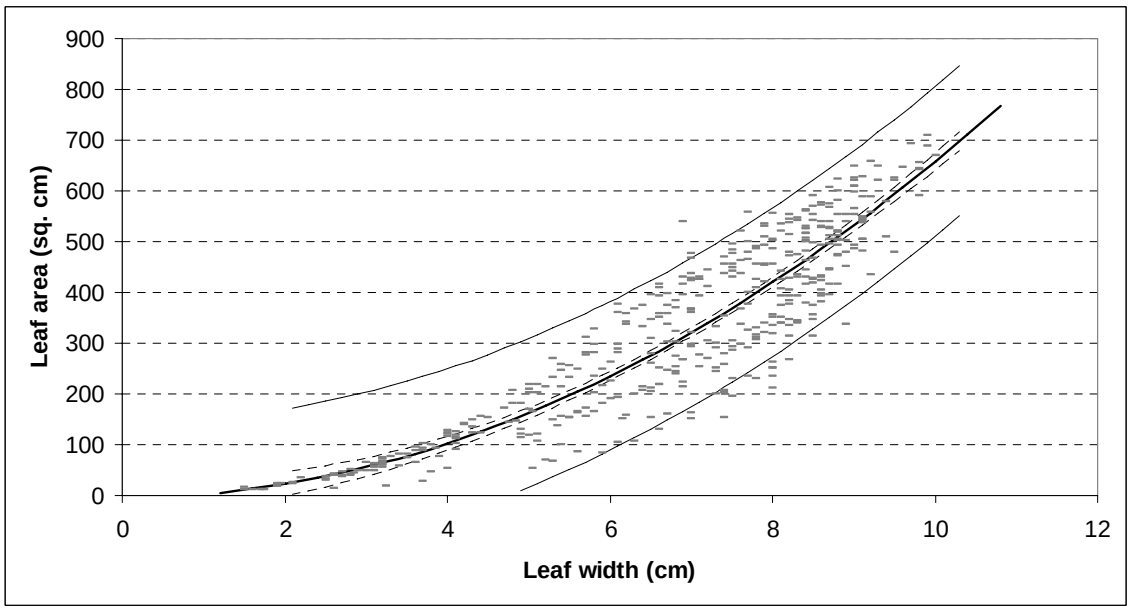


Fig. C.4. Quadratic regression of individual leaf area against leaf width in high density sample plants. The regression line ($y = a + bx + cx^2$) is shown with 95% confidence interval (dashed) and 95% prediction interval lines. Coefficient values are a = -4.99 ± 28.84, b = 0.64 ± 10.21, c = 6.57 ± 0.84; R^2 = 0.828, mean square error = 5530, F = 1057; df = 2, 440; P < 0.001.

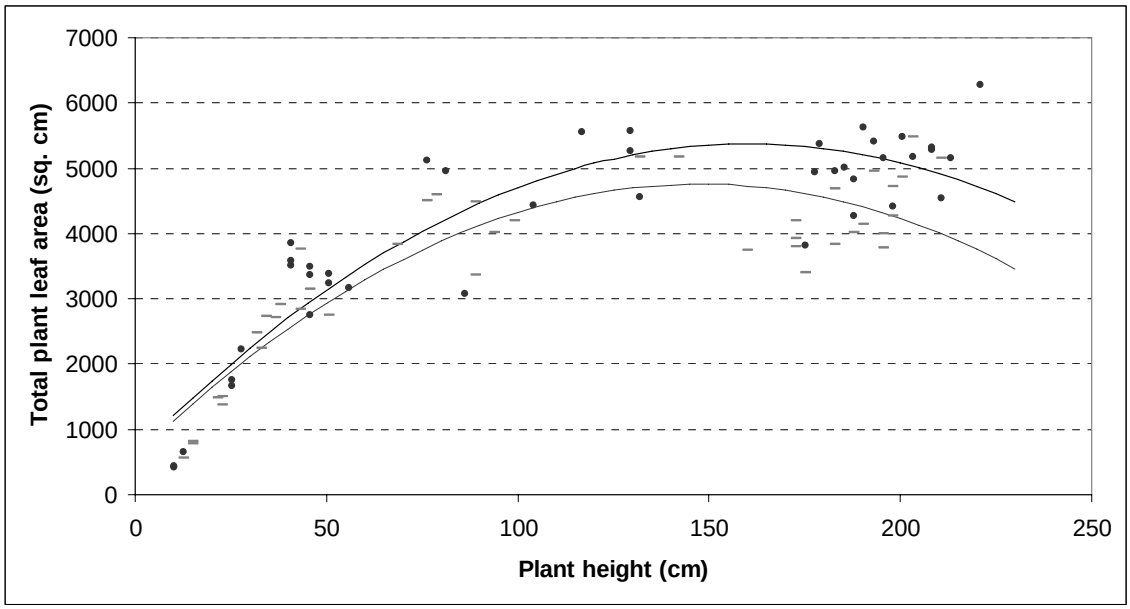


Fig. C.5. Quadratic regression ($y = a + bx + cx^2$) of total plant leaf area against leaf width in low (dots) and high (dashes) density sample plants. The solid line represents the low-density plant regression: $a = 346.0 \pm 265.4$, $b = 70.0 \pm 6.44$, $c = -0.23 \pm 0.028$; $R^2 = 0.887$, mean square error = 272120, $F = 137$; df = 2, 35; $P < 0.001$. High-density regression (dashed line): $a = 384.0 \pm 276.4$, $b = 63.7 \pm 7.06$, $c = -0.23 \pm 0.032$; $R^2 = 0.820$, mean square error = 299955, $F = 82.2$; df = 2, 36; $P < 0.001$.

