INTERACTIONS AMONG MAIZE PHENOLOGIES, TRANSGENIC \textit{Bacillus thuringiensis} MAIZE AND SEED TREATMENT FOR MANAGEMENT OF PESTS AND DISEASES OF MAIZE

DISSERTATION

Presented in Partial Fulfilment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of The Ohio State University

By

Motshwari Obopile, BSc (Hons), M.S.

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The Ohio State University

2009

Dissertation Committee:

Dr. Ronald B. Hammond, Advisor
Dr. Celeste Welty
Dr. David J. Horn
Dr. Pierce A. Paul
Dr. Peter R. Thomison

Approved by

____________________________

Dr. David J. Horn
Advisor

Graduate Program in Entomology
Planting date is critical in maize production because it influences the availability of growing degree days required to mature the maize crop, and the amount of pests on the crop throughout the season. The interactions among maize phenologies, transgenic \textit{Bacillus thuringiensis} (Berliner) maize(Bt) and seed treatment were studied from 2006 to 2008 based on the following objectives: (1) To evaluate the potential use of planting dates and varying maize maturity in combination with transgenic maize and seed treatment to manage corn rootworm (\textit{Diabrotica virgifera virgifera} LeConte), (2) To evaluate the potential use of planting dates and varying maize maturity in combination with transgenic maize on oviposition and subsequent larval injury to maize by European corn borer [\textit{Ostrinia nubilalis} (Hübner)], (3) To evaluate the potential use of planting dates and varying maturity of maize in combination with transgenic hybrids on stalk and ear rots incidence and severity following European corn borer infestation.

Corn rootworm larval feeding was reduced by delayed planting and using transgenic Bt maize and seed treatment. When populations of corn rootworms were high, root lodging occurred on untreated maize and lodging declined in late plantings. Significant yield reduction associated with root injury occurred only in 2007 at Wooster on untreated early planted corn. Increased adult beetles feeding on maize caused silk clipping, resulting in poor seed set and reduced yield. Yield from short season hybrids were comparable to full season hybrids especially in late plantings. The European corn borer study showed higher numbers of egg masses from second generation moths on late planted corn. Injuries on stalks and leaves were significantly higher on non-transgenic corn, and increased with delayed planting. The benefit of Bt maize was more significant when planting was delayed than was early when planting.
Planting Bt hybrids with resistance to European corn borer lowered both stalk rots and ear rots compared with non-Bt genotypes especially on late plantings. Compared with short season hybrids, significant reduction in stalk and ear rots occurred in full season hybrids. In summary, the results suggest that in management of European corn borer and associated diseases (rots), when planting is delayed due to unforeseen circumstances and populations would cause economic damage, planting short season Bt hybrids could be beneficial. For corn rootworms, using a seed treatment or transgenic Bt maize might be beneficial where populations are known to be high and planting is early.
DEDICATION

To my father, Obopile Dihemo-A Ntshobo (1924 – 2004). Robala ka Kagiso Mokwena
ACKNOWLEDGMENTS

I am very grateful to my graduate Committee members: Dr. Celeste Welty, Dr. David J. Horn, Dr. Pierce A. Paul, Dr. Peter R. Thomison and Dr. Ronald B. Hammond, thank you all for your guidance. I thank Ron Hammond my major advisor for agreeing to be my advisor again. Thanks Ron for working with me in the fields and contributing to my graduate studies (Master’s degree 1996-1998, and PhD, 2005-2009). I also thank Peter Thomison and Pierce Paul for contributing respectively to the agronomic and plant disease aspects of this study. I appreciate the help and all the support of Judith A. Smith for keeping me focused throughout my stay at Wooster.

I am grateful to Brenda Franks and Shirley Holmes for their administrative support and being so kind. Thanks to my dear friends Dr. Rich Gary and Nicola Gallagher for providing me with free accommodation at Columbus, OH when doing course work. Thanks to Christine, Cloe, Sonia, Tony and Midge for showing me Ohio and the USA. To my all friends at Wooster, Alfred Alumai, Valdir Correa, Rodrigo Chorbadjian, Sougata Bardhan, Claudia Kuniyoshi and Karla Medina Nagendra Subedi, Abdul Rauf and many others.

Special thanks to all my brothers and sisters (9 names need more space!!) for their encouragements. Thanks to my wife Gobonaone, and to Bryan and Latie. Most important of all thanks to my mother Oneile Obopile and my late father, Obopile Difhemo for making sure that I attend Primary and secondary school, despite all the financial limitations of that time. I am grateful to Botswana College of Agriculture for
funding my PhD studies. Salaries and research support were also provided in part by State and Federal funds appropriated to the Ohio Agricultural Research and Development Center, The Ohio State University. We thank Monsanto Company for providing seeds.
VITA

July 1993…………………………………………………………BSc. (Hons) Plant Biotechnology
University of London,
Wye College,
Wye, Near Ashford-Kent, UK.

September 1998………………………………………………M.S. Entomology,
The Ohio State University
Columbus Ohio, USA

September 2005- Present………………………………….Graduate Student
The Ohio State University
Ohio Agricultural Research
and Development Center
Wooster, Ohio USA

Work experience

2002-present…………………………………………………Lecturer
Department of Crop Science
and Production.
Botswana College of Agriculture.
Gaborone, Botswana

1998 -2002………………………………………………….Research Entomologist
Department of Agricultural
Research, Gaborone, Botswana.

1993 - 1996. …………………………………………………..Assistant Agricultural Research
Officer, Department of
Agricultural Research, Gaborone, Botswana.
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FIELD OF STUDY

Major Field: Entomology
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CHAPTER 1

1. INTRODUCTION

Maize is grown in many countries on every populated continent. In 2007, maize was grown on more than 148 million hectares worldwide, with the majority grown as field maize (USDA, NASS 2007). Globally, maize production totaled more than 705,000 million metric tons in 2006-2007 (USDA, NASS 2007), with 38% of the world production coming from the USA (267,598 million metric tons). As a crop, maize production is more than double that of any other crop. Maize yields have steadily increased since the 1950s, although a high degree of variability indicates that factors such as the weather, irrigation practices, and plant nutrition have a major impact on yields (USDA, NASS 2008). To achieve its yield potential, maize must receive optimum water and nutrients and be protected against pests, such as insects, diseases, and weeds, and other factors that may reduce yield.

Selection of an optimum planting date to ensure physiological maturity before fall frost is a major management consideration for maize producers (Saseendran et al. 2005). Optimum maize planting dates are regional and vary across the conterminous USA because of differences in climate and length of growing seasons in areas where the crop is produced (Bruns 2003). Thus, for optimization of yield, planting at the appropriate time to fit the hybrid maturity length and growing season is critical. Factors that can
delay planting include weather conditions (e.g. excessive soil moisture) and equipment breakdowns which can push planting beyond the optimum dates of late April to early May in the Eastern USA Corn Belt (Nielsen et al. 2002, Jarvis 1986). Additionally, cornfields planted during the optimum time frame often require replanting at later dates when weather stresses or pests cause excessive plant mortality.

Accurate knowledge of the planting window of any particular hybrid at a particular location is critical when selecting hybrid seed for planting when normal planting is delayed or for replanting when crop stand is not optimal (Benson 1990). Replanting or delayed planting will shorten the effective growing season for the maize plants. A shorter growing season due to delayed planting means fewer available growing degree days (GDDs) to mature the maize crop. In late planting situations, the goal is to allow the maize crop to utilize the season as much as possible yet reach physiological maturity before the occurrence of a killing frost (Nielsen et al. 2002). Full season hybrids (>105 days relative maturity) can be planted in the Eastern Corn Belt as late as early June (Nielsen et al. 2002). However, planting such full season hybrids in late May or early June often results in slow grain drying which results in higher grain moisture content at harvest because conditions at maturity (September/October) are less favorable for grain drying. As a result most farmers allow maize grain to field dry to grain moisture of 20% or less prior to harvesting to save on drying cost. The setback is that during such field drying, the crop is subjected to weathering conditions, which can result in a reduction in grain yield and quality. The potential yield of maize is reported to decline by 10-20% if planting is delayed until 20 May and 1 June respectively (Benson 1995).
The reduction in yield potential of late planted maize often forces growers to make the economic decision of switching to soybean because it is generally more profitable than late planted maize (Nielsen et al., 2002). However, by switching to an alternative crop like soybean, the producer faces a potential loss in revenue. Therefore, if given more time within the growing season, growers would prefer to grow maize to meet their regular markets. Switching to soybean production may not be sustainable because of various factors, including the emerging threats of Asian soybean rust (*Phakopsora pachyrhizi* Sydow) and soybean aphid (*Aphis glycines* Matsumura). The cost of producing late planted soybean is expected to be higher because of the increased use of insecticide and fungicide applications, thereby negatively impacting profit.

Planting short season maize hybrids (<105 days relative maturity) when planting late may facilitate earlier harvest and minimize the cost of drying the grain. Indeed, short season maize hybrids with improved agronomic performance have been developed for shorter growing seasons. These hybrids may be more competitive than commonly grown full season hybrids within a zone of adaptation. Short season hybrids might also offer grain producers a better economic alternative than soybean as a late planted crop.

1.1 Pests and disease problems

Late planting subjects maize plants to heavier infestations of certain pests and diseases which can result in yield loss (Wiatrak et. al. 2005). The major pests of maize in the US Corn Belt are European corn borer, *Ostrinia nubilalis* (Hübner), western corn rootworm, *Diabrotica virgifera virgifera* LeConte, and northern corn rootworm, *Diabrotica barbari* Smith & Lawrence. Yield losses and control expenditures associated
with European corn borer cost farmers more than $1 billion annually (Mason et al. 1996). Crop losses and control costs attributed to corn rootworms have also been reported to reach $1 billion annually in the United States (Metcalf, 1986). The major diseases of maize are grouped into leaf blights, stalk rots, ear rots, and viral diseases (OSU Extension, 1998). One of the most important foliar diseases of maize is gray leaf spot which has been reported in most of the maize producing areas of USA (Latterell and Rossi, 1983). Stalk rot is considered the most persistent and destructive stalk diseases of maize throughout the world (Gatch et. al. 2002). Stalk rot in maize may result in annual losses estimated at 5 to 10 percent (Lipps et al. 2001). The most common stalk rot pathogens in the USA include *Gibberella zeae* (Schewein), *Collectotrichum graminicola* (Ces.), and *Fusarium verticilliodes* (Sacc) Nierenberg (Kommendahl and Windels 1981, White 1999). The ear rot diseases that occur in Ohio include Gibberella, Fusarium, Diplodia, and Aspergillus, but Gibberella ear rot is considered the most important.

### 1.1.1 European corn borer

*Biological and life cycle.* European corn borer has several ecotypes capable of completing one to four generations across different latitudes. However, throughout most parts of the Corn Belt, two generations occur (Mason et al. 1996). European corn borer overwinters as late instar larvae in maize stalks. The overwintering larvae pupate in the spring, and then emerge as moths that prefer to deposit their egg masses on the underside of leaves of whorl stage of earliest planted and tallest maize plants in an area (Pilcher and Rice 2001). Each egg mass appears like a small mass of fish scales that may include 15-20 eggs. The eggs hatch into early instar larvae that initially feed on foliage, causing
windowpane injury on the tender central whorl. This feeding subsequently leads to shot hole injury on the emerging foliage (Willson and Eisley 2001). As larvae reach the third and fourth instar, they tunnel into midribs and stalks. The larvae will then complete larval development to the fifth instar, and then pupate. These individuals will emerge as adult moths in late July and early August.

The larval generation that occurs in spring on whorl stage maize is called the first brood. The period of time required for larval growth from egg hatch to pupation is about 29-33 days depending on the prevailing temperatures. During the period of larval development, a significant proportion of larvae perish due to natural elements such as rain or predation by beneficial insects. Larval mortality occurs primarily during the early first, second, and third instars, when the larvae are most susceptible to adverse weather and predators. Late fourth and fifth instars are less likely to be killed by weather or predators, but are more susceptible to parasitism by various beneficial parasitic wasps (Willson and Eisley 2001).

There are various beneficial organisms including predators and parasitoids, and pathogens that infect European corn borer (Coll and Bottrell 1991, Lewis and Bing 1991). Four relatively abundant predators that feed on eggs and young instars are the twelve-spotted lady beetle, _Coleomegilla maculata_ (DeGeer) (Coleoptera: Coccinellidae), the insidious flower bug, _Orius insidiosus_ (Say) (Hemiptera: Anthocoridae), and common green lacewing, _Chrysoperla carnea_ Stephens (Neuroptera: Chrysopidae) (Sparks et al. 1966, Jarvis and Guthrie 1987). The most important parasitoids of European corn borer are _Lydella thompsoni_ Herting (Diptera: Tachinidae), _Macrocentrus grandii_ Goidanich (Hymenoptera: Braconidae), _Simpiesis viridula_ (Hymenoptera: Eulophidae), and
Eriborus terebrans Gravenhorst (Hymenoptera: Ichneumonidae). In corn, these parasitoids are considered specialists on European corn borer larvae (Coll and Bottrell 1991). Two entomopathogens that infect O. nubilalis are the microsporidium, Nosema pyrausta (Paillot) (Microspora: Nosematidae) (Lewis and Cossentine 1986, Siegel et al. 1987), and the fungus, Beauveria bassiana (Balsamo) Vuillemin (Deuteromycotina: Hyphomycetes) (Bing and Lewis 1993). However, despite these management approaches and biotic mortality factors, European corn borer remains a persistent pest of maize in the Midwest. Larvae of the first brood pupate in the stalk and emerge as adult moths in late July and early August. Adults of this summer flight prefer to deposit their eggs on late planted corn, which is typically less physiologically mature (R1 stage, silking) and more succulent than earliest planted maize (Ritchie et al. 1993, Mason et al. 1996). The larvae hatching from this generation are referred to as second brood or second generation. They will overwinter as late instar larvae in stubble (Willson and Eisley 2001).

Damage caused by European corn borer. European corn borer moths exhibit preference to certain stages of maize development during oviposition (Mason et al. 1996). First generation larvae feeding on whorl-stage maize leaves and later within the stalk can cause 5-6% yield loss per larva (Bode and Calvin, 1990). Second generation larvae feeding on the ear, ear shanks and stalk can cause 2-3% yield loss per larva during the reproductive stage of maize growth (Bode and Calvin, 1990). Yield losses caused by European corn borer injury are due to a combination of stalk injury by both the first or second brood larvae, ear dropage from second brood injury to shanks and ears, and enhancement of stalk rot due to microbial infection of injured stalk (Jarvis et al. 1984). The severity of ear drop and stalk breakage depends on the incidence and location of
Management of European corn borer. The historical management options for first generation larvae included planting intermediate resistant hybrids containing DIMBOA, or planting a susceptible hybrid early and scouting to predict the larval population density, and then using established economic thresholds (ET) applying an insecticide as needed (Pilcher and Rice 2001). For second generation larvae, management attempts are minimal; however scouting and applying a well timed insecticide spray was the typical recommendation for management of European corn borer (Mason et al. 1996). Detection of European corn borer larvae in 75% or more of plant stand warrants treatment if an average of 1 larva per stalk can be prevented from completing development of the first brood. Treatment for second brood would be warranted if 50% or more of the plants have eggs or early larva (OSU Extension 2005).

However, a survey indicated that 70-80% of Iowa and Minnesota maize growers had never used insecticides to manage European corn borer (Rice and Ostlie 1997). Heinemann et al. (1992) suggested that the main reason for inaction with regards to insecticide application by growers is the difficulty in proper timing of spray application to obtain an economic benefit. Rice and Ostlie (1997) suggested that some reasons why growers did not manage European corn borer included undetected yield losses caused by European corn borer, reluctance to scout, and insecticide concerns. Alternative methods have been employed that are simpler to use, easier to incorporate into the farming system, and able to reduce economic losses caused by European corn borer. In considering
practical alternatives, growers have a history of using cultural management technologies rather than insecticides when managing European corn borer (Rice and Ostlie 1997).

Adjustment of planting dates. Adjusting planting dates has been recommended as a cultural method to control European corn borer. Jarvis et al. (1986) demonstrated that the yield of full season hybrids in both European corn borer infested and non-infested plots were much greater than those of short season hybrids. The losses due to European corn borer were greater in short season hybrids when planted late. Jarvis et al. (1986) recommended that growers should plant full season hybrids early. They recommended that if short season hybrids are planted late, European corn borer must be closely monitored. These recommendations were made before the development of transgenic hybrids which protect maize from feeding injury by European corn borer.

Transgenic Maize (Bt). Field maize has been genetically engineered to be resistant to the European corn borer, southwestern corn borer, and southern corn stalk borer (Koziel et al. 1993, Armstrong et al. 1995). The crystalline protein (cry) produced by the plant is derived from the bacterium *Bacillus thuringiensis* (Berliner) (Bt) (Koziel et al. 1993). Cry1Ab protein binds to specific receptors in the midgut of the larvae, disrupting the digestive tract of targeted insect so that the pest stops eating and mortality occurs (DeGooyer 2006). The Environmental Protection Agency (EPA) approved the use of maize hybrids that carry the *Bacillus thuringiensis* (Berliner) subsp. *kurstaki* Cry gene in August 1995.

Transgenic maize in 1996 contained genetic event 176 (Cry1Ab protein, NatureGard or KnockOut) (Pilcher et al. 2002). Event 176 expresses the toxin in green plant tissue, pollen and the stalk, but not in the silk and kernels. This genetic event
provided full protection against first-generation larvae but only partially controlled second-generation larvae (DeGooyer 2006). In 1997, maize hybrids that contained genetic event Bt11 or MON810 (Cry1Ab protein, YieldGard) were marketed. Events BT11 and MON810, result in ‘full season’ expression in leaf, pollen, tassel, silk and kernel tissue (Fearing et al. 1997; Andow and Hutchison, 1998). These two genetic events provided full-season protection against both first and second generations of European and southwestern corn borer larvae. Plantings of Bt maize grew from 8 percent of U.S. maize acreage in 1997 to 26 percent in 1999, then fell to 19 percent in 2000 and 2001, before climbing to 29 percent in 2003 and 49 percent in 2007 (USDA, NASS 2007).

Insect resistance management. Despite the commercial success of Bt crops, there is widespread concern that the advantages provided by them will be short-lived because the constitutive expression of Bt toxins in plant tissues, perhaps leading to the selection and multiplication of rare Bt-resistant insects. Thus, the U.S. Environmental Protection Agency mandated that seed companies create, and growers implement, an insect resistance management (IRM) program to delay the evolution of resistance by European corn borer (U.S. EPA 2001). The strategy selected is known as the high-dose/structured refuge plan (Ostlie et al. 1997). Refuges are comprised of non-Bt crop plants that are used to maintain Bt-susceptible insects in the population. Transgenic insecticidal plants express a very high toxin dose.

In the Corn Belt farmers are required to plant a refuge of 20% non-transgenic maize (Onstad and Buschman 2006) on a farm. The non-transgenic maize must be placed within 0.5 mile (0.80 km) of Bt maize (preferably within 0.25 mile or closer). The non Bt
maize refuge can be treated with insecticides (excluding Bt based insecticides) for control of corn borers if treatment is economically justified. In the cotton growing areas, at least 50% of the maize acreage on a farm must be planted as a non-Bt maize refuge because some insects that feed on both plants could spread to Bt cotton after developing resistance to Bt maize (USEPA 2001). There is a potential double exposure of maize earworm, a polyphagous pest to both Bt maize (Cry1Ab, Cry1F) and Bt cotton (Cry1Ac) during the same growing season which can result in development of resistance to Bt genes (Caprio 1998, USEPA 2001, Storer et al. 1999). The refuge is intended to produce susceptible insects that can mate with resistant insects to increase the chances that offspring with the resistance allele will remain heterozygous for resistance. By definition, high-dose transgenic insecticidal plants are expected to kill such heterozygous offspring.

*Combination of planting dates and Bt corn.* The combination of adjustment of planting dates and using transgenic maize as a trap crop for egg recruitment has been modeled as a strategy for managing European corn borer (Alstad and Andow 1995, Ostlie et al. 1997) as a way to manage part of the pest population (Pilcher and Rice 2001). Although the use of planting dates has not been widely used in the past (Rice and Ostlie 1997, Pilcher and Rice 1998), the integration of Bt maize into such a pest management system may provide an opportunity to better use cultural management tactics, allowing planting date separations of Bt and non-Bt maize to be a viable alternative (Pilcher and Rice 2001). Bt maize planted early might gain an economic benefit from protection against the first generation, and when planted late, will gain the greatest economic advantage from protection against the second generation (Pedigo and Rice 2006, Pilcher and Rice 2003).
1.1.2 Corn rootworms

Biology and life cycle. The western corn rootworm and northern corn rootworm are important pests of maize that now occur throughout much of the US Corn Belt. When assessed in 1995, corn rootworms were responsible for the largest expenditure by growers for insect management in maize production systems (Pike 1995). Rootworms overwinter as eggs that are deposited by adult females in the soil during the late summer of the previous growing season (Willson and Eisley 2001). Survival and development of larval instars of corn rootworms depend largely on the availability of maize roots as a food source.

Corn rootworms have one generation per year with larvae present from May through July. Adults are abundant from July through September, the time when most maize plants are at their reproductive stages (silking to physiological maturity) (Heinrichs et al., 2006). The females lay their eggs on the upper 5 to 20 cm of soil among the roots of maize during summer and early fall, usually in maize fields where adults feed. Thus, most problems are in maize following corn. The western corn rootworm and northern corn rootworm undergo diapause in the egg stage during winter and eggs hatch the next spring in late May and early June (Heinrichs et al., 2006).

Damage caused by corn rootworm. After hatching in spring, larvae first feed on young roots of maize plants which are usually at vegetative stages (VE to VT stages). After the larvae finish feeding, pupation occurs in mid-June to late July and adults emerge a short time later. Plants with rootworm injury are susceptible to drought, root
diseases, and reductions in yield. Feeding on the root tissue by corn rootworm reduces the ability of the plant to absorb water and nutrients from the soil (Reidel 1990). Injured plants are also prone to lodging, resulting in reduced yield (Spike and Tollefson 1991). Yield losses also may result from difficulties associated with mechanical harvesting of lodged plants.

Adults also feed on foliage and silk of maize causing significant injury and interference with pollination by excessive feeding on silk, which is called silk clipping (Al-Deeb 2003). When the population of adult beetles is high during the pollination period and silks are chewed into husks, poorly filled ears may result due to lack of pollination. Late planted maize is more likely to be damaged by adults because silking more coincides with higher densities of adults. The economic threshold for corn rootworms in continuous maize fields is to apply corn rootworm control measure next year if an average 0.75 beetle per plant are found during samplings in August-mid September (Wright et al. 2003).

Management of corn rootworms. Current pest management options to manage rootworm injury have consisted of crop rotation, application of soil insecticides, manipulation of planting dates, use of transgenic hybrids, and insecticide seed treatments. Crop rotation has displaced soil insecticides in many areas, and is an effective corn rootworm management tool (Chandler et al. 2000). Growers employ the use of crop rotation with soybean and other crops to interrupt the life cycle of the corn rootworm. Since corn rootworms feed primarily on roots of maize plants, by rotating the field out of maize production the food source is eliminated. Thus, corn rootworm historically is only damaging in continuous maize production systems. However, years of annual maize and
soybean rotation has resulted in selection for a western corn rootworm population that deposits eggs in soybean or other crop fields (Levine and Oloumi-Sadeghi 1996), making crop rotation less effective as a control option for western corn rootworm in first year corn. Also, a subset of northern corn rootworm population is now capable of prolonging their diapause for a year, and surviving for more than one winter as eggs, thereby overcoming the pest management benefits of crop rotation (Levine et al. 1992, Steffey et al. 1992).

The use of soil-applied insecticides delivered at planting was a primary tool to limit yield loss associated with corn rootworm larval feeding (Chandler et al. 2000). The insecticides are applied to treat soil against rootworm on continuous maize cropping systems. Although soil insecticides protect maize roots from feeding damage, they do not always effectively manage corn rootworm larval populations (Gray et al. 1992). Large numbers of beetles are frequently produced in the fields treated with soil insecticides. Additionally, corn rootworm populations have developed resistance to numerous classes of insecticides (Chandler 2003) including chlorinated hydrocarbons (Ball & Weekman 1962) and organophosphates (carbaryl and parathion-methyl) (Meinke et al. 1998). Soil insecticides also provided control of any secondary soil pests that happened to be present.

Manipulation of planting dates has been suggested as management options for controlling corn rootworms (Carlson and Gauge 1989). Maize planting dates are reported to influence the relative availability of corn rootworm larvae (Bergman and Turpin 1984). By planting the crop earlier in spring, maize pollination will be nearly completed during beetle emergence. The lack of fresh silks reduces the number of beetles that are attracted
to the maize fields to oviposit. Inadequate food supply at egg hatch caused by delayed planting can results in reduction in corn rootworm population due to mortality of early hatching larvae (Bergman and Turpin 1984).

Two recent developments in corn rootworm management are the use of transgenic hybrids and seed treatments. In 2003, U.S Environmental Protection Agency granted Monsanto Company a registration to sell transgenic maize hybrids expressing the Cry3Bb1 protein that protects maize against western corn rootworm, northern corn rootworm, and Mexican corn rootworm (EPA 2003). This event (referred to as MON 863) constitutively expresses the Cry3Bb1 toxin derived from Bacillus thuringiensis (Bt) in both root and above ground tissue, although only rootworm larvae are affected by the toxin (EPA 2003). Unlike Mon 810 (CryBb1gene) which is a high-dose event for European corn borer control (in which survivorship of corn borer larvae is rare), event MON 863 (Cry3 Bb1gene) for rootworm is expressed at a low-to-moderate dose resulting in plentiful adult survival and emergence (Vaughn et al. 2005). The overall survival of corn rootworm larvae that feed on transgenic maize roots is estimated to range from 17 to 62% (EPA 2002). The expression of Cry3Bb1 protein is believed to be greatest in the root tips where newly hatched larvae initially feed. Penetration into roots by first instar is not very common; rather, they graze on the outer portions of roots tissue. This feeding behavior is thought to slow down the larval development and ultimately delay adult emergence (Gray and Steffey 2005).

In 2005 EPA also registered Herculex RW hybrids (event DAS-59122-7, Cry34Ab1/Cry35Ab1) to provide root protection against corn rootworm larvae (EPA 2005). The maize hybrids were commercialized by Dow AgroSciences LLC and Pioneer
Hi-Bred International Inc., under the trade name Herculex™ RW (Herman et al. 2007). Syngenta has developed MIR604 corn, which expresses a modified Cry3A protein (mCry3A) for corn rootworm control which was registered in 2006 by EPA (EPA 2006). MIR604 maize is sold as Agrisure™ RW hybrid maize (Raybould et al. 2007).

Although larval feeding on roots is the primary mechanism for exposure, concentrations of Cry3Bb1 protein have been reported to range from 30 to 93 in the leaf, from 49 to 86 in grain, from 30 to 93 in pollen and from 3.2 to 66 in roots (EPA 2003). However, adult rootworms are not significantly deterred by the presence of Cry3Bb1 and ingestion of toxin does not adversely affect adult longevity (Nowatzki et al. 2006). As with other transgenic Bt crops, the risk of resistance development is perceived as being high, especially for MON 863, which does not express a high dose of toxin.

The use of Bt maize was developed as an alternative control tactic to soil insecticides, becoming an integral part of an Integrated Pest Management (IPM) program for corn rootworm (Vaughn et al. 2005). It is recommended in Ohio that control interventions using Bt maize or soil insecticides be applied only when maize follows maize, or on first year maize following soybean if the western corn rootworm variant is considered a potential problem based on sampling or other observations (Hammond 2005, Willson and Eisley 2001). Although transgenic rootworm maize does not offer total protection against larval feeding, data suggest that the quantity of root injury is minor and should not translate into economic yield loss (Gray and Steffey 2005). An added benefit of transgenic rootworm maize is that root protection is not dependent on planting time, weather influences, calibration of application equipment, or soil conditions for optimum performance by soil insecticides. However, the narrow spectrum of activity
by Bt hybrids against corn rootworm larvae can also be considered a limitation when secondary pests (e.g., white grubs, wireworms, seedcorn maggots, grape colaspis) are present in economically damaging densities (Rice, 2004). Thus, all transgenic maize for rootworm control also comes with a low rate of an insecticide seed treatment for control of these secondary soil pests.

The other recent development for rootworm control has been insecticide seed treatments. In the last few years, advances in seed coating technology have allowed insecticides and fungicides to be applied to the seed on both maize and soybean, enabling farmers to buy pre-treated seed that is ready to plant (Jarvi et al. 2006). The use of seed treatments had been confined to the use of non-systemic products that prevented stand losses that were applied as planter-box treatments to manage early pests. The target pests included seedcorn maggot, seedcorn beetles, and wireworm, all pests that cause seed injury and stand losses (Willson and Eisley 2001). The newer commercially-applied seed insecticides attempted to go beyond the traditional early season protection role because they have systemic action. On corn, they protect against early season insects such as wireworms and seedcorn maggots, and some are also labeled for use against flea beetles and black cutworms. These seed treatments, including clothianidin (Poncho®) and thiamethoxam (Cruiser®), have two labeled rates, a low rate for non-rootworm pests and a high rate that is intended to suppress damage from rootworm larvae. As a result, new seed treatments provide protection against a full range of pests causing potential stand losses plus suppression of grub and rootworm pests causing root-system injury. However, studies show that the high rate of insecticide seed treatments does not protect
maize roots from corn rootworm larval damage very well when infestations of corn rootworm larvae are heavy (Steffey et al. 2005).

1.1.3 Diseases of Maize

The major diseases of maize are grouped into leaf blights, stalk rots, ear rots, and viral diseases (OSU Extension 1998). The most common leaf blight diseases are gray leaf spot, Stewart's bacterial leaf blight, rust and northern corn leaf blight. These diseases occur in most areas where maize is grown, but the occurrence varies from year to year depending on weather conditions and the susceptibility of the hybrid planted (OSU Extension 1998). Yield loss caused by leaf blight diseases is caused by loss of green leaf tissue, which results in fewer kernels and lightweight grain. In this review the ear rots and stalk rots are discussed in detail because they relate to European corn borer injury that is been studied.

Stalk rots. Stalk rot is one of the most persistent and destructive diseases of maize throughout the world (Gatch et al. 2002). Stalk rots in maize may result in annual losses estimated at 5 to 10 percent (OSU Extension 1998). Numerous fungi have been implicated in stalk rot of corn, whose effects include reduced yields, premature death of plants, and lodged stalks (Jarvis et al. 1986). Yield losses also occur due to reduced grain fill caused by pathogens invading maize stalks prior to physiological maturity (Koehler 1960). Destruction of the pith tissue also predisposes the plants to lodging. The predominant members of the stalk rot complex in the United States are *Gibberella zeae* (Schwein.) Petch, *Colletotrichum graminicola* (Ces.), and *Stenocarpella maydis* (Berk.).
Other stalk pathogen species are members of the genus *Fusarium*, namely *F. verticillioides* (Sacc.), *F. proliferatum* (T. Matsushima), and *F. subglutinans* (Wollenweb. and Reinking) (Kommendahl and Windels 1981).

Injury due to feeding by European corn borer is one stress that can promote the progression of stalk rot (White 1999), contributing to the development of stalk rot in several ways. By tunneling stalk tissue, larvae create points for fungal invasion, serve as vectors of some pathogens into the stalk, and cause physiological stress that can predispose maize to stalk rot development (Bergstrom and Nicholson 1999, Jarvis et al. 1986). This relationship between European corn borer damage and stalk rot is an important factor in pest management decisions because the yield loss attributed to European corn borer often is due in part to subsequent fungal decay of tissue injured by larvae (Keller et al. 1986).

*Ear rots.* Gibberella, Diplodia, Fusarium, and Aspergillus ear rot diseases occur in Ohio, with Gibberella ear rot being the most important. The Gibberella pathogen enters from the silk end of the ear when cool, wet weather persists for several weeks through late silking of the crop. The occurrence of a whitish to pinkish mold on the ear tip is diagnostic feature for Gibberella rot. On shelled grain, the symptoms may be seen as a pinkish coloration in some of the kernels. The disease is also important because the fungus frequently produces toxins that make the maize unfit for feeding. Some maize hybrids are less susceptible than others to Gibberella ear rot.

Diplodia ear rot caused by the fungus *Stenocarpella maydis* (Berk) is also an important disease in many maize growing regions in the world (Dorrance et al. 1998). Ears affected by *Diplodia* are covered with a thick mat of white fungal growth. Ears are
usually colonized from shank up into the ear, and losses are due to reduced seed weight and seed viability.

Fusarium ear rot caused by several species of *Fusarium*, is common, but only individual kernels are affected on ears. Kernel infection by any of the *Fusarium* species can reduce yield and quality, and can result in mycotoxins being accumulated in the grain (Kommendahl and Windels 1981). Insect activity has long been associated with *Fusarium* infection of maize kernel and stalks (Hopmans and Murphy 1993). Injury caused by European corn borer feeding is often the initial infection sites for Fusarium species (Chiang and Wilcoxson 1961). Larvae can also act as vectors of *F. monoliforme*, carrying the fungus from plant surface into maize ears. Infestation by European corn borer can increase the symptoms of Fusarium ear rot as well as increase symptomless kernel infection (Sobek and Munkvold 1999).

Aspergillus ear rot of maize caused by *Aspergillus flavus* Link:Fr., is an economic concern to producers and consumers because it is associated with aflatoxin, a naturally occurring toxin and one of the strongest carcinogens found in nature (Castegnaro and Mcgregor 1998). Aflatoxins are toxic secondary metabolites produced by the filamentous fungi *A. flavus* and *A. parasiticus*. Aflatoxin level may vary among genotypes (Darrah et al. 1987) due to effectively reducing lepidoptera ear infestation, therefore reducing aflatoxin contamination of grain (Williams et al. 1998). Also Mcmillian et al. (1988) noted lower concentrations of aflatoxin in European corn borer resistant hybrids compared with conventional ones.
1.2 Justification and Objectives

There is a continued interest in selection of optimum planting dates to expose maize plants to maximum growing degrees days (Nielsen et al. 2002). When faced with scenarios that prevent planting maize at optimum times, growers often must decide whether to switch to earlier-maturity hybrids or to a different crop like soybean to minimize the risks associated with late plantings. Late planting subjects maize plants to different biotic stresses, including heavier infestation of pests and diseases which can result in yield loss (Wiatrak et al. 2005). When planting late, different pest and disease management interventions are required.

Various pest management strategies are available when planting late, including rotations, use of transgenic hybrids, and pesticides. These strategies can be used in compatible combinations in an IPM program for major pests of corn. Pilcher and Rice (2001) showed that Bt maize hybrids resist damage by European corn borer, and therefore can be of economic value in late plantings. However, their results were based on full season hybrids, which are not suitable for delayed plantings because their yield potential is reduced by a shortened season. Full season hybrids mature later than normal, get harvested late, and are susceptible to being killed by frost. All these have a negative impact on yield, and often force growers to switch to soybean. Short season maize hybrids with improved agronomic potential are now available. However, their responses to pest pressure need to be evaluated under these delayed planting scenarios. There is a need to develop pest management strategies that will minimize yield losses.

The overall objective of this study was to examine the impacts of varying planting dates and the newer pest management tactics of transgenic maize and seed treatments on
maize with different maturities. Specific objectives were: (1) to evaluate the potential use of planting dates and varying maize maturity in combination with transgenic maize and seed treatment to manage corn rootworm (*Diabrotica virgifera virgifera* LeConte), (2) to evaluate the potential use of planting dates and varying maize maturity in combination with transgenic maize on oviposition and subsequent larval injury by European corn borer (*Ostrinia nubilalis* (Hübner)), (3) To evaluate the potential use of planting dates and varying maturity of maize in combination with transgenic hybrids on maize disease incidence and severity following European corn borer infestation.

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CHAPTER 2

INTERACTION AMONG PLANTING DATES, TRANSGENIC MAIZE AND SEED TREATMENT ON CORN ROOTWORM DAMAGE AND MAIZE GRAIN YIELD.

2.1 ABSTRACT

A study was conducted from 2006 to 2008 at Hoytville and Wooster, Ohio to evaluate the potential use of planting dates in combination with transgenic maize and seed treatments to manage corn rootworm (Diabrotica virgifera virgifera). Three planting dates (early, middle, and late), targeting late April/early, mid-May and early June respectively were used. We planted six hybrid treatments consisting of two hybrids with seed treatment, two transgenic hybrids, and two untreated hybrids, each set represented by one short and one full season maturity hybrid. Root injury by rootworm larval feeding was significantly reduced by planting maize in early June. The use of transgenic maize and seed treatment also significantly reduced root injury by rootworm larvae. When populations of rootworm larvae were high, significant lodging and stunted growth were observed on untreated maize and declined when planting was delayed. When a high population of adult beetles fed on maize silk, significant clipping occurred that resulted in
poor seed set. The influence of planting date on grain yield was inconsistent from year
to year. In 2006 silk clipping by corn rootworm and Japanese beetles caused reduction in
yield on late planted corn. Higher root injury in 2007 at Wooster caused significant
reduction in yield on untreated early planted maize than late planting. Grain yields from
short season hybrids were comparable to full season hybrids especially on later plantings.
These results show that the use of a seed treatment and transgenic maize might be
beneficial only when rootworm population is high and planting is early.