APPENDIX D.

Selected R and SAS Statistical Code

Chapter 2.

Poisson Regression

Analysis of plot distance, direction, and planting date effects on colored egg mass data (R)

```
# read in 2004 colored egg mass data
# column headers are “total” (season-wide egg mass total), “dir” (plot direction),
# “dist” (plot distance), and “pd” (planting date)
color04=read.table("E:/colored04.txt", header=T, sep="\t")

# identify variables
total=data$Total
dir=data$Dir
dist=data$Dist
pd=data$PD

# specify poisson regression model
g<-glm(total~factor(dir)+factor(dist)+factor(pd), family="poisson")
summary(g)
```

Analysis of plot distance, direction, and planting date effects on colored egg mass data (SAS)

```
data hexagon;
input direction $ distance $ PD mass;
datalines;
A     A     1     3
A     A     2     1
……
F     D     3     0
;
proc genmod;
class direction distance PD;
model mass = direction distance PD / dist=poisson link=log type3;
run;
```

Rose Diagram Generation (R)

```
# load CircStats library
library(CircStats)
# read in 2004 daily mean wind directions
wind=read.table("G:/04wind_directions.txt", header=F)
# list directions
wind
is.vector(wind)
# specify dot attributes and bins (number of distinct directions that are shown around the circle)
rose.diag(wind,bins=18,pts=TRUE,cex=1,dotsep=50,shrink=1.2)
```

Appendix A.

Generalized Linear Mixed Model Approach for Longitudinal Data (SAS)

Analysis of egg mass coloration on first and last day of egg-laying by individual European corn borer females

id = individual moth
trt = dye color treatment (red or blue)
time = first or last day of laying
color = color rating (1-5)

```
data color;
input id trt time color;
datalines;
1     1     1     1
1     1     2     .
……
129   0     1     4
129   0     2     4
;
run;
proc nlmixed data=color qpoints=100;
parms b0=0 b1=0 b2=0 b3=0 sd=1 thres1=1 thres2=1 thres3=1;
Z = b0 + b1*trt + b2*time + b3*trt*time + u;
if (col=1) then P = 1 / (1 + EXP(-(0-Z)));
else if (col=2) then P = (1/(1 + EXP(-(thres1-Z)))) - (1/(1 + EXP(-
0Z))));
else if (col=3) then P = (1/(1 + EXP(-(thres1+thres2-Z)))) - (1/(1 +
EXP(-(thres1-Z))));
else if (col=4) then P = (1/(1 + exp(-(thres1+thres2+thres3-Z)))) -(1/(1
+ EXP(-(thres1+thres2-Z))));
else if (col=5) then P = 1 - (1/(1 + exp(-(thres1+thres2+thres3-Z))));
LL = LOG(P);
MODEL col ~ general(LL);
random u ~ normal(0,sd*sd) subject=id;
ESTIMATE 'Threshold2' thres1;
ESTIMATE 'Threshold3' thres2;
ESTIMATE 'Threshold4' thres3;
run;
```

Analysis of egg mass coloration on first, third and fifth day of egg-laying by individual European corn borer females

```
data color;
input id trt time col;
datalines;
1     0     1     1
4     0     1     4
1     0     3     1
4     0     3     4
1     0     5     1
4     0     5     3
……
124   1     1     1
127   1     1     2
124   1     3     1
127   1     3     2
124   1     5     .
127   1     5     3
;
run;
proc nlmixed data=color;
      parms b0=0 b1=0 b2=0 b3=0 sd=1 i1=1 i2=1 i3=1;
      eta = b0 + b1*trt + b2*time + b3*trt*time + u;
      if (col=1) then p = probnorm(-eta);
      else if (col=2) then
         p = probnorm(i1-eta) - probnorm(-eta);
      else if (col=3) then
         p = probnorm(i1+i2-eta) - probnorm(i1-eta);
      else if (col=4) then
         p = probnorm(i1+i2+i3-eta) - probnorm(i1+i2-eta);
      else p = 1 - probnorm(i1+i2+i3-eta);
         ll = log(p);
      model col ~ general(ll);
      random u ~ normal(0,sd*sd) subject=id;
      estimate 'thresh2' i1;
      estimate 'thresh3' i1 + i2;
      estimate 'thresh4' i1 + i2 + i3;
      estimate 'icc' sd*sd/(1+sd*sd);
      run;
```

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