2.2 INTRODUCTION

The western corn rootworm, *Diabrotica virgifera virgifera*, and northern corn
rootworm, *Diabrotica barberi* are important pests of maize throughout the Midwest.
Levine and Oloumi-Sadeghi (1991) estimated that 50-60% of the total maize acreage in
the United States was treated annually with soil insecticides for corn rootworm control.
Costs associated with managing corn rootworms in continuous maize are annually one of
the largest expenditures for insect management (Metcalf 1986, Steffey et al. 1994).
Major economic damage is caused by larval feeding on maize roots. Larvae hatch in
spring from overwintering eggs deposited by adult females in the soil during the late
summer of the previous season. Larvae feeding on young maize roots during the
vegetative stages make plants susceptible to drought, lodging, and reduced yield. After
the larvae finish feeding, pupation occurs in mid-June to late July and adults emerge a
short time after that. Adult feeding on silk can then interfere with pollination when silk
clipping is excessive (Spike and Tollefson 1991, Al-Deeb 2003). Yield losses also can
result from difficulties associated with mechanical harvesting of lodged plants. When the
density of adult beetles is high during the pollination period and silks are chewed down into the husks, poorly filled ears may result due to lack of pollination.

Pest management options for corn rootworm consist of crop rotation, application of soil insecticides, insecticidal seed treatments, and transgenic maize hybrids. Until the advent of transgenic maize hybrids, the use of soil-applied insecticides delivered at planting remained the primary tool to limit yield loss associated with corn rootworm larval feeding on continuous corn. Survival and development of rootworm larvae depend largely on the availability of maize roots as a food source. Thus, crop rotation is able to break the normal rootworm cycle when soybean or another crop followed corn. Indeed, crop rotation displaced soil insecticides in many areas, and was for the most part an effective corn rootworm management tool. However, years of annual maize and soybean rotation resulted in selection for western corn rootworm variant that deposits eggs in fields where soybean is grown (Levine and Oloumi-Sadeghi 1996). Oviposition in soybean fields made crop rotation ineffective as a control option for corn rootworm in first year corn. Additionally, a variant of northern corn rootworm became capable of prolonging diapause and surviving for more than one winter as eggs, thereby also overcoming the benefits of crop rotation as a pest management option (Levine et al. 1992, Steffey et al. 1992). Varying maize planting dates is reported to influence the relative abundance of corn rootworm larvae (Bergman and Turpin 1984). Lack of an adequate food supply, i.e., maize roots, at egg hatch causes larval mortality resulting in changes in corn rootworm population dynamics and reduction in larval feeding damage (Bergman and Turpin 1984).
Currently, the use of transgenic hybrids and seed treatments are the most widely used tactics in corn rootworm management. Transgenic maize hybrids expressing the Cry3Bb1 protein that protects maize against corn rootworms were first registered for use in 2003 (EPA 2003). The transgenic hybrids constitutively express the Cry3Bb1 toxin derived from *Bacillus thuringiensis* (Bt) in both root and above ground tissue, although only rootworm larvae are affected by the toxin (EPA 2003). In 2005 EPA also registered Herculex RW hybrids (event DAS-59122-7, Cry34Ab1/Cry35Ab1) to provide root protection against corn rootworm larvae (EPA 2005). The maize hybrids were commercialized by Dow AgroSciences LLC and Pioneer Hi-Bred International Inc., under the trade name Herculex™ RW (Herman et al 2007). Syngenta has developed MIR604 corn, which expresses a modified Cry3A protein (mCry3A) for corn rootworm control which was registered in 2006 by EPA (EPA 2006). MIR604 maize is sold as Agrisure™ RW hybrid maize (Raybould et al 2007). The use of Bt maize was developed as an alternative method or as a component of an integrated pest management to manage corn rootworm (Vaughn et al. 2005).

Although transgenic rootworm maize does not offer total protection against larval feeding, the quantity of root injury is minor and does not usually translate into economic yield loss. An added benefit of transgenic rootworm maize is that root protection does not depend upon planting time, weather influences, calibration of application equipment, or soil conditions for optimum performance. However, the narrow spectrum of activity against corn rootworm larvae is considered a limitation when secondary pests (e.g., white grubs, wireworms, seedcorn maggots, grape colaspis) are present in economically
damaging densities (Rice 2004). Currently all transgenic rootworm maize hybrids supplied by seed companies come with a low rate of a seed treatment applied to them.

In the last few years advances in seed coating technology have allowed insecticides to be applied to maize seed, enabling farmers to buy commercially-applied treated seed (Jarvi et al. 2006). These seed treatments were initially intended to reduce plant stand losses associated with secondary soil pests, including seedcorn maggots, seedcorn beetle, and wireworm that can cause seed injury and stand losses (Willson and Eisley 2001). Two of these systemic seed treatments, clothianidin (Poncho®) and thiamethoxam (Cruiser®), now are available at two labeled rates, a low rate for the secondary pests and a higher rate intended to suppress damage from corn rootworm larvae. Thus, these seed treatments may prevent stand loss caused by secondary soil pests and reduce root injury by corn rootworms. However seed treatments are not recommended in most Midwest States when infestations of corn rootworm larvae are high because they do not protect maize roots from larval injury (Steffey et al. 2005).

While most growers are now using transgenic hybrids or seed treatments, there is little information on the interactions of these transgenic hybrids and seed treatments with maize cropping systems. One of the most important decisions growers face is selection of an optimum planting date to ensure physiological maturity before fall frost. Factors such as weather conditions (e.g. excessive soil moisture) and equipment breakdowns which can push planting beyond the optimum dates of late April to early May in Eastern USA Corn Belt (Nielsen et al. 2002, Jarvis 1986). Late planting often subjects maize plants to heavier infestation of pests and diseases which can result in yield loss (Wiatrak et al. 2005).
Knowledge of the impact of planting dates is critical when selecting hybrid seed for late plantings. Planting short season hybrids late in the season may reduce frost damage by maturing earlier than full season hybrids. Late planting also reduces availability of maize roots to eclosing larvae, which can survive only a few days without becoming established on a suitable host and reduce rootworm larvae (Branson, 1989). In this study, we evaluated the performance of maize hybrids of different maturities planted on various dates under various pest management options. The objectives of the study were 1) to evaluate the potential use of planting dates in combination with transgenic maize hybrids and seed treatments to manage corn rootworm and 2) to compare performance of short season hybrids with full season hybrids (transgenic vs. non transgenic). Our first hypothesis was that maize phenology influences adult egg laying pattern and injury to roots. The second hypothesis was that the yield from short season hybrids will be equal to or higher than full season hybrids when planted late and corn rootworm infestations are high.

2.3 MATERIALS AND METHODS

The experiment was carried out at the Wooster Campus (40° 46´N, 81° 55´W) of the Ohio State University Ohio, Agricultural Research and Development Center in Ohio and the Western Agricultural Research Station near South Charleston, Ohio (39° 51´N, 83° 40´ W) from 2006 to 2008. To enhance the natural infestation of rootworms, the experiments were conducted in fields previously planted with late planted maize that served as a trap crop. Adult corn rootworms are reportedly attracted to fresh pollen and
silk in fields planted with trap crop (Hill and Mayo 1974). The land was prepared by plowing in the spring followed by disc harrowing to break up the sod. Experimental design was a $3 \times 6$ treatment factorial replicated four times in a randomized complete block arranged in split plot layout. The first factor (main plot) was planting date, randomized within each replication. There were three planting dates (early, mid, and late) targeting late April/early May, late May and early June (Table 2.1). The second factor (subplot) consisted of treated seed, a transgenic hybrid, and an untreated hybrid assigned randomly within planting date.

Individual plots, 10 m long and 4 rows wide on row spacing of 0.76 m, were planted on the various dates at a seeding rate of 79 000 seeds ha$^{-1}$. Each treatment had one short and one full season maize hybrid making a total of six treatments (Table 2.2). The maize hybrids used in this study were commonly used hybrids, selected because they have comparable agronomic characteristics. The study aimed to represent normal situations in the farmer’s field; thus, isolines which are often planted in similar studies were not used because they were not normally-grown hybrids. The hybrids changed from 2006 to 2007 because some of them were not available from the company that supplied us with seed.
<table>
<thead>
<tr>
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<th>Middle</th>
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<td></td>
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</tr>
<tr>
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</tr>
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<td></td>
<td>2008</td>
<td>17 October</td>
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Table 2.1. Planting and harvest dates at Wooster and South Charleston, Ohio from 2006 to 2008.
<table>
<thead>
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<tr>
<td>Dekalb DKC61-73</td>
<td>Untreated</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2.2. Maize hybrids planted at Wooster and South Charleston from 2006 to 2008.
The seed treatment was clothianidin (Poncho™ 1250) (Gustafson LLC, Dallas TX) commercially applied at 1.25 mg a.i. per seed. All the Bt maize hybrids were obtained from DeKalb seed company, and were treated with the low rate Poncho™ 250 for protection from secondary soil pests. Poncho™ 250 is not labeled for control of corn rootworm, and is not considered to affect rootworm larvae; thus, it was believed that it would have no effect on the experiment.

**Data collection**

The maize growth stage (Ritchie et al. 1993) of each treatment was recorded weekly from early vegetative stages to reproductive stages to estimate growing degree days available for maize to reach flowering (R1) and R6 (black layer) stages. Different growth stages of maize would show the relationship between maize phenology and rootworm developmental stages. To evaluate root injury, five plants were randomly dug from each plot in each replication. The root samples were collected from the two outside rows so that the two middle rows could be used for recording plant stands and grain yield after crop physiological maturity. The roots were soaked in water, then the soil was washed off and roots evaluated for larval injury using the node injury scale of Oleson et al. (2005). The node injury scale is based on a scale of values ranging from 0-3 (0 = no injury and 3=3 nodes missing). The percentage of root lodging was determined by counting the number of plants leaning more than 30° in the middle rows and dividing the number by the total number of plants and multiplying by 100. Injury from early season pests was also recorded each year throughout the study when observed. Plant height was measured only in 2007 at Wooster when corn rootworm plants showed severe stunting caused by corn rootworm larval feeding of the roots. The heights were measured on 13
July when early planted maize was on VT stage and middle and late planting on V14 and V11 respectively.

The plants were examined for activities of adult corn rootworm and Japanese beetles which feed on leaves, silks, and tassels. Ten randomly selected plants were examined from the tassel to below ear, and both rootworm and Japanese beetles found were recorded on a weekly basis until September. The number of plants showing silk clipping was recorded and expressed as a proportion of 10 sampled plants. Due to higher infestations of corn rootworm at Wooster in 2007 that resulted in increased silk clipping, shriveled or ears with poor kernel set were recorded during 2007 and 2008 to determine the effect of this clipping.

At the end of adult activity, eggs of both western corn rootworm and northern corn rootworm were sampled. Due the labor intensive nature of egg sampling and limited resources, egg density samplings were collected initially at South Charleston in 2006 and the following years at Wooster only. Four random soil cores were collected at the depth of 10-20 cm; two cores between maize rows and two near the plant base using golf course cup-cutter (10 cm diameter; 20 cm deep) (Pierce & Gray, 2006). The samples were bagged, labeled, and stored at 0°C until examination. Eggs were separated from the soil by using a combination of washing, floatation in a concentrated magnesium sulfate solution and sieving through a 60-mesh sieve with 25°C tap water leaving the eggs on the screen surface.

Grain yield

After physiological maturity, the plant population was recorded for each plot on 3 meter section of 2 middle rows. Plant populations were determined during the later part
of grain filling by counting plants within the two center rows of each plot. Plots were hand harvested by collecting ears from 3 meter sections of 2 middle rows of each treatment per replication. The dates of harvesting during the three years of study are shown in Table 1. Bulk yields were measured by weighing the shelled dry grain production in 2006 and unshelled ears during 2007 and 2008 from a 3-m row section per treatment per replication. Grain moisture content for each treatment was determined using a Seedburo Model GMA 128 Grain Moisture Analyzer (Seedburo Equipment Co., Chicago, IL). Grain yields were adjusted at respective moisture levels for each treatment.

Data analysis

The percentage infestation of plants by early pests and silk clipping were transformed using the arcsine square \[\text{arcsine } \sqrt{\frac{\text{percent } x}{100}}\] to stabilize variance (Fry, 1993). Counts of eggs and adult Japanese beetles were transformed using log \((x+1)\) transformation (Quinn and Keough 2002, Horn, 1988). Data on adult corn rootworm were square root \((x + 0.05)\) transformed because they are known to have random distribution (Hammond et al. 2002). Percentages were arcsine transformed because they are bounded at 0% and 100% and log transformation does not work well for such data as they change each end of the distribution differently (Quinn and Keough 2002). Data on beetle numbers were analyzed as repeated measure designs using the repeated statement in mixed model procedure (PROC MIXED) (SAS Institute, 2003). The procedure adjusted for the serial autocorrelation among the repeated samples on each sampling date (Littell et al. 1996). Selection of the covariance structure was done using the Akaike’s Information Criterion (AIC) (Akaike 1974). The first-order autoregressive covariance structure was used in the analysis because it modeled the repeated measure error structure.
better than other covariance structures. Replication and replication x planting date were considered random while planting date, hybrids and sampling dates were fixed effects. The analysis tested significance for the following treatment effects: planting dates, hybrids, sampling dates, and the interaction between planting dates x hybrids x sampling dates.

Data on yield, percentage clipping, and number of eggs, plant height and number poorly filled ears were analyzed using mixed model analysis (PROC MIXED) (SAS Institute 2003). Planting dates and hybrids were considered fixed. Replications and interactions were assumed to be random effects. The interaction between planting date and hybrids was tested. To compare long vs. short season and transgenic vs. non transgenic hybrids, treated vs. untreated seed, contrast analysis was performed using CONTRAST option within PROC MIXED. Multiple comparisons were made on least square means of the fixed effects using the PDIFF option of the LSMEANS statement in SAS. All the comparisons were based on Fisher’s protected least significant difference and considered significant at [P-value of] $\alpha = 0.05$.

To understand the relationship between maize grain yield, maize pest damage, and phenology, we used a multiple regression analysis, with yield as the dependent variable and root injury, silk clipping, poor seed setting, root lodging, and growing degree days as independent (predictor) variables. The best-fit models were selected using stepwise procedures and the best subsets regression in Minitab® Release 15 (Minitab Inc., 2006). The selection criteria were based on adjusted coefficient of determination ($r^2$), mean square error (MSE), and Mallow’s Cp (Quinn and Keough 2002). The selection was based on small mean square error (MSE), highest adjusted $r^2$ values, and a smallest
Cp values (Fry 1993). Multicollinearity was diagnosed by assessing the bivariate correlations and the variance inflation factor (VIF) to identify predictor variables that could be correlated. Variance inflation factor values greater than ten suggest severe multicollinearity (Chatterjee and Hadi, 2006).

### 2.4 RESULTS

**Root injury**

In 2006, a significant reduction in root injury caused by corn rootworm larval feeding was observed at South Charleston ($F = 28.36; df = 2, 51; P < 0.0001$) and Wooster ($F = 4.45; df = 2, 45; P = 0.017$) (Fig. 2.1A and B). The root injury was significantly affected by hybrid treatments at both locations (South Charleston: $F = 8.19; df = 5, 51; P < 0.0001$, Wooster: $F = 3.08; df = 5, 45; P = 0.018$). Significant planting date × hybrids interaction effects on root injury were observed at South Charleston ($F = 6.69; df = 10, 51; P < 0.0001$) and Wooster ($F = 2.67; df = 10, 45 P = 0.012$). A significant reduction in root injury occurred at South Charleston on transgenic Bt hybrids ($F = 28.19; df = 1, 51$, $P < 0.0001$) and hybrids with seed treatment ($F = 21.74; df = 1, 51; P = < .0001$) compared with untreated hybrids. A similar trend was observed at Wooster; significantly lower injury was observed on Bt hybrids ($F = 8.23; df = 1, 45; P = 0.006$) and hybrids with seed treatment 1250 ($F = 7.41; df = 1, 45; P = 0.009$) than untreated hybrids (Fig. 2.1B).

Multiple comparisons within planting showed no significant differences between transgenic Bt hybrids and hybrids with seed treatment (Fig. 2.1A and B). Comparison of least square means within each planting date showed significantly higher injury on
untreated hybrids on early planted full season than short season hybrid at South Charleston (Fig. 2.1A). In contrast, higher root injury on untreated hybrids occurred on early planted short season hybrids than on full season hybrids at Wooster (Fig. 2.1B). However, no significance occurred between the untreated hybrids on early June planted maize (Fig. 2.1A and B). When maize was planted on early June, no significant differences occurred among treatments indicating that the effect of Bt maize and seed treatment was largely lessened by later planting (Fig. 2.1A and B).

In 2007, a similar pattern was observed (Fig. 2.1C and D), but root injury was much higher at Wooster than during the previous year, resulting in root lodging (Fig. 2.2A) (see root lodging section). Root injury due to larval feeding at each location was significantly lower on late planted maize than early and middle plantings (South Charleston: $F = 13.69; \text{df} = 2, 9; P = 0.002$, Wooster: $F = 31.98; \text{df} = 2, 51; P <0.0001$). Hybrid treatment significantly affected root injury at both locations (South Charleston: $F = 24.33; \text{df} = 5, 45; P <0.0001$, Wooster: $F = 44.19; \text{df} = 5, 51; P <0.0001$). Significant planting date × hybrid interaction effects on root injury was observed at South Charleston ($F = 6.87, \text{df} = 10, 45; P <0.0001$) as well as at Wooster ($F = 12.73, \text{df} = 10, 51; P <0.0001$). Contrast analysis revealed a significant decrease in root injury on transgenic Bt hybrids ($F = 99.96; \text{df} = 1, 45; P <0.0001$) and hybrids with seed treatment ($F = 80.96; \text{df} = 1, 45; P <0.0001$) compared with untreated hybrids at South Charleston.
Fig. 2.1. Mean root injury ratings from hybrids with or without Bt or seed treatment for each planting date. Letters associated with planting date indicate that a significant difference occurred, with hybrid means having the same letter not significantly different (Fisher protected LSD, \( \alpha = 0.05 \)). PD = planting date; PDH = planting date \( \times \) hybrid interaction; *, ** and *** denote significance at \( P \leq 0.05 \), \( P \leq 0.01 \) and \( P \leq 0.001 \) respectively. SS = short season hybrid, LS = full season hybrid, UN = untreated hybrid, ST = seed treatment with clothianidin and Bt = hybrid with Bt gene.
Similar results were observed at Wooster; significantly less injury was observed on Bt hybrids ($F = 167.35; \text{df} = 10, 51; P < 0.0001$) and hybrids with seed treatment ($F = 161.49; \text{df} = 10, 51; P < 0.0001$) than untreated hybrids (Fig. 2.1C). Early planted hybrids with seed treatment had significantly higher injury than transgenic Bt hybrids at Wooster (Fig. 2.1D) but no such differences were observed at South Charleston (Fig. 2.1C). Multiple comparisons of least square means within each planting date showed higher injury on untreated full season hybrids planted on 23 May at Wooster (Fig. 2.1D). As in 2006, delayed planting significantly reduced root injury thus negating the effects of both seed treatment and Bt maize (Fig. 2.1C and D).

In 2008, relatively more injury was observed at South Charleston than in preceding years, causing plants in untreated plots to lodge (Fig. 2.2B) (see root lodging section). Feeding by corn rootworm larvae was significantly reduced by delaying planting until early June at South Charleston ($F = 9.82; \text{df} = 2, 6; P = 0.013$) and Wooster ($F = 5.14; 2, 6; P = 0.050$) (Fig. 2.1E and F). Root injury was significantly affected by hybrid treatments at both locations (South Charleston: $F = 8.73; \text{df} = 5, 45; P < 0.0001$; Wooster: $F = 6.38; \text{df} = 5, 45; P = 0.0001$). Significant planting date × hybrid interaction effects on root injury occurred at South Charleston ($F = 2.60; \text{df} = 10, 45; P = 0.014$) and Wooster ($F = 5.01; \text{df} = 10, 45; P < 0.0001$). Root injury was significantly reduced by the use of transgenic Bt hybrids ($F = 36.33; 1, 45; P < 0.0001$) and seed treatment ($F = 25.05; 1, 45; P < 0.0001$) at South Charleston compared with untreated non- Bt hybrids (Fig. 2.1E). Similar results were observed at Wooster; significantly lower injury occurred on Bt hybrids ($F = 19.92; \text{df} = 1, 45; P < 0.0001$) and hybrids with seed treatment ($F = 17.95; \text{df} = 1, 45; P = 0.0001$) than untreated hybrids.
(Fig. 2.1F). There were no significant differences between transgenic Bt hybrids and hybrids with seed treatment within each planting date at both locations (Fig. 2.1E and F).

Comparison of least square means within each planting date showed that significantly higher injury on untreated hybrids occurred on middle planted full season hybrids than on short season hybrids at South Charleston (Fig. 2.1E). At Wooster, higher injury on untreated hybrids occurred on full season hybrid planted early (Fig. 2.1F). When planting was delayed until early June, no significances occurred between the two untreated hybrids with respect to root injury (Fig. 2.1E and F). Early planted hybrids with seed treatment had significantly more root injury than transgenic Bt hybrid at South Charleston (Fig. 1F), but no such differences were observed at Wooster (Fig. 2.1F). At each location, delaying planting until early June resulted in no significant differences among treatments suggesting that late planting lessened the effect of Bt maize and seed treatment (Fig. 2.1E and F).

Lodging and plant height

During this study root lodging caused by rootworm larval feeding on maize roots only occurred in 2007 at Wooster and in 2008 at South Charleston (Fig. 2.2A and B). Lodging was significantly correlated with root injury rating at South Charleston (r = 0.74; n = 72; P <0.0001) and also at Wooster (r = 0.66812; n = 72; P <0.0001). The effect of planting date, Bt corn, and seed treatment on the percentage of plants that lodged showed a similar trend at both locations (Fig. 2.2A and B).
Fig. 2.2. Mean percentage of maize plants that root lodged in plots with or without seed treatment or Bt maize for each planting date. Letters associated with planting date indicate that a significant difference occurred, with hybrid means having the same letter not significantly different (Fisher protected LSD, $\alpha = 0.05$). PD = planting date; PDH = planting date × hybrid interaction; *, ** and *** denote significance at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$ respectively. SS = short season hybrid, LS = full season hybrid, UN = untreated hybrid, ST = seed treatment with clothianidin and Bt = hybrid with Bt gene.
There was a significant reduction in the percentage of plants that lodged when planting was delayed at South Charleston ($F = 16.07 \ df = 2, 6; P = 0.004$) and also at Wooster ($F = 60.65, df = 2, 6; P = 0.0001$). The percentage of root lodging differed significantly among hybrids at each location (South Charleston: $F = 12.48; df = 5, 45$; $P<0.0001$; Wooster: $F = 59.54, df = 5, 45$; $P <0.0001$). The planting date × hybrid interaction was highly significant at South Charleston ($F = 12.48; df = 10, 45; P<0.0001$) as well as at Wooster ($F = 59.54; df = 10, 45; P<0.0001$).

A significant reduction in root lodging was observed on Bt maize hybrids ($F = 14.77; df = 1, 45; P = 0.0004$) and hybrids with seed treatment ($F = 44.31; df = 1, 45; P <0.0001$) at South Charleston and Wooster (Bt: $F = 220.64; df = 1, 45; P <0.0001$, Seed treatment: $F = 73.55; df = 1, 45; P<0.0001$) when compared to untreated hybrids. Multiple comparisons within planting dates showed significant lower percentage of lodging on short season compared to full season hybrids (Fig. 2.2A and B). Although root lodging was significantly reduced by both Bt maize and seed treatment, the effects were largely negated by delayed planting (Fig. 2.2A and B).

The variation in plant height among hybrid treatments within different planting dates occurred only at Wooster in 2007 on untreated hybrids (Fig. 2.3A and B). The heights were collected 13 July when early planted maize was on VT stage and middle and late planting on V14 and V8 respectively. The significant differences in plant height associated with planting dates ($F = 44.03, df = 2, 6, P = 0.0003$) were due to the obvious reason that earlier planted maize was taller than later planted maize when data was collected. Our main interest was to show the differences among hybrids within each planting date and when averaged across planting date (Fig. 2.3A and B). Significant
Fig. 2.3. Effects of root injury caused by corn rootworm larval feeding on plant height from different hybrid treatments. Means within Fig. 2.3A followed by the same letter are not significantly different. Means with the same letter within a planting date in Fig. 2.3B are not significantly different. Fisher protected LSD ($\alpha = 0.05$) was used for both figures. SS = short season hybrid, LS = full season hybrid, UN = untreated hybrid, ST = seed treatment with clothianidin and Bt = hybrid with Bt gene.
Adult corn rootworm populations

Both the northern and western corn rootworm adults were counted collectively throughout this study, although the numbers of northern corn rootworm adults were generally very low compared with western corn rootworm. In 2006 the numbers of adult corn rootworm that emerged and infested maize plants were influenced by date of planting, but not by hybrid at both South Charleston (F = 0.65; df = 5, 209; P = 0.661) and Wooster (F = 2.21; df = 5, 364; P = 0.053). The beetle counts were therefore averaged across hybrid treatments to obtain least square means for planting at each sampling date (Fig. 2.4A). At South Charleston, significantly more corn rootworms infested late planted maize than middle and early planting (F = 23.70; df = 2, 71; P <0.0001) (Fig. 2.4A). Significant differences were also detected among sampling date (F = 163.95; df = 7, 66; P <0.0001) and planting date × sampling date interaction (F = 19.55; df = 14, 70.6, P <0.0001).

Examination of the planting date × sampling date interaction indicated some variations during the phenological growth stages of plants, characterized by peaks in populations. The first significant peak of beetle populations at South Charleston occurred on 19 July when early planted short season hybrids were in brown silk and full season hybrids were in green silk stages (Fig. 2.4A). There was not a significant peak associated with the middle planting at South Charleston in 2006. A significant population increase occurred when the late planted short season hybrid reached silking stage on 1 August, and the highest peak in beetles coincided with the peak of silking by full season hybrids on 8 August (Fig. 2.4A). After 18 August the beetle numbers declined sharply while silk dried and maize plants reached dough and physiological maturity.
At Wooster the same trend was observed where significantly more beetles were found on late planted maize than early and middle plantings \( (F = 13.73, \text{df} = 2, 9; P = 0.002) \) (Fig. 2.4B). Corn rootworm adult numbers were significantly affected by date of sampling \( (F = 88.02; \text{df} = 9, 113 \, P < 0.0001) \) and sampling date × hybrid interaction \( (F = 22.75; \text{df} = 18, 113; P < 0.0001) \). Multiple comparisons of least square means showed a significant peak in the number of beetles on early planted maize on 3 August than middle and late plantings (Fig. 2.4B). This was a period when early planted full season hybrids and middle planted short season hybrid plants reached the silk stage, while late planted maize was still in a vegetative stage. The second significant peak of population occurred on 11 August when late planted short season hybrids were in the silk stage (Fig. 2.4B). The third slightly lower peak occurred on 22 August that was associated with silking of late planted full season hybrids (Fig. 2.4B). After 22 August, the beetle numbers declined rapidly when maize silk dried and maize plants matured.

In 2007, the numbers of corn rootworm beetles at South Charleston were not significantly affected by planting date \( (F = 1.28; \text{df} = 2, 14; P = 0.310) \) and hybrid treatments \( (F = 1.22; \text{df} = 5, 282; P = 0.302) \), but by sampling date \( (F = 89.57; \text{df} = 7, 104; P < 0.0001) \) and the planting date × sampling date interaction \( (F = 46.87; \text{df} = 14, 104; P < 0.0001) \).
Fig. 2.4. Mean number of corn rootworms at South Charleston and Wooster from 2006 to 2008. Letters associated with sampling date indicate that a significant difference was obtained, with planting date means having the same letter not significantly different (Fisher protected LSD, \( \alpha = 0.05 \)). R1/2 denotes that full season hybrids were in R1 and short season hybrids in R2 for a respective sampling date. R1 = green silk stage; R2 = brown silk (blister) stage.
Three distinct peaks with significantly higher populations of corn rootworm beetles were evident for each planting date, and coincided with the peak of 50% silking (Fig. 2.4C). The first peak in the beetle population occurred on 10 July when short season hybrids were in the silking stage. The second peak happened on 30 June when full season hybrids planted mid-May and short season hybrids planted early June were in the silk stage (Fig. 2.4C). The last peak occurred on 8 August coinciding with peak silking of late planted full season hybrids.

Relatively higher numbers of corn rootworm were observed at Wooster than at South Charleston in 2007 (Fig. 2.4C and D). The beetle population was significantly higher on earlier planted maize than middle and late plantings (df = 2, 12; 71.97; P <0.0001). Significant differences in beetle numbers were also detected among hybrid treatment (F = 4.19, df = 5, 297 P = 0.001), sampling date (F = 486.8; df = 7, 117; P <0.0001), planting date × hybrid (F = 2.11; df = 10, 297; P = 0.024), sampling date × hybrid (F = 2.23; df = 35, 117; P = 0.0007), and planting date × sampling date interactions (F = 69.21; df = 14, 117; P = <.0001). The significant difference in the number of beetles between hybrids was due to an increased number of beetles that were observed on untreated and the treated non-Bt hybrid, DKC61-73 (Fig. 2.5A).

Multiple comparisons within planting showed that a significantly higher number of beetles infested early planted maize on 19 July, a period when more than 50% of short season hybrids were on silking stage while middle and late planted maize was still on vegetative stage (Fig. 2.4D). The population remained significantly higher on early planted maize even when middle planted short season hybrids were reaching silking stage on 25 July. A small peak of population that coincided with a peak of silking on full
season hybrids happened on middle planted maize resulting in no significant differences between early and middle plantings. However the beetle population in late planted maize remained significantly lower than the in other two plantings until 10 August when a slight peak occurred but did not significantly affect beetle numbers among the three planting dates for the rest of remaining sampling dates (Fig. 2.4D).
Fig. 2.5. Mean number of corn rootworm adults from Wooster in 2007 and South Charleston in 2008. Means averaged across planting date and time to show differences among hybrid treatments. Means followed by the same letter within a figure are not significantly different (Fisher protected LSD (α = 0.05)). SS = short season hybrid, LS = full season hybrid, UN = untreated hybrid, ST = seed treatment with clothianidin and Bt = hybrid with Bt gene.
In 2008 a significantly higher number of beetles was obtained on late planted maize than middle and early plantings ($F = 13.94; \text{df} = 2, 12; P = 0.0007$) (Fig. 2.4E). The beetle numbers varied significantly among hybrid treatments ($F = 5.71; \text{df} = 5, 191; P < 0.0001$), sampling date ($F = 57.76; \text{df} = 4, 98; P < 0.0001$), and planting date × sampling date ($F = 32.57; \text{df} = 8, 98; P < 0.0001$) interactions. No significant planting date × hybrid ($F = 1.80; \text{df} = 10, 191; P = 0.062$) and sampling date × hybrid ($F = 0.64; \text{df} = 20, 98; P = 0.873$) interactions effects were observed. An increase in the number of beetles obtained from untreated non-Bt hybrid, DKC61-73 caused the significant differences among hybrid treatments (Fig. 2.5B). During the last week of July and first week of August, when hybrids from middle and early planting were on silking stage, the numbers of beetles did not vary between early and middle planting, but were significantly lower on late planted maize that was still on vegetative stage (Fig. 4E). A significant peak of the beetle population on late planted maize happened on 14 August when short season hybrids were in brown silking and full season hybrids were in green silking. On the remaining sampling date, the number of beetles declined towards the end of August when maize plants were maturing (Fig. 2.4E).

At Wooster the numbers of corn rootworm beetles did not vary significantly among planting dates ($F = 0.41; \text{df} = 2, 6.77; P = 0.680$) and hybrid treatments ($F = 1.70; \text{df} = 5, 162; P = 0.1376$), but differed between sampling date ($F = 61.44; \text{df} = 3, 107; P < 0.0001$) and the planting date × sampling date interaction ($F = 25.18 =; \text{df} = 6, 107; P < 0.0001$) (Fig. 2.4F). When counting started during the first week of August, significantly higher numbers of beetles were found on early and middle plantings compared to late planted maize (Fig. 2.4F). At this time, most maize in middle and early plantings was in
the silk stage and early planted maize was showing mostly brown silk, whilst middle planted maize showed green and brown on short season and full season hybrids respectively (Fig. 2.4F). A slight peak in the number beetles occurred on middle planted maize on 12 August that was significantly higher than in late plantings. A major increase in the numbers of beetles on late planted maize occurred on 12 August when short season hybrids were in silk stage and peaked at a period when 50% of full season hybrids were in the silk stage (Fig. 2.4F). Counting stopped on 27 August when beetles numbers showed a significant decline and maize silk dried.

**Egg density**

Egg density was not correlated with corn rootworm adult density in 2006 (r = 0.20, P = 0.421) and 2007 (r = 0.187; P = 0.456), but a significant correlation was observed in 2008 (r = -0.64; P = 0.004). In 2006, the highest egg density was obtained from late planted maize plots, but did not differ significantly from middle and early plantings (F = 1.67; df = 2, 51; P = 0.1981) (Fig. 2.6A). There was neither a significant hybrid treatment (F = 0.64, df = 5, 51; P = 0.6714) nor planting date × hybrid interaction effects on egg density (Fig. 2.6A). In 2007, egg density was significantly higher on late planted maize than early planting (F = 12.99, df 2, 45; P < 0.0001) (Fig. 2.6B). Hybrid treatment did not significantly affect egg density (F = 0.61, df = 5, 45; P = 0.691), but planting date × hybrid interaction was significant (F = 2.33; df = 10, 45; P = 0.026). In 2008 late planted maize plots had significantly more eggs than earlier plantings (F = 4.37; df 2, 45; P = 0.0184). Egg density was not affected by either hybrid treatment (F = 0.40; df 5, 45; 0.847) or planting date × hybrid interaction (F = 0.62; df = 10, 45; P = 0.786) (Fig. 2.6C).
Fig. 2.6. Mean egg density (eggs/liter) collected from three planting dates of different hybrid treatments. PD = planting date, PDH = planting date × hybrid interaction. *, and *** denote significance at $P \leq 0.05$ and $P \leq 0.001$ respectively. SS = short season hybrid, LS = full season hybrid, UN = untreated hybrid, ST = seed treatment with clothianidin and Bt = hybrid with Bt gene.
Early pests

Injuries caused by early pests were recorded collectively as early pest injury and occurred in considerable proportions during 2006 at South Charleston and Wooster (Fig. 2.7A and B). However in 2007 and 2008 injuries by early pests were negligible (≤ 0.2 plants per replication for early planting and ≥ 0.1 for middle and late planting) and are therefore not reported. At South Charleston the main damage to leaves was caused by the common stalk borer (*Papaipema nebris* (Guenee) and true armyworm (*Pseudaletia unipuncta* (Haworth) that invaded maize fields in 2006. The other visible damage was caused by black cutworm, *Agrotis ipsilon* (Hufnagel) which showed plants cut either at or below ground level.

Analysis of data showed significant reduction in damage when planting was delayed than during normal early planting at South Charleston (F = 121.28; df = 2, 6; P <0.0001) (Fig. 2.7A). Hybrid treatment had a significant effect on early pest injury (F = 8.65; df = 5, 45; P <0.0001). Since the two Bt maize hybrids were obtained from a seed company with the low rate of clothianidin (Poncho™ 250), a significant protection of secondary soil pests occurred with them compared to untreated hybrids (F = 13.23; df =1, 45; P = 0.0007). However, the protection was significantly lower than seed treatment with higher rate of clothianidin (1.25 mg a.i. per seed) (F = 41.28; df =1, 45; P <0.0001) (Fig. 2.7A). Hybrid with seed treatment in this paper referred to treatment with clothianidin at rate of 1.25 mg a.i. per seed. A significant planting date × hybrid interaction occurred (F = 3.98; df =10, 45; P <0.0006). Contrast analysis for a linear trend showed a significant planting date by seed treatment interaction (F = 41.28; df = 1 45; P <0.0001) indicating a linear response of early pest injury. However no such
significant linear response was observed among the planting date × Bt maize interaction (df = 2, 45, F = 0.11 P = 0.8934) indicating reduced effects of lower rate clothianidin compared to higher rate. While the seed treatment was able to reduced incidence of early pests, the effects were largely lessened by later plantings when no significant differences occurred among the hybrids (Fig. 2.7A). Incidence of early pests was negatively correlated with plant stand at South Charleston (r = -0.38; P = 0.0009) but not at Wooster (r = 0.06; P = 0.60).

Early pest injury at Wooster in 2006 was mostly caused by maize flea beetle, *Chaetocnema pulicaria* (Melsheimer) and slugs (*Agriolimax* sp). Because neither seed treatment nor Bt maize hybrid are registered to control slugs and maize flea beetles, no significant reduction in injury occurred among hybrid treatment (df = 5, 54, F = 1.33, P = 0.266) (Fig. 2.7B). Incidence of foliar injury was reduced on late planted maize compared to middle and early plantings (F = 135.64; df = 2, 54; P <0.0001); however, no planting date × hybrid interaction effects were observed (F = 0.37; df = 10, 54; P = 0.956).
Fig. 2.7. Incidence of early pest injury on maize planted at South Charleston and Wooster in 2006. Letters associated with planting date indicate that a significant difference occurred, with hybrid, with means having the same letter not significantly different (Fisher protected LSD, $\alpha = 0.05$). PD = planting date; PDH = planting date $\times$ hybrid interaction; *** denote significance at $P \leq 0.001$. SS = short season hybrid, LS = full season hybrid, UN = untreated hybrid, ST = seed treatment with clothianidin and Bt = hybrid with Bt gene.
Japanese beetle populations

The peak of populations of Japanese beetles during the three years of this study was generally associated with the period when maize plants were reaching or already in silking stages (Fig. 2.8). In 2006, the numbers of Japanese beetles that infested maize at South Charleston was significantly higher on early and late planted maize than middle planting ($F = 5.65; \text{df} = 2, 175; P = 0.004$) (Fig. 2.8A). The population also varied among hybrid treatments ($F = 2.52; \text{df} = 5, 261, P = 0.030$), sampling date ($F = 69.15; \text{df} = 4, 261, P < 0.0001$) and planting date × sampling date interaction ($F = 77.48; \text{df} = 8, 261, P < 0.0001$). Three distinct peaks with significantly higher populations of Japanese beetles occurred at South Charleston on each planting date, and were generally associated with a time when at least 50% of maize plants were in silking stage (Fig. 2.8A). The first significant peak of Japanese beetle population occurred in 19 July on early planted corn. At this time, full season hybrids were in brown silk (blister/R2) while short season hybrids were in green silk stage (R1) (Fig. 2.8A). The second peak, which was associated with middle plantings happened on 1 August when full season hybrids reached silking stages and short season hybrids were already in that stage (Fig. 2.8A). Significantly higher numbers of Japanese beetles were obtained during the peak of 8 August on late planted maize compared to early plantings, coinciding with peak silking by late planted full season hybrids.

At Wooster, the number of Japanese beetles was not significantly affected by planting date ($F = 2.20; \text{df} = 2, 6; P = 0.192$) and hybrid treatments ($F = 1.93; \text{df} = 5, 261 P = 0.090$), but was by sampling date ($F = 12.41; \text{df} = 4, 261; P < 0.0001$) and planting date × sampling date interaction ($F = 6.49; \text{df} = 8, 261; P < 0.0001$). Within planting date
multiple comparisons showed that significantly higher numbers of Japanese beetles were
found on early planted maize than middle and late planting on 28 July. During this
period early planted maize was in R1 stage while short season were R2 stage. As the
season progressed the population declined to less than one beetle per plant with no
significant differences occurring among the planting dates (Fig. 2.8B).

In 2007, significantly higher numbers of Japanese beetles were observed on early
and late planted maize than on middle planting at South Charleston (F = 18.05; df = 2, 6;
P = 0.003) (Fig. 2.8C). No significant differences occurred among hybrid treatments (F =
0.83; df = 5, 369; P = 0.532), but significant differences were observed between planting
date × hybrid interaction (F = 2.86; df = 10, 369; P = 0.002), sampling date (F = 44.67; df
= 6, 369; P <0.0001), sampling date × planting date (F =40.06 ; df = 12, 369; P <0.0001)
and sampling date × hybrid interaction (F = 40.06; df = 12, 369; P <0.0001). Three
distinct peaks with significant higher populations of Japanese beetles were apparent for
each planting date, coinciding with time when maize was in silking stage (Fig. 2.8C)
similar to 2006. The first peak of beetle population occurred on 18 July when early
planted full season hybrids reached silking stage. The second peak happened on 8
August when middle planted full season and short season hybrids were in silking stage
(Fig. 2.8C). The last peak occurred on 17 August when late planted full season hybrids
were in brown silk stages.

The beetle population was significantly higher on late planted maize than early
and middle plantings at Wooster (F = 22.46; df = 2, 139; P <0.0001). Beetle numbers
were not affected significantly by hybrid treatment (F =1.50; df = 5, 139 P = 0.1949) but
were by planting date × hybrid (F = 2.17; df = 10, 139; P = 0.023), sampling date (F =
20.92; \(df = 6, 80; P < 0.0001\), sampling date × hybrid (\(F = 2.02; df = 30, 80; P = 0.0067\)),
and planting date × sampling date interactions (\(F = 15.03; df = 12, 80; P < 0.0001\)).
The numbers beetles on early planted maize did not differ significantly among planting dates
(Fig. 2.8D). The significance occurred on 10 August where higher numbers of beetles
were found in middle planting compared to early and late plantings. At this time maize
in early and middle plantings had passed the silking stage except full season hybrids on
middle plantings that were still in brown the silk stage (Fig. 2.8D). The last peak
occurred when late planted full season hybrids were at the peak of silking on 15 August.

In 2008, the numbers of Japanese beetles found infesting maize at South
Charleston were not significantly affected by planting date (\(F = 0.91; df = 2, 7.2; P =
0.444\)) and hybrid treatments (\(F = 1.49; df = 5, 193; P = 0.1952\)), but by sampling date (\(F
= 12.04; df = 4, 103; P < 0.0001\)) and planting date × sampling date interaction (\(F
= 16.36; df = 8, 103; P < 0.0001\)). When counting started, early planted maize of both
maturities and middle planted short season hybrids were already in silk stages. At this
time, significantly higher numbers of Japanese beetles were found on early planted maize
than middle and late plantings (Fig. 2.8E). A significant peak of Japanese beetle
population on middle planted maize occurred on 5 August when full season hybrids were
in brown silk stage.

The population on late planted maize showed a significant increase on 14 August
when short season hybrids were in brown silk and 50% of full season hybrids were in
green silking. The population then peaked on 20 August when full season hybrids were in
brown silk stage (Fig. 2.8E). Again, this was similar to the other two years.
Fig. 2.8. Mean number of Japanese beetles at South Charleston and Wooster from 2006 to 2008. Letters associated with sampling date indicate that a significant difference was obtained, with planting date means having the same letter not significantly different (Fisher protected LSD, $\alpha = 0.05$). R1/2 denotes that full season hybrids were in R1 and short season hybrids in R2 for a respective sampling date. R1 = green silk stage; R2 = brown silk (blister) stage.
Unlike at South Charleston, significantly higher numbers of Japanese beetles were observed on middle and early plantings than in the late planting at Wooster in 2008 ($F = 3.94; df = 2, 122; P = 0.022$) at (Fig. 2.8B). No significant differences occurred among hybrid treatment ($F = 0.57; df = 5, 122; P = 0.723$), planting date $\times$ hybrid ($F = 1.24; df = 10, 122; P = 0.271$), or sampling date $\times$ hybrid ($F = 0.69; df = 15, 60; P = 0.789$). However, significance occurred among sampling dates ($F = 13.84; df = 3, 60; P < 0.0001$) and sampling date $\times$ planting date interaction ($F = 7.39; df = 6, 60; P < 0.0001$). Within planting dates, comparisons revealed that the population of Japanese beetles did not vary between early and middle planted corn, but was significantly higher than late planted maize (Fig. 2.8F). At this time all maize on early and middle plantings was already in silk stages. A significantly higher numbers of Japanese beetles on late planted maize were obtained on 18 August, a time when at least 50% of full season hybrids were in the silking stage (Fig. 8F).

**Silk clipping**

In 2006, the proportion of plants with silk clipping caused by corn rootworm and Japanese beetle feeding was significantly higher on early planted maize than middle and late plantings at South Charleston ($F = 23.18; 2, 45; P < 0.0001$) while at Wooster injury was significantly higher on late planted maize ($F = 3.93; df = 2, 45; P = 0.0267$) (Table 2.3). The proportion of plants with silk clipping did not vary among hybrids at either location (South Charleston: $F = 1.41; df 5, 45; P = 0.237$; Wooster: $F = 1.56; df = 5, 45; P = 0.190$) (Table 3). No planting date $\times$ hybrid interaction effects were observed at South Charleston ($F = 1.39; df = 5, 45; P = 0.217$) or at Wooster ($F = 0.71, df = 5, 45; P = 0.706$). The proportion of plants with silk clipping was not significantly affected by
either transgenic Bt hybrid \( (F = 1.61; \text{df} = 1, 45; P = 0.211) \) or seed treatment \( (F = 0.04; \text{df} = 1, 45; P = 0.846) \) at South Charleston and similarly at Wooster \( (Bt: F = 0.03; \text{df} = 1, 45; P = 0.858, \text{seed treatment: } F = 0.03; \text{df} = 1, 45; P = 0.854) \). There was no significant difference between hybrid relative maturities at South Charleston \( (F = 2.80; \text{df} = 1, 45; P = 0.101) \) but at Wooster a significantly higher proportion of plants with silk clipping was obtained from short season hybrids \( (F = 9.69; \text{df} = 1, 45; P = 0.003) \). The proportion of plants with silk clipping among hybrids ranged from 3% to 17% at South Charleston and 6% to 31% at Wooster (Table 2.3).

In 2007, a significantly higher proportion of plants with silk clipping was obtained on early planted maize at South Charleston \( (F = 20.70; \text{df} = 2, 6; P = 0.0020) \), compared with virtually no clipping recorded (silk length must be reduced to 1.27 or less) on late and middle planting (Table 2.3). A significantly higher proportion of plants with silk clipping was found on late planted maize at Wooster than early planting \( (F = 60.65; \text{df} = 2, 6; P = 0.0001) \). Hybrid treatments did not have significant effects on the proportion of plants with silk clipping at South Charleston \( (F = 1.24; \text{df} = 5, 45; P = 0.309) \), but significant differences occurred at Wooster \( (F = 59.54; \text{df} = 1, 45; P < 0.0001) \). There was no significant planting date \( \times \) hybrid interaction effects at South Charleston \( (F = 1.24; \text{df} = 10, 45; P = 0.295) \), but a significant interaction was found at Wooster \( (F = 59.54; \text{df} = 5, 45; P < 0.0002) \).

The proportion of plants with silk clipping in 2007 was not significantly affected by either transgenic Bt maize \( (F = 1.40; \text{df} = 1, 45; P = 0.243) \) or seed treatment \( (F = 1.93; \text{df} = 1, 45; P = 0.172) \) compared to untreated hybrids at South Charleston. Hybrid maturity rating did not affect the proportion of plants with silk clipping at South
Charleston (F = 3.87; 1, 45; P = 0.0553) and Wooster (F = 1.18, df = 1, 45; P = 0.284).
The proportion of plants with silk clipping ranged from 2% to 11% at South Charleston
and from 8% to 29% at Wooster (Table 2.3).

In 2008, a significantly higher proportion of plants with silk clipping was
obtained on late planted maize at South Charleston (F = 33.50, 2, 45; P <0.0001), but not
at Wooster (F = 0.14; df = 2, 45; P = 0.870). Hybrid treatments had significant effects on
the proportion of plants with silk clipping at South Charleston (F = 2.3; df = 5, 45 P =
0.054), but not at Wooster (F = 2.27; df = 5, 54, P = 0.060). No planting date × hybrid
interaction occurred at either location (South Charleston: F = 0.59; df = 10, 45; P = 0.810,
Wooster: F = 1.58; df = 10, 54; P = 0.1387).

In 2008 the proportion of plants with silk clipping was significantly lower on
transgenic Bt maize (F = 4.56, df = 1, 45; P = 0.038) and seed treatment (F = 5.89; df = 1,
45; P = 0.019) compared to untreated hybrids at South Charleston. At Wooster, a
significantly lower proportion of plants had silk clipping on Bt hybrids (F = 5.14; df = 1,
54, P = 0.027), but no such differences were observed among hybrids with seed
treatment: F = 0.12; df = 1, 54; P = 0.729) when compared to untreated ones. Hybrid
maturity rating did not affect the proportion of plants with silk clipping at either location
(South Charleston: F = 3.62; df = 1, 45; P = 0.064; Wooster: F = 1.30; df = 1, 54; P =
0.260). The proportion of plants with silk clipping ranged from 8% to 28% at South
Charleston and 1% to 10% at Wooster (Table 2.3).
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<td>0.08 ± 0.03b</td>
</tr>
<tr>
<td></td>
<td>Dekalb DKC61-69VTR Bt (LS)</td>
<td>0.08 ± 0.04a</td>
<td>0.29 ± 0.07a</td>
</tr>
<tr>
<td></td>
<td>Dekalb DKC61-73 ST (LS)</td>
<td>0.11 ± 0.06a</td>
<td>0.25 ± 0.08a</td>
</tr>
<tr>
<td></td>
<td>Dekalb DKC61-73 UN (LS)</td>
<td>0.06 ± 0.04a</td>
<td>0.24 ± 0.06a</td>
</tr>
<tr>
<td>2008</td>
<td>Planting Date</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Early May</td>
<td>0.08 ± 0.02b</td>
<td>0.05 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>Mid May</td>
<td>0.08 ± 0.02b</td>
<td>0.05 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>Early June</td>
<td>0.38 ± 0.07a</td>
<td>0.06 ± 0.02</td>
</tr>
<tr>
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<td>Hybrid</td>
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<td></td>
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<tr>
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<td>Dekalb DKC52-40 Bt (SS)</td>
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<td>0.01 ± 0.01</td>
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<td>0.10 ± 0.04</td>
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<td>0.09 ± 0.03</td>
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<td>0.06 ± 0.02</td>
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<td>Dekalb DKC61-73 UN (LS)</td>
<td>0.28 ± 0.09a</td>
<td>0.05 ± 0.02</td>
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</tbody>
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Table 2.3. Proportion of plants with silk clipping caused by corn rootworm and Japanese beetles from 2006 to 2008. Means followed by the same letter within a column are not significantly different (Fisher protected LSD ($\alpha = 0.05$)). Columns for each location are separated by year, planting date and hybrid. ‡Means averaged across hybrids. §Mean averaged across planting dates. SS = short season hybrid, LS = full season hybrid, UN = untreated hybrid, ST = seed treatment with clothianidin and Bt = hybrid with Bt gene.
**Poor seed setting**

In 2007, the percentage of plants with poor seed set was significantly correlated with the proportion of plants with silk clipping at South Charleston ($r = -0.30$, $P = 0.012$) and at Wooster ($r = -0.25$, $P = 0.038$). Significant correlation was also observed between root injury and percentage of plants with poor seed set at Wooster ($r = 0.61$, $P < 0.0001$) but not at South Charleston ($r = 0.14$, $P = 0.230$). The percentage of plants with poor seed set was significantly higher on middle and late planting than early planting at South Charleston ($F = 10.43$; df = 2, 45; $P = 0.0002$), while at Wooster, the percentage of plants with poor seed set was higher on early and middle plantings than late ($F = 22.60$; df = 2, 45; $P = 0.0016$) (Fig. 2.9A and B). There was no planting date × hybrid interactions effects at either location (South Charleston; $F = 1.23$; df = 10, 45; $P = 0.298$; Wooster: $F = 1.06$; df = 10, 45; $P = 0.415$).

There was no significant hybrid treatment effect observed at South Charleston ($F = 1.89$; df = 5, 45; $P = 0.115$), but a significant difference occurred at Wooster ($F = 12.11$; df = 5, 45; $P < 0.0001$). Contrast analysis revealed that Bt maize did not affect the percentage of plants with poor seed set ($F = 0.40$; df = 1, 45; $P = 0.531$), but a significant reduction occurred on hybrids with seed treatment ($F = 6.53$; df = 1, 45; $P = 0.014$) at South Charleston. At Wooster, significantly reduction in the percentage of plants with poor seed setting was obtained from both Bt hybrids ($F = 40.27$; 1, 45; $P < 0.0001$) and hybrids with seed treatment ($F = 37.74$; df = 1, 45; $P < 0.0001$) than untreated ones. There was no significant difference between short and full season hybrids at South Charleston ($F = 0.91$; df = 1, 45; $P = 0.346$), but significantly higher percentages of short season hybrids had poor seed set ($F = 4.24$; df = 1, 45 $P = 0.045$).
Fig. 2.9. Mean percentage of plants with poor seed set at South Charleston and Wooster 2007 and 2008. Letters associated with planting date indicate that a significant difference occurred, with hybrid means having the same letter not significantly different (Fisher protected LSD, $\alpha = 0.05$). PD = planting date; PDH = planting date $\times$ hybrid interaction; *, ** and *** denote significance at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$ respectively. SS = short season hybrid, LS = full season hybrid, UN = untreated hybrid, ST = seed treatment with clothianidin and Bt = hybrid with Bt gene.
In 2008, the percentage of plants with poor seed set was again significantly correlated with the percentage of plants with silk clipping at South Charleston \((r = 0.50; P < 0.0001)\) and also at Wooster \((r = 0.24, P = 0.046)\). No significant correlation occurred between the percentage of plants with barren ear tips and root injury at South Charleston \((r = -0.01; P = 0.913)\), but significance occurred at Wooster \((r = 0.33; P = 0.005)\). Late planted maize had a significantly higher percentage of plants with poor seed set than early and middle plantings at South Charleston \((F = 14.18; \text{df} = 2, 51; P < 0.0001)\) (Fig. 2.9C). Conversely, a higher percentage of plants with poor seed set were found on early planted maize than middle and late plantings at Wooster \((\text{df} = 2, 54; F = 16.91; P < 0.0001)\) (Fig. 2.9D). At each location, hybrid treatment had significant effects on the percentage of plants with poor seed set (South Charleston: \(F = 3.51; \text{df} = 5, 51; P = 0.0085\), Wooster: \(F = 21.13; \text{df} = 5, 54; P < 0.0001\)). A significant planting date × hybrid interaction did not occur at South Charleston \((F = 1.51, \text{df} = 10, 51; P = 0.163)\), but it did at Wooster \((F = 11.38; \text{df} = 10, 54; P < 0.0001)\). At South Charleston, percentage of plants with poor seed set was significantly higher on untreated hybrids than Bt hybrids \((F = 11.87; \text{df} = 1, 51 P = 0.001)\) and hybrids with seed treatment \((F = 3.96; \text{df} = 1, 51; P = 0.050)\); similar results occurred at Wooster (Bt: \(F = 35.40; \text{df} = 1, 54; P < 0.0001\); seed treatment: \(F = 45.23; \text{df} = 1, 54; P<0.0001\)). Relative hybrid maturity had no significant effects at South Charleston \((F = 0.21; \text{df} = 1, 51; P = 0.652)\), but significantly more short season hybrids on early and middle plantings had poor seed set at Wooster \((\text{df} = 16.96; \text{df} = 1, 54; P = 0.0001)\) (Fig. 2.9D).
Plant populations

Plant population per hectare ranged from 58,000 – 77,000, 71,000 – 80,000, and 75,000 – 85,000 plants in 2006, 2007, and 2008, respectively. In 2006, plant population at harvest was significantly lower on early planted maize than early and middle plantings at South Charleston (F = 3.22; df = 2; 51; P = 0.048) and at Wooster (F = 6.95; df =2; 6; P = 0.027). Hybrid treatment significantly affected the plant population at both locations (South Charleston: F = 11.36; df = 5, 51; P <0.0001, Wooster: F = 10.71; df = 5, 45; P <0.0001). No significant planting date × hybrid interaction was observed at South Charleston (F = 0.51; df = 10, 51; P = 0.872), but it was at Wooster (F = 2.42; df = 10, 45; P = 0.021). There was a significantly higher plant population in Bt hybrids (F = 12.36; df = 1, 51; P = 0.0009) and seed treatment (F = 47.90; df = 1, 51; P<0.0001) than untreated hybrids at South Charleston. However at Wooster Bt hybrids had no significant effects on plant population (F = 3.65; df = 1, 45; P = 0.063), but significantly more plants were obtained from seed treatment (F = 11.51; df = 1, 45; P = 0.002) than untreated hybrids. Relative maturity did not significantly affect plant population at South Charleston (F = 2.40; df =1, 51; P = 0.128) but higher plant populations were found on full season hybrids than short season at Wooster (F = 26.45; df = 1, 45; P<0.0001). The plant population was significantly correlated with incidence of early pests at South Charleston (r = -0.38; P = 0.0009) and at Wooster (r = -0.28; P = 0.019). No significant differences in plant population were observed in 2007 and in 2008 at both locations and; statistics are therefore not reported.
Grain yield

In 2006, maize planted in early June had a significant reduction in yield compared to when planted in late April at South Charleston \((F = 11.86, \text{df} = 2, 45; P < 0.0001)\) (Fig. 2.10A). Yield was significantly affected by hybrid treatments \((F = 11.86; \text{df} = 5, 45; P < 0.0001)\), but no significant planting date \(\times\) hybrid interaction occurred \((F = 0.84; \text{df} = 10, 45; P = 0.591)\). Transgenic Bt maize \((F = 7.39; \text{df} = 1, 45; P = 0.009)\) and seed treatment \((\text{df} = 12.72; \text{df} = 1, 45; P = 0.0009)\) had significantly higher yield compared to untreated hybrids. Significantly higher yields were obtained from full season hybrids compared with short season hybrids \((F = 43.36; \text{df} = 1, 45 P < 0.0001)\). At Wooster, no significant effects were detected among planting date \((F = 0.96; \text{df} = 2, 6 = 0.434)\), hybrid treatment \((F = 0.79; \text{df} = 5, 45; P = 0.563)\) and planting date \(\times\) hybrid interaction \((F = 0.62; \text{df} = 10, 45; P = 0.786)\) (Fig. 2.10B). Multiple comparison and contrast analysis also revealed no significant differences among treatments (Fig. 2.10B). Significant correlation was observed between plant population and yield at South Charleston \((r = 0.30, P = 0.010)\) but not at Wooster \((r = 0.17; P = 0.15)\).

Multiple regression analysis for 2006 data showed significant effects of silk clipping and root injury on yield at South Charleston \((F = 10.68; \text{df} = 2; r^2 = 0.24; P<0.0001)\) but not at Wooster \((F = 0.72; \text{df} = 2; r^2 = 0.02, P = 0.492)\). The significance of the regression model indicated that at least one predictor variable was significantly related to yield at South Charleston but not at Wooster. A significant inverse relationship between silk clipping and yield was detected at South Charleston (Table 2.4), indicating that the adult beetle population feeding on silk had a significant impact on yield. Only 23.6\% of the variation in yield was explained by both adult corn rootworms and larval
feeding at South Charleston. Addition of root injury did not improve the fit of the model at both locations, indicating that larval feeding was too low to cause economic damage in 2006 (Table 2.4).

In 2007, planting date did not affect grain yield at South Charleston ($F = 1.01; df = 2, 9; P = 0.403$), but significantly higher yields were obtained on late planted maize than early planting at Wooster ($F = 23.10, df = 2, 6; P = 0.002$). No significant planting date $\times$ hybrid interaction effects were observed at either location (South Charleston: $F = 0.49; df = 10, 45; P = 0.891$, Wooster: $F = 1.91; df = 10, 45; P = 0.069$). Grain yield was significantly affected by hybrid treatment at South Charleston ($F = 6.53; df = 5, 45; P = 0.0001$) and Wooster ($F = 16.52; df = 5, 45; P <0.0001$). There was no significant yield increase from maize protected by Bt gene ($F = 2.27; 1, 45; P = 0.139$) or seed treatment ($F = 0.28; df = 1, 45; P = 0.596$) compared with untreated hybrid at South Charleston, but a significant increase in yield was obtained from Bt maize ($F = 54.17; df = 1, 45; P <0.0001$) and seed treatment ($F = 60.07; P = 1, 45; P <0.0001$) at Wooster (Fig. 2.10D). At both locations, a significant yield advantage occurred on full season than short season hybrids (South Charleston: $F = 29.17; df = 1, 45; P <0.0001$, Wooster: $F = 4.73; df = 1, 45; P = 0.035$).

Multiple comparisons within each planting date showed that the significant differences in yield between the maturity groups did not occur among hybrids protected by both Bt gene and seed treatment (Fig. 2.10C and D). A significant reduction in yield in short season hybrids was therefore mainly due to untreated short season hybrid for each planting date at both locations (Fig. 2.10C and D). No significant differences among hybrids occurred on late planted maize probably due to reduced larval feeding by
corn rootworms associated with late planting (Fig. 2.10D). Statistical analysis showed that a regression model was significant at both South Charleston (F = 4.54; df = 2; r² = 0.12; P = 0.014) and Wooster (F = 43.26 df = 2; r² = 0.56; P<0.0001).

Root injury and poor seed set accounted for only 11.6 % of variation in yield response at South Charleston and 55.6 % at Wooster. At each location, a significant inverse relationship was observed between yield and the percentage of plants with poor seed setting (Table 2.4), again a possible reflection of adult feeding. Yield was inversely related to root injury at Wooster but not at South Charleston (Table 2.4), indicating that larval feeding significantly reduced yield at Wooster but not at South Charleston.

In 2008, planting date did not significantly affect grain yield at either location (South Charleston: F = 3.60; df = 2, 6 P = 0.094 Wooster: F = 1.26; df = 2, 6; P = 0.349) (Fig. 2.10E and F). At South Charleston, no significant differences occurred among hybrid treatment (F = 0.76; df = 5, 45; P = 0.580) and planting date × hybrid interactions (F = 1.58; df 10, 45; P = 0.143). However at Wooster, significance occurred among hybrid (F = 4.19; df = 5, 45; P = 0.003) and planting date × hybrid interaction (df = 3.07; df = 10, 45; P = 0.005). Significant differences among hybrid treatment at Wooster were due to increased yield among Bt hybrids (F = 8.56; df = 1, 45; P = 0.005) and seed treatment (F = 6.33; df = 1, 45; P = 0.016) compared with untreated hybrids, and full season vs. short season hybrids (F = 7.35; df = 1, 45; P = 0.001). Multiple comparisons using planting date × hybrid interaction showed that these differences were mainly due to a significant yield reduction on the early planted short season hybrid, DKC52-47 that was untreated (Fig. 2.10F). There were no differences observed as planting was delayed.
At both locations, the regression model showed significant effects of independent variables on yield (South Charleston: $F = 8.55$; $df = 3$; $P < 0.0001$; Wooster: $F = 13.54$; $df = 2$; $P < 0.0001$), and explained 27.1% of the variation in yield at South Charleston and 28.5% at Wooster. There was not a significant relationship between yield and root injury at either location (Table 2.4). However a significant inverse relationship was again observed between poor seed setting and grain yield at both locations (Table 2.4). Growing degree days were inversely related to yield only at Wooster, indicating that yield was impacted negatively by delayed planting at Wooster but not at South Charleston (Table 2.4).

**Phenology and thermal time.**

Data on growing degree days (GDDs) required by maize hybrids to reach silking (R1) and black layer or physiological maturity (R6) is shown in Table 2.5. The measured GDDs were for the most part, not consistent with those provided by the seed company (Table 2.5). Except for short season hybrids planted in 2006 at South Charleston and in 2006 and 2007 at Wooster, measured GDD intervals after planting (AP) to R1 for both maturity groups were greater than the respective company values (Table 2.5). On the contrary, measured GDD intervals after planting to R6 were less than those provided by the seed company for all hybrids planted at both locations over the three years (Table 2.5). Regression analysis using day of planting during the year as the independent variable and growing degree days required for maize to reach physiological maturity (R6) as the dependent variable showed significance at both locations (Table 2.6). The results indicate that delayed planting was significantly associated reduced growing degree days at each location for both short and full season hybrids.
Fig. 2.10. Mean maize grain yield obtained at South Charleston and Wooster from 2006 to 2008. Letters associated with planting date indicate that a significant difference occurred, with hybrid means having the same letter not significantly different (Fisher protected LSD, $\alpha = 0.05$). PD = planting date; PDH = planting date × hybrid interaction; *, ** and *** denote significance at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$ respectively. SS = short season hybrid, LS = full season hybrid, UN = untreated hybrid, ST = seed treatment with clothianidin and Bt = hybrid with Bt gene.
<table>
<thead>
<tr>
<th>Location, year</th>
<th>Predictor variable</th>
<th>( \beta \pm SE )</th>
<th>t</th>
<th>( P )</th>
<th>MSE</th>
<th>Adj. ( r^2 )</th>
<th>Cp</th>
<th>VIF</th>
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<td>S. Charleston, 2006</td>
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<td>12.59 ± 0.31</td>
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<td>% silk clipping</td>
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<tr>
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</tr>
<tr>
<td></td>
<td>Root injury</td>
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Table 2.4. Results of multiple-regression showing relationship between grain yield various independent variables associated with variation in yield. ‡predictor variables excluded when high multicollinearity occurs (>10 VIF values) and/or had higher Cp and MSE values with low adjusted \( r^2 \). VIF = variance inflation ratio; MSE = mean square error; \( C_p \) = Mallow’s \( C_p \) value. \( \beta \) = coefficient, SE = standard error
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</table>

Table 2.5. Effect of planting date on thermal time phenological events within frosted† and unfrosted environments.

DAP = Days after planting to R1 (silking) and R6 (black layer or physiological maturity).

§GDD values in parenthesis are those stated by seed supplier

‡The GDDs values represent means across two hybrids for each short and full season relative maturity ratings.

Short = 101 to 102 relative maturity days. Full = 111 relative maturity days.
<table>
<thead>
<tr>
<th>Environment</th>
<th>Year</th>
<th>Maturity§</th>
<th>Regression†</th>
<th>r²</th>
<th>Significance‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Charleston</td>
<td>2006</td>
<td>Short</td>
<td>Y = -0.125x² + 29.73x + 688.84</td>
<td>0.93</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Full</td>
<td>Y = -0.202x² + 51.14x – 633.08</td>
<td>0.83</td>
<td>**</td>
</tr>
<tr>
<td></td>
<td>2007</td>
<td>Short</td>
<td>Y = -4.386x + 3005.85</td>
<td>0.99</td>
<td>***</td>
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<tr>
<td></td>
<td></td>
<td>Full</td>
<td>Y = -6.073x + 3419.54</td>
<td>0.99</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td>2008</td>
<td>Short</td>
<td>Y = -0.130x² + 34.38x + 173.11</td>
<td>0.82</td>
<td>**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Full</td>
<td>Y = 114x² + 30.05x + 320.21</td>
<td>0.81</td>
<td>**</td>
</tr>
<tr>
<td>Wooster</td>
<td>2006</td>
<td>Short</td>
<td>Y = -0.174x² + 42.53x – 140.00</td>
<td>0.94</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Full</td>
<td>Y = -0.285x² + 73.46x – 2174.21</td>
<td>0.87</td>
<td>**</td>
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<tr>
<td></td>
<td>2007</td>
<td>Short</td>
<td>Y = -0.218x² + 59.62x – 1563.56</td>
<td>0.58</td>
<td>**</td>
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<tr>
<td></td>
<td></td>
<td>Full</td>
<td>Y = -0.302x² + 82.72x – 3045.87</td>
<td>0.56</td>
<td>**</td>
</tr>
<tr>
<td></td>
<td>2008</td>
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<td>Y = -3.250x + 2749.3</td>
<td>0.96</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Full</td>
<td>Y = -4.723x + 3025.79</td>
<td>0.98</td>
<td>***</td>
</tr>
</tbody>
</table>

Table 2.6. Effects of delayed planting on thermal intervals [(growing degree days (GDDs) from planting to kernel black layer

*** Significant at P = ≤ 0.001 and ** significance at = P ≤ 0.01
† Y = GDD from planting to R6; x = day of year
‡ Significance of linear regression models
§ Data from each maturity group was combined and average used. Short = 101 to 102 relative maturity days. Long = 111 relative maturity days.


2.5 DISCUSSION

Our study showed that maize phenology significantly influenced the amount of injury to maize caused by rootworm larvae feeding on roots, adult beetle feeding on silk, and injury by early season pests. Root injury was significantly reduced by delayed planting and the use of Bt maize and seed treatment. The results showed that delaying planting until early June reduced root injury to insignificant levels. The reduction in corn rootworm larval feeding on maize roots as planting is delayed was also observed by Musick et al. (1980) and Hoffmann et al. (2000). The effects of delayed planting in relation to corn rootworm egg hatch patterns presumably reduces availability of maize roots and larval colonization sites resulting in mortality of the earliest eclosing larvae (Bergman & Turpin 1984). The effect of Bt maize and seed treatment on root injury by larval feeding were mitigated by delayed planting. Therefore, when planting is delayed, the use of seed treatment and Bt maize to manage corn rootworm would not be economically justified because of higher natural mortalities associated with the late planting.

When the population of corn rootworm larvae was high, injury on hybrids with seed treatment was significantly higher than on Bt hybrids. These results corroborate the concern in most Midwest States that when infestations of corn rootworm larvae are high, seed treatment may not effectively protect maize roots from larval injury (Steffey et al. 2005). However in this study, root injury was still significantly much lower with seed treatment compared to untreated hybrids (Fig. 2.1). Protection of maize from corn rootworm by using Bt hybrids and seed treatment did not translate into yield advantage
except in 2007 at Wooster where higher root injury among untreated hybrids occurred. This was the only time where a significant inverse relationship between root injury caused by rootworm larval feeding and yield occurred (Table 2.6).

Corn rootworm larval feeding on roots caused significant plant lodging twice, first at Wooster in 2007 and then in South Charleston in 2008. Like root injury, the percentage of plants that lodged was higher on early planted corn, but became significantly reduced as planting was delayed. As in other studies (Riedell and Evenson 1993, Allee and Davis 1996), hybrid choice affected the amount of lodging. In our study, within planting date comparisons revealed that early planted short season hybrids with no-Bt gene and seed treatment had a significantly lower percentage of plant that root lodged than their full season counterparts. However, these differences were not observed between short vs. full season as planting was delayed. Large root systems have been suggested to reduce lodging among hybrids (Riedell and Evenson 1993). We did not measure morphological characteristics, including the root systems of the hybrids in this study, and therefore cannot attribute the differences in lodging between short vs. long hybrids to such traits.

Population dynamics of adult corn rootworm and Japanese beetles were influenced by growth stages of corn. Generally, late planted maize had significantly higher populations than early planted corn, probably because corn rootworm adult emigrated out of early planted maize into late planted corn. Naranjo (1991) reported that the net seasonal emigration of adult corn rootworm out of early planted towards late-planted maize was 62%. Immigration into the late planted maize could have contributed to a significant increase in eggs laid on late planted maize plots than earlier plantings.
This finding corroborates previous reports that late planted maize attracts beetles that disperse from early planted maize when maturity advances to feed on newer silk and consequently lay eggs (Spencer et al. 2009, Pierce and Gray 2006). For field trapping purposes, planting a trap crop later in the season would attract dispersing beetles and lay eggs on the trap crops (Hill and Mayo 1974).

Corn rootworm adults are attracted preferentially to maize silks (Chiang 1973), and peak adult populations coincided with peak silking of maize in the field. Since hybrids of different relative maturities were planted at different dates, temporal effects of maize phenology on adult distribution were observed. Adult corn rootworms aggregated where the silks were available in the cornfield because distribution of silk is not uniform on any given date within a field (Park and Tollefson 2006; Toepfer et al. 2007). The adult beetles that clustered around silking plants caused significant silk clipping; however, this was not consistent from year to year and between locations.

At Wooster, more clipping occurred on non-Bt maize compared with Bt hybrids and hybrids with seed treatment, possibly because of higher beetles numbers that emerged stayed in plots after emerging because they are known to stay in the areas where they emerged during the peak of population if conditions are favorable (Park and Tollefson 2006). Silk clipping was positively correlated with density of corn rootworm beetles indicating significant effects of feeding.

The impact of early season pests was significant only in 2006 when they caused increased damage on early planted corn. When injuries by soil pests was prevalent, seed treatment with clothianidin significantly reduced damage by early pests, but the effect was largely mitigated by late planting because of fewer injuries during that period.
Hoffmann et al. (2000) also observed that the benefit of soil insecticides to control soil pests was significant when maize was planted early than late. When considering yield advantage, earlier planted maize showed significant increases in yield when maize was protected by seed treatment, but significant differences were not observed on later planted corn. A significant negative correlation was observed between early pest injury and the plant population at South Charleston, but not at Wooster. This observation suggested that soil pests largely contributed to reduction of plant stand at South Charleston but not at Wooster. When stand reducing pests injuries were low, such as at Wooster in 2006, the use of seed treatment to control early pests was not beneficial.

Poor seed setting observed in this study could have been caused by both insect and drought stress at reproductive stages of maize (Culy et al. 1992). Maize is particularly sensitive to water deficits that coincide with the tasseling-silking period, causing a marked decreased in seed set (Schusler and Westgate 1991). While drought stress may have contributed to poor seed set, significant correlations between poor seed setting and silk clipping indicated that corn rootworm and Japanese beetles contributed to poor seed set, especially when populations were high. In 2007, higher poor seed setting was observed on non-Bt compared with Bt and hybrid with seed treatment. A significant correlation between poor seed setting and root injury was observed indicating that injury to roots perhaps partly contributed to poor seed setting. Larval feeding by corn rootworm reduces root volume and uptake of water and nutrients (Reidel 1990) and can induce stress on plants resulting in poor seed setting. Evidence of stress was observed among untreated hybrids at Wooster in 2007, especially during a dry spell that occurred in June (personal observation) (Appendix A). A significant reduction in plant height on
untreated hybrids indicated that plant growth among untreated hybrids was largely due to stress related to root injury.

The influence planting date had on grain yield was inconsistent from year to year (Fig. 2.10). The use of Bt and seed treatment improved yield significantly in four of six comparisons (2006 at South Charleston, and in 2007 at both locations and in 2008 at Wooster). Significant differences in yield on early planted maize at South Charleston were largely due to stand reduction caused by early pests on untreated hybrids. The suggestion that reduction of plant stand by early pests affected yield is supported by significant correlations observed between early pest injury and plant populations, and between yield and stand counts at South Charleston. Among the three planting dates, higher yields were obtained from full season hybrids compared with short season hybrids at South Charleston.

Yield reduction observed on late planted maize at South Charleston in 2006 might have been associated with significantly higher silk clipping in the late planting. Greater silk clipping causing a yield loss is supported by higher numbers of adults beetle on late planted maize which were positively correlated with silk clipping. While root injury was generally reduced by later planting, the high beetle pressure on silking plants could have contributed to reduction in yield relative to earlier plantings. Roth et al. (1995) found that silk clipping in late planted maize resulted in a 15% yield reduction relative to early planted maize while in sweet maize yield reduction ranging from 30 to 40% were reported (Hoffman et al. 2000).

In 2007, protection of maize by the use of seed treatment and Bt maize did improve yields at South Charleston when compared to untreated hybrids. However,
planting full season hybrids resulted in yield advantage over short season hybrids among
the three planting dates. The percentage of plants with poor seed set and root injury
accounted for only 11% of variation in yield, which is logical given the lower number of
beetles that emerged and fed on maize at South Charleston in 2007 (Fig. 2.3C). In 2007,
unusually higher yields were obtained from late planted maize than middle and early
planting at Wooster. A significant yield advantage of using Bt hybrids and hybrids with
seed treatment were realized on earlier and middle planted corn, but no significant
differences were observed on late planted corn. Late planted maize did not incur
significant root injury by corn rootworm larvae when compared with earlier planted corn.
When planting is delayed, late planted maize evades injury because most larvae that
hatch starve to death due to lack of maize roots (Bergman and Turpin 1984). The lower
yield on early and middle plantings might also be explained by high populations of corn
rootworm on early and middle planted maize that significantly increased silk clipping,
resulting in high poor seed set on early and middle planted corn. This was supported by
results of multiple regression analysis that showed that poor seed setting and root injury
negatively impacted yield at Wooster in 2007.

In 2008, significant differences in yield were observed only at Wooster which was
caused by a significant yield reduction on early planted short season hybrid, DKC52-47
that was untreated (Fig. 2.10F). The reduction in yield on this hybrid was mostly
associated with higher poor seed setting (Fig. 2.9). This explanation is also supported by
regression model that showed a negative linear yield response to poor seed setting and
growing degrees days in 2008 at Wooster. Reduced growing degrees days might explain
the significant lower yield obtained from short season hybrids compared with full season
hybrids at Wooster in 2008. While there is some evidence that yield reduction was associated with corn rootworm injuries in some years of this study, it is also probable that such differences were perhaps because of the yield potential of the hybrids, and not due to protection of rootworm injuries by seed treatment or Bt corn. This is important especially where the differences occur within the same maturity group, otherwise the differences would be due to the hybrid’s ability to utilize growing season. Nevertheless, there is potential for yield loss due to root injury and silk clipping to occur in some years as happened at Wooster in 2007. Monitoring the population of corn rootworm to predict the damage in the following year will be vital in making decision to use pest management tactics. This study showed that the use of seed treatment and transgenic maize might be beneficial only when rootworm populations are predicted to be high and planting is early. When planting late because of unforeseen circumstances, such as unfavorable environmental conditions, the use of seed treatment and transgenic hybrids to control corn rootworm injury to roots might not be beneficial because of reduced injury associated with delayed planting.

2.6 REFERENCES


Levine, E., and H. Oloumi-Sadeghi. 1996. Western corn rootworm (Coleoptera: Chrysomelidae) larval injury to corn grown for seed production following soybeans grown for seed production. Journal of Economic Entomology. 89:1010-1016


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US EPA. Environmental Protection Agency. 2005. Bacillus thuringiensis Cry34AB1 and Cry34Ab1 proteins and the genetic material necessary for their production (plasmid insert PHP 17622) in Event DAS-59122-7 corn, 133 pages.


CHAPTER 3

THE INFLUENCE OF MAIZE PHENOLOGY AND TRANSGENIC BT MAIZE ON OVIPOSITION AND INJURY TO MAIZE BY EUROPEAN CORN BORER

3.1 ABSTRACT

A study was conducted to determine the influence of planting date and transgenic maize on oviposition by *O. nubilalis* and subsequent larval injury on corn. Transgenic and non-transgenic maize hybrids with different maturity ratings (short vs. full season) were planted on three planting dates (early, middle, and late), targeting late April/early, mid-May and early June respectively. The study was carried out at the Northwest Agricultural Research Station near Hoytville and at Wooster, Ohio from 2006 to 2008. Egg masses, growth stages, injuries to leaves and stalks, number of larvae and grain yield were compared among planting dates and between Bt and non-Bt genotypes of different relative maturity ratings. Egg mass density did not differ significantly between transgenic and non-transgenic corn. However significant differences were observed among planting dates, sampling dates, and sampling date x planting date interactions. Generally higher numbers of egg masses from second generation moths were deposited on late planted maize than middle and early plantings. Injuries on stalks and leaves were significantly higher on non-transgenic corn, and increased with delayed planting. The influence of egg mass density on number of larvae per plant and tunneling length was
inconsistent among the years and locations. Significantly higher yields were obtained from full season than short season hybrids when maize was planted early. Delayed plantings grain yield from short season hybrids were comparable to full season hybrids. The benefit of Bt maize was significant when planting was delayed due to reduced impact of increased tunneling associated with late planting. When planting is delayed due to heavy moisture or unforeseen circumstances and European corn borer population is known to cause economic damage in an area, selection of short season hybrids with the Bt gene could be beneficial.

### 3.2 INTRODUCTION

The European corn borer, *Ostrinia nubilalis* (Hubner), is considered one of the most damaging insect pests of maize (*Zea mays* L.) throughout North America. Yield losses and control expenditures associated with European corn borer cost farmers more than $1 billion annually (Mason et al. 1996). In much of the Corn Belt, the European corn borer is bivoltine with two distinct periods of moth flights lasting 4-6 weeks each; one in the spring and one in the summer (Sappington 2005). European corn borers overwinter as full-grown larvae within maize stalks, pupating and emerging as adults in the spring. The moth prefers to deposit its egg masses on the underside of leaves of whorl stage maize of earliest planted and tallest maize plants in an area (Ritchie et al. 1993, Mason et al., 1996).

The eggs hatch into early instar larvae that initially feed on foliage, causing windowpane injury on the tender central whorl. Foliar feeding may lead to shot hole injury in on the emerging leaves (Willson and Eisley 2001). As larvae reach the third and fourth instar, they tunnel into midribs and stalks. First generation larval feeding on
whorl-stage leaves and within the stalk may cause 5-6% yield loss per larva per plant (Bode and Calvin 1990). Second generation larvae feed on the ear, ear shanks and stalk causing 2 -3% yield loss per larva per plant during the reproductive stage of maize growth.

Pest management options for first-generation larvae have included planting moderately resistant hybrids, or planting susceptible hybrids early and then scouting to predict the larval population density. Using established economic injury levels (EIL), growers can apply an insecticide when needed (Pilcher and Rice 2001). For second generation larvae, management attempts are minimal, although scouting and applying a well timed insecticide spray has been the typical recommendation (Mason et al. 1996). However, Rice and Ostlie (1997) reported that 70-80% of Iowa and Minnesota maize growers have never used insecticides to manage European corn borer.

Alternative methods are needed that are simpler to use, easier to incorporate into farming systems, and have the ability to reduce economic losses. In considering practical alternatives, growers have a history of using various cultural management technologies (Rice and Ostlie 1997). Stalk destruction by plowing or turning maize stubble in fall or early spring is considered an effective cultural practice that reduces overwintering larvae of European corn borer (Mason et al. 1996, Rice and Ostlie 1997). Adjusting planting dates has also been recommended as a method to control European corn borer. Jarvis et al. (1986) demonstrated that yield of full season hybrids in both European corn borer infested and non-infested plots were much greater than those of short season hybrids. Losses were greater in short season hybrids when planted late. They recommended that growers should plant full season hybrids early; if short season hybrids are planted late,
European corn borer infestations should be closely monitored. These recommendations were made before the development of transgenic hybrids.

Transgenic field maize has been genetically engineered to express an insecticidal protein from *Bacillus thuringiensis* (Bt) to prevent damage by stalk-boring pests, in particular European corn borer, southwestern corn borer, and southern cornstalk borer (Koziel et al. 1993). Adjustment of planting dates and using transgenic maize as a trap crop for egg recruitment has been proposed as a strategy for managing European corn borer (Alstad & Andow 1995, Ostlie et al. 1997). Adjustment of planting dates to take advantage of Bt maize and European corn borer behavior was suggested as a way to manage part of the pest population (Pilcher and Rice 2001). Pilcher and Rice (2003) showed that early planted Bt maize can be protected against the first generation and when planted late will gain the greatest economic advantage from protection against the second generation. This work did not include short season hybrids which are suitable for shortened growing season during late planting situation compared to full season hybrids. Although the use of planting dates has not been widely used in the past (Rice and Ostlie 1997, Pilcher and Rice 1998), the integration of Bt maize into a pest management system may provide an opportunity to better use this cultural management tactic.

In this study we evaluated transgenic and non-transgenic hybrids of different maturities planted on sequential dates along with the use of various management tactics. The objective of this study was to determine possible interactions among planting date, maize maturity, and management tactics on egg laying by European corn borer, followed by larval injury and ultimately maize yield. Our first hypothesis was that maize phenology and transgenic Bt maize influence European corn borer oviposition, and injury
to maize and yield. The second hypothesis was that the yield from short season hybrids will be equal to or higher than full season hybrids when planted late and European corn borer infestations are high.

3.3 MATERIALS AND METHODS

The experiment was carried out at the Northwest Agricultural Research Station near Hoytville (41° 12´ N, 83° 45´ W) and on the Wooster Campus (40° 46´N, 81° 55´W) of the Ohio Agricultural Research and Development Center, The Ohio State University from 2006 - 2008. The land was prepared plowing in the spring followed by disc harrowing to break up the sod. The field was previous planted with a crop of soybean. The treatment design at each location was a 3 x 4 treatment factorial replicated four times in a randomized complete block experimental design arranged in split plot layout. The first factor (main plot) was planting date, randomized within each replication. There were three planting dates (normal/early, mid & late) targeting late April/ early May, late May and early June each year (Table 3.1). The second factor (subplot) was 4 hybrids (Table 3.2) assigned randomly within the planting date (main plot). The 4 hybrids represented two maturity groups: short and full season hybrids.

Each maturity group was then represented by two Bt hybrids and two non-Bt hybrids. Both Bt and non Bt maize seed were treated with clothianidin (Poncho, Gustafson LLC, Dallas TX) at rate of 0.25 mg a.i. per seed to control secondary soil pests. This seed treatment, applied to all hybrids, does not offer control of European corn borer. The maize hybrids used in this study were commonly used hybrids, selected
because they have comparable agronomic characteristics. The study aimed to represent normal situations in the farmer’s field; thus, isolines which are often planted in similar studies were not used because they were not normally-grown hybrids. Individual plots were 10 m by 12 rows wide. Plots were planted using row spacing of 0.76 m at a seeding rate of 79,000 seeds ha\(^{-1}\). The growing degree days required for maize plants to reach R1 (silking stage) and R6 (black layer) were calculated based on dates that each maize maturity reached respective reproductive stages. The maize growth stage (Ritchie et. al. 1993) of each planting date was recorded weekly during vegetative stages to reproductive stages.

**Insect data collection**

Moth flight patterns were monitored with pheromone traps using Iowa or Z strain of lure. The trap was located adjacent to the maize crop. The pheromone trap operated 24 hours a day from 6 June to 21 September in Wooster and 13 June to 22 September in Hoytville during 2006. In 2007 trapping in Wooster started on 29 May and ended in 22 September. In Hoytville trapping started on May 30 and ended in 26 September 2007. In 2008 trapping started on 13 May in Wooster and ended in 19 September while in Hoytville started on 22 July and ended on 25 September. European corn borer moths collected by the traps were counted weekly.
<table>
<thead>
<tr>
<th>Location</th>
<th>Year</th>
<th>Early</th>
<th>Middle</th>
<th>Late</th>
</tr>
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<tbody>
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<td>7 June</td>
</tr>
<tr>
<td></td>
<td>2007</td>
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<td></td>
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<td>2006</td>
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</tr>
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<th>Middle</th>
<th>Late</th>
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</tr>
<tr>
<td></td>
<td>2008</td>
<td>np</td>
<td>14 October</td>
<td>14 October</td>
</tr>
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Table 3.1. Planting and harvest dates at Wooster and Hoytville, Ohio from 2006 to 2008. np = Not planted due to continuous high soil moisture during late April until mid May 2008.
<table>
<thead>
<tr>
<th>Year</th>
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<th>Bt Event</th>
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<th>Maturity GDDs</th>
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<td>Non Bt</td>
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<td></td>
<td>Dekalb DKC63-80</td>
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<td>2790</td>
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</tbody>
</table>

Table 3.2. Maize hybrids planted at Wooster and Hoytville, Ohio from 2006 to 2008
Maize was exposed to natural infestation of European corn borer populations. Sampling for pests began in a plot as plants from the same planting date emerged. Egg masses were counted weekly during both the first and second generation flight of European corn borer. Eggs masses were counted on all leaves of whorl-stage plants for first generation, and three leaves above and below the ear leaf during the second generation flight. Egg masses were counted on 10 randomly selected plants in rows 3 and 10 (5 plants from each row).

Assessment of foliar injury by first generation corn borers was done by counting the number of plants showing windowpane feeding and shot holes on emerging foliage on 20 randomly selected plants. Second-generation borer injury was determined by splitting stalks after physiological maturity and measuring tunnel length (centimeters). Stalks of 10 plants (five from each row 3 and 10) were taken from each plot by excising the plants at the base with corn knives. The stalks were brought to the laboratory and dissected longitudinally. The proportions of plants infested with corn borer larvae, the cumulative lengths of larval tunnels in the stalks, and number of tunnels per stalk were recorded. The proportion of stalks broken due to corn borer damage was also recorded. The broken stalks due to corn borers were identified by checking for frass and tunneling. At each dissecting period, the number of live larvae found in a stalk was recorded. The injury estimates were recorded from the two middle rows so that plant populations and grain yield could be recorded from those rows after physiological maturity.

Grain yield data

After physiological maturity, the plant population was recorded for each plot on 3 meter section of 2 middle rows. Plant populations were determined during the later part
of grain filling by counting plants within the two center rows of each plot. Plots were hand harvested by collecting ears from 3 meter sections of 2 middle rows of each treatment per replication. The dates of harvesting during the three years of study are shown in Table 1. Bulk yields were measured by weighing the shelled dry grain production in 2006 and unshelled ears during 2007 and 2008 from a 3-m row section per treatment per replication. Grain moisture content for each treatment was determined using a Seedburo1 Model GMA 128 Grain Moisture Analyzer (Seedburo Equipment Co., Chicago, IL). Grain yields were adjusted at respective moisture levels for each treatment.

Statistical analysis

Data on percentage of infested plants, broken stalks and damaged ears were transformed using the arcsine square root \([\text{arcsine} \sqrt{(\text{percent } x/100)}]\) to stabilize variance (Fry, 1993). Counts of egg masses and stem tunneling were transformed using log \((x+1)\) transformation (Quinn and Keough 2002). Data on egg masses were analyzed as repeated measure designs using repeated statement in mixed model procedure (PROC MIXED) (SAS Institute, 2003). The procedure adjusted for the serial autocorrelation among the repeated samples on each sampling date (Littell et al. 1996). The Akaike’s Information Criterion (AIC) (Akaike 1974) was used in selecting the covariance structure that best modeled the egg mass data. A repeated measure was performed with heterogeneous autoregressive or Toeplitz covariance structures, which modeled the repeated measure error structure better than other covariance structures. In the mixed model procedures, replication and replication × planting date were considered random while planting date, hybrids and sampling dates were fixed effects. The analysis tested
significance for the following treatment effects: planting dates, hybrids, sampling dates and the interaction between planting dates × hybrids × sampling dates.

The analyses of the data on foliar and stalk damage, ear damage, and grain yield were performed with PROC MIXED in SAS (SAS Institute 2003) to generate Restricted Maximum Likelihood (REML) variance component estimates. Planting dates and hybrids were considered fixed. Replications and replication × planting date were assumed to be random effects. Multiple comparisons were made on least square means of the fixed effects using the PDIFF option of the LSMEANS statement. All the comparisons were based on Fisher’s protected least significant difference and considered significant at P-value of $\alpha = 0.05$. To compare long vs. short season and transgenic vs. non transgenic hybrids, contrast analysis was performed using CONTRAST option within PROC MIXED.

To investigate the relationship between egg masses, stalk tunneling length, and number of live larvae, regression analyses was performed with egg mass as an independent variable and tunneling and number of larvae as dependent variables. Other factors (number of stalk tunnels, percentage of broken stalks and percentage of infested stalks) we omitted from regression analysis because of high correlation with tunneling length and number of larvae per plant (Table 5). Relationship between egg masses and leaf injury was not determined due to extremely low densities throughout the study (Fig. 2).

The relationship between maize grain yield, European corn borer damage (stalk tunneling length; number of stalk tunnels; percentage leaf injury; percentage of broken stalks; percentage of infested stalks) and growing degree days was investigated using
multiple regression analysis, with yield as the dependent variable and above-mentioned European corn borer damage including growing degree days as independent (predictor) variables. The best-fit models were selected using stepwise procedures and the best subsets regression in Minitab® Release 15 (Minitab Inc., 2006). The selection criteria were based on adjusted coefficient of determination ($r^2$), mean square error (MSE), and Mallow’s Cp (Mallow 1973). The selection was based on small mean square error (MSE), highest adjusted $r^2$ values, and a smallest Cp values (Fry 1993). Multicollinearity was diagnosed by assessing the bivariate correlations and the variance inflation factor (VIF) to identify predictor variables that could be correlated. Variance inflation factor values greater than ten suggest severe multicollinearity (Chatterjee and Hadi, 2006).

3.4 RESULTS

Moth flight pattern

The moth flight pattern showed bivoltine generations during the three years of this study (Fig. 3.1). The first moth flight had its peaks in June, with the populations declining during July. The second moth flight peaked in August and declined towards the end of September or early October. In 2006, the numbers of moths during the first flight were slightly higher at Wooster than at Hoytville (Fig. 3.1A). At both locations the number of moths caught during the second flight were similar, being about half compared to the number of moths during the first flights (Fig. 3.1A).
In 2007, the number of moths per trap during the first flight was higher at Hoytville than at Wooster (Fig. 3.1B). In contrast, the number of moths caught during the second flight at Wooster was much higher than in Hoytville, with a sharp peak (95 moths) through the last week of August (Fig. 3.1B). The number of moths caught during the first flight was almost half lower than in 2006 at both locations (Fig. 3.1A and B). Moths trapped during the second flight in 2007 was six times higher in number than in 2006 for each location (Fig. 3.1B).

In 2008, the trap located at Hoytville caught only two moths during the entire season (data not presented). Because moths laid eggs masses on maize plants at Hoytville in 2008 the poor moth catch could be due to the fault in trap, probably having used an expired lure. The number of moths caught at Wooster during the first flight was comparable to that in 2006 (Fig. 3.1C). Almost twice the numbers of moths were caught in the second flight compared to the first flight.
Fig. 3.1. European corn borer moth flight patterns at the Northwest Research station near Hoytville and Wooster Campus in (A) 2006, (B) 2007 and (C) 2008. The trap located at Hoytville caught only two moths during the entire season of 2008 (data not presented).
Egg mass density and ovipositional preferences

Because of the different planting dates, vegetative growth stages were available from early June through mid-August, while reproductive stages were present from mid-July to late August. These differences provided a range of plant growth stages for European corn borer oviposition throughout the growing season (Fig. 3.2). Egg mass densities did not differ significantly between transgenic (Bt) and non-transgenic hybrids throughout this study indicating no preference in moth oviposition between these two treatments. Because there were no significant differences in egg mass density between Bt and non Bt maize hybrids, means were averaged across hybrids to show ovipositional preferences on different planting dates over time during the three years (Fig. 3.2).

The numbers of egg masses from first flight moths were higher on earliest planted maize (late April/early May) than those planted on mid May and early June over three years at both locations (Fig. 3.2). However, significant differences in egg mass density from first flight moths were observed only at Wooster in 2006 and 2007 (Fig. 3.2B and D) on 6 July and 27 June, respectively. Eggs masses for first generation moths were not observed at Hoytville during 2008 because early planting was not done due to heavy soil moisture during late April to mid-May.

In 2006, higher egg mass densities from second generation moths were observed on late planted maize at Hoytville, which resulted in significant differences among planting date (F = 5.30; df = 2, 34; P = 0.001) (Fig. 3.2A, Table 3.3). Egg mass density was not significantly affected by planting date × hybrid (F = 0.73; df = 6, 285) but by hybrid (F = 3.48, df = 3, 209; P = 0.017), sampling date (F = 31.93; df = 7, 75; P = <0.0001), planting date × sampling date (F = 8.90; df=14, 75; P <0.0001) and sampling
date × hybrid interactions (F = 1.83; df = 21, 75 P = 0.031). A comparison of least square means using planned orthogonal contrasts showed that the significant difference between hybrids observed at Hoytville was attributed to higher number of eggs masses laid on full season hybrids (F = 6.31; df = 1, 27; P = 0.021) and was not due to transgenic hybrid effects because no significant differences were found between Bt and no-Bt hybrids (F = 0.09; df = 1, 27; P = 0.768). A multiple comparison of least square means for planting date × sampling date interaction indicated that egg mass densities at Hoytville were significantly higher on early planted maize than middle and late plantings on 23 July (Fig. 2A). In contrast, significantly more eggs masses were laid on late planted maize than early and middle plantings during the peak of 23 August (Fig. 3.2A). From 4 to 9 August, no significant differences in egg masses densities between middle and late plantings were observed. No significant differences between early and middle plantings were detected from 14 August to 9 September when counting was terminated.

At Wooster, the mean egg mass density in 2006 was significantly greater on early planted maize than middle (23 May) and late plantings (F = 3.78; df = 2, 285; P = 0.024) (Fig. 2B, Table 3.3). Significant differences in egg mass density were also observed among sampling dates (F = 17.09; df = 7, 285; P = <0.0001) and planting date × sampling date (F = 4.52; df = 14, 285; P <0.0001) but not among hybrids (F = 0.51; df = 3, 285; P = 0.675), planting date × hybrid (F = 0.73; df = 6, 285; P = 0.629) and sampling date × hybrid interactions (F = 21; 285; F= 0.85; P = 0.663). Similar trends in egg mass population dynamics were observed at Wooster where significantly higher densities occurred on earlier planted maize than from middle and late plantings during mid-July (Fig. 3.2B). Less separation occurred in number of eggs masses laid during the first week
of August (Fig. 3.2B). Significantly more eggs masses were laid on late planted maize during 15 and 28 of August than on early and middle plantings.

At both locations the highest peak of egg masses from second generation occurred when maize plants reached silking (R1) or blister stages (R2) for each planting date treatment (Fig 3.2A and B). The egg mass density typically showed a decline when maize from each planting reached R3 (milk) stage.

In 2007, statistical tests showed significantly higher number of egg masses on late planted maize than middle and early plantings at Hoytville (F = 7.24; df = 2, 12; P = 0.0088) and at Wooster (F = 14.36; df = 2, 12; P <0.0001) (Table 3, Fig. 3.2C and D). The lowest numbers of eggs were obtained from mid-May planted maize at both locations. Egg mass density varied among sampling dates at Hoytville (F = 4.35, df = 11, 45 P = 0.0002) and Wooster (F = 3.63; df = 12, 45; P <0.0001).
Fig. 3.2. Mean egg masses per plant during first and second flight periods at Hoytville and Wooster Campus from 2006 to 2008. Letters associated with sampling date indicate that a significant difference was obtained, with planting date means having the same letter not significantly different (Fisher protected LSD, α = 0.05). R1 = green silk stage; R2 = brown silk (blister) stage.
Interactions were observed between sampling dates × planting dates at Hoytville (F = 2.98; df = 22, 45; P = 0.001) and at Wooster (F = 2.57, df = 6, 45; P = 0.019). At Hoytville no significant differences were detected between hybrids (F = 0.73; df = 3, 150; P = 0.537), planting date × hybrid (F = 1.18; df = 6, 150, P = 0.322) and sampling date × hybrid (F = 1.47; df = 33, 45; P = 0.114) interactions. At Wooster, no significant differences between hybrids were observed (F = 1.75; df = 3, P = 0.156) but planting date × hybrid (F = 2.57; df = 6, 465; P = 0.019) and sampling date × hybrid (F = 1.60; df = 36, 465; P = 0.018) interactions were significant Fig. 3.2C&D).

General patterns of egg mass density in the 2007 experiment were similar to those in 2006. A significantly higher number of egg masses was laid on early planted maize than middle and late plantings during 26 July at Hoytville (Fig. 3.2C) and 19 July at Wooster (Fig. 3.2D). In contrast during the middle of season, significantly more egg masses were laid on late planting than early and middle plantings as shown by the highest peak on 7 August at Hoytville and 21 August at Wooster (Fig. 3.2C and D). From the end of July until egg mass sampling was stopped at the end of September, no significant differences in egg mass densities between early and middle plantings were observed except at Wooster on 21 August (Fig. 3.2C and D). At each location, more egg masses laid by second generation moths occurred when maize plants reached reproductive stages (silking (R1) or blister stages (R2) (Fig 3.2C and D) and declined at the beginning of the R3 (milk) stage.
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<th>Year</th>
</tr>
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</tr>
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</tr>
<tr>
<td></td>
<td>Middle</td>
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</tr>
<tr>
<td></td>
<td>Late</td>
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</tr>
<tr>
<td>Wooster</td>
<td>Early</td>
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</tr>
<tr>
<td></td>
<td>Middle</td>
<td>0.03±0.004b</td>
</tr>
<tr>
<td></td>
<td>Late</td>
<td>0.04±0.006ab</td>
</tr>
</tbody>
</table>

Table 3.3. Mean egg masses (±SE) per plant for each planting date average across sampling date and hybrid. *np* = Not planted due to continuous high soil moisture during late April until mid May 2008. Means with the same letter on a column for each location are not significantly different (Fisher protected LSD (α = 0.05)).
In 2008, maize planted on 22 May at Hoytville had numerically more egg masses than early June planted maize during July, but this difference was not significant ($F = 3.13; df = 1; 142; P = 0.079$) (Fig. 3.2E) (Table 3.3). There were no significant differences in egg mass density among hybrids ($df = 3, 142; F = 1.75, P = 0.156$), planting date × hybrid ($F = 0.95, df = 3, 142; P = 0.420$), planting date × sampling date ($F = 0.33; df = 8, 142; P = 0.951$) or by sampling date × hybrids ($F = 1.36, df = 24, 142; P = 0.136$) interactions; however, significance was observed between sampling date ($df = 8, 142; F = 2.75, P = 0.007$). Averaged across planting dates and hybrids, the number of egg masses sampled on 16 July (first peak) was significantly lower ($t = -2.48; df = 142; P = 0.0144$) than those sampled on 21 August (last peak). The last peaks of egg masses from both plantings happened when maize plants were reaching reproductive stages (Fig. 3.2E).

At Wooster, significantly more egg masses were deposited on late planted maize than middle and early planting ($F = 5.50; df = 2, 321; P < 0.005$) (Fig. 3.2F, Table 3.3). The lowest mean egg mass density was recorded on middle planting. Additional tests showed significant differences between sampling date ($df = 8, 321; F = 8.19, P < 0.0001$) and planting date × sampling date interaction ($F = 3.93; df = 16, 321; P < 0.0001$) but not between hybrids ($F = 0.41; df = 3, 321; P = 0.749$), planting × hybrid ($F = 0.97; df = 6, 321 P= 0.443$) and sampling date × hybrid interactions ($F = 1.23 df = 24; 321 P = 0.212$) (Fig. 3.2F). As in 2006 and 2007, the higher numbers of eggs masses at Wooster occurred when plants reached silking (R1) or blister (R2) stages (Fig. 3.2F. Significantly more egg masses were laid on early and middle plantings on 18 July than late planted.
However, late planted maize had significantly more egg masses during the peak of 21 August than early and middle plantings (Fig. 3.2F).

**Leaf and stalk injury**

*Leaf injury.* In 2006, significant planting date × hybrid interaction occurred at both Hoytville ($F = 2.44; df = 6, 27; P = 0.05$) and Wooster ($F = 4.05; df = 6, 27; P < 0.0003$) Fig. 3.3A and B). A reduction in percentage of plants with injured leaves was observed on later planted maize than early planting at Hoytville and Wooster (Fig. 3.3A and B). Leaf injury was caused by larvae from first generation moths which are known to deposit eggs on tallest and earliest planted corn. A significant treatment effect on the percentage of plants with leaf injury was detected between hybrids at Hoytville ($F = 7.47; df = 3, 27; P < 0.0009$) and Wooster ($df = 3, 27; F = 11.46, P < 0.0001$). This difference was due to significantly lower leaf injury on Bt hybrids than non-Bt hybrids at both locations ($Hoytville: F = 22.40; df = 1, 27; P = < 0.0001$, Wooster: $F = 34.31; df = 1, 27; P < 0.0001$). No significant hybrid effects were detected between short and full season hybrids at either ($Hoytville: F = 0.01; df = 1, 27; P = 0.94$, Wooster: $F = 0.04; df = 1, 27; P = 0.839$).

Similar results were observed in 2007 (Fig 3.3C and D). The interaction among planting date × hybrid was significant at Hoytville ($F = 2.71 df = 6, 24 P = 0.038$) and Wooster ($F = 6.27; df = 6, 25; P = 0.0004$). The percentage of plants with leaf injury at each location was higher on early planted maize than middle and late planting at both locations (Fig. 3.3C and D).
Fig 3.3. Mean percentage of plants with leaf injury caused by European corn borer larvae from 2006 to 2008 at Hoytville and Wooster. Means with the same letter within a planting date are not significantly different (Fisher protected LSD ($\alpha = 0.05$). PD = planting date; PDH = planting date by hybrid interaction. *, ** and *** denote significance at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$ respectively. S = short season and L = full season.
Injury to leaves in 2007 differed significantly among hybrids at Hoytville (F = 30.36; df = 3, 24; P<0.000) as well as Wooster (F = 19.65; df = 3, 25; P<0.0001). The difference was again due to significantly higher percentage injured plants on non-Bt hybrids compared to Bt hybrids (Hoytville: F = 78.33; df = 1, 24; P = 0.0001, Wooster: F = 57.36; df = 1, 25; P = 0.0001). Multiple comparisons showed that the increase in leaf injury on full season hybrids occurred on early and middle planted non Bt hybrid at Hoytville (Fig. 3.3C). At Wooster significantly higher leaf injury occurred on early planted maize (Fig. 3.3D).

In 2008, statistical tests showed a high planting date × hybrid interaction effects on leaf injury at Wooster (F =7.28; df = 6, 33; P< 0.000) but not at Hoytville (F = 2.61; df = 1, 16; P= 0.092). Only two plantings were done at Hoytville and maize planted on 22 May had significantly higher leaf injury than early June planted maize (F = 6.61; df = 1, 16; P = 0.022) (Fig. 3E). At Wooster early planted maize had a higher percentage of plants with foliar injury than middle and late plantings (Fig. 3.3F). Examination of planting date by hybrid interaction showed that leaf injury did not differ among non-Bt maize hybrids at Hoytville (Fig. 3.3E). A Wooster, significantly higher leaf injury occurred on full season hybrid on middle planting compared to short season hybrid (Fig. 3.3F).

**Stalk tunneling.** Throughout this study stalk tunneling from European corn borer larval which was mostly caused by second generation European corn borer occurred on non-transgenic hybrids (Fig. 3.4), but none on the Bt transgenic hybrid. In 2006, planting date × hybrid interaction effects were not significant at either location (Hoytville: F = 0.62; df = 6, 33; P=709, Wooster: F =1.26; df = 6, 33; P< 0.303). Tunneling length per
stalk was also not significantly affected by date of planting at either location (Fig. 3.4A and B) (Hoytville: $F = 1.19; \ df = 2, \ 33; \ P = 0.317$, Wooster: $F = 1.04; \ df = 2, \ 33; \ P = 364$). Significance between hybrids occurred (Hoytville: $F = 36.46; \ df = 3, \ 33; \ P < 0.0001$, Wooster: $F = 349.94, \ df = 3, \ 33; \ P < 0.0001$). At both locations the Bt gene provided complete protection from stalk tunneling injury in all the plantings as confirmed by highly significant differences between Bt and non Bt hybrids (Hoytville: $F = 108.95; \ df = 1, \ 33; \ P < 0.0001$, Wooster: $F = 1046.53, \ df = 1, \ 33; \ P < 0.0001$). A comparison between short season and full season hybrids showed no significant effects on stalk tunneling at Hoytville ($F = 0.01; \ df = 1, \ 33; \ P = 0.974$). Multiple comparisons showed that late planted full season non-Bt hybrid had significantly longer tunneling than the short season hybrid at Wooster (Fig. 3.4B).
Fig. 3.4. Mean tunneling length per stalk among hybrids planted on different dates from 2006 to 2008 at Hoytville and Wooster. Letters associated with planting date indicate that a significant difference occurred, with hybrid means having the same letter not significantly different (Fisher protected LSD, $\alpha = 0.05$). PD = planting date; PDH = planting date by hybrid interaction; ** and *** denote significance at $P \leq 0.01$ and $P \leq 0.001$. S = short season and L = full season.
In 2007, significant planting date × hybrid interaction was observed at both locations (Hoytville: $F = 8.35; \text{df} = 6, 18; \text{P} = 0.0002$, Wooster: $F = 5.65; \text{df} = 6, 35; \text{P} = 0.0003$). An increase of 52% in stalk tunneling occurred at Wooster and 62% at Hoytville on maize planted in early June than when planted earlier in May. Significant differences were also detected among hybrids at Hoytville ($F = 112.73; \text{df} = 3, 18; \text{P}<0.001$) and at Wooster ($F = 357.35; \text{df} = 3, 35; \text{P}<0.001$). A complete reduction in stalk tunneling was observed on Bt hybrids (Hoytville: $F = 1069.23, \text{df} = 1, 18; \text{P}<0.0001$, Wooster: $F = 1069.23, \text{df} = 1, 35; \text{P}<0.0001$) compared to non-Bt hybrids (Fig. 3.4C and D). There was significantly more stalk tunneling on full season hybrids compared to short season hybrids planted on 8 June at Hoytville ($F = 6.77; \text{df} = 1, 35; \text{P} = 0.018$) but not at Wooster ($F = 0.64; \text{df} = 1, 35; \text{P} = 0.4301$) (Fig. 3.4C).

In 2008, date of planting had a significant effect on hybrid response to tunneling injury as shown by a significant planting date × hybrid interactions at both locations (Hoytville: $F = 7.35; \text{df} = 3, 14; \text{P}=0.0034$, Wooster: $F = 7.35; \text{df} = 6, 33; \text{P}<0.0001$ (Fig. 3.4E and F). At an increase of more than 100% in stalk tunneling occurred on maize planted early June than when planted early May at Wooster (Fig. 3.4F). At each location, tunneling length differed significantly among hybrids (Hoytville: $F = 28.52; \text{df} = 3, 16; \text{P}<0.0001$, Wooster: $F = 104.55; \text{df} = 3, 33; \text{P}<0.0001$) (Fig. 3.4E and F). These differences again were because of a higher reduction in feeding on Bt hybrids at Hoytville ($F = 85.38; \text{df} = 1, 16; \text{P}=<0.0001$) and Wooster ($F = 312; \text{df} = 1, 33; \text{P}<0.0001$). Multiple comparisons showed that short season non-Bt hybrid planted in 22 May at Hoytville had longer tunneling than the full season hybrid (Fig. 3.4E). In contrast when maize was planted in 9 June full season non-Bt hybrid had longer tunneling than
the short season hybrid (Fig. 3.4E). At Wooster, short season non-Bt hybrid had longer tunneling than the full season hybrid when maize was planted in 9 June (Fig. 3.4F).

Generally an increase in egg mass density resulted in an increase in number of larvae and stalk tunneling length (Table 3.4). Both stalk tunneling length and number of larvae were not significantly influenced by egg mass density at either location in 2006, but significance occurred in 2007 (Table 3.4). An inconsistency occurred where more egg masses laid on late planted maize in 2006 at Hoytville was associated with a decrease in tunneling length, consequently a negative regression slope. In 2008 stalk tunneling and number of larvae per plant were independent of egg mass density at Hoytville but increased significantly with an increase in egg masses at Wooster. The number of stalk tunnels, percentage of broken stalks and percentage of infested stalks were highly correlated with tunneling length and number of larvae per plant (Table 3.5).
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<th>Slope ± SE</th>
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Table 3.4. Relationship between mean egg masses per plant, mean tunneling length (cm) and number of larvae per stalk for second generation at two locations from 2006 to 2008. §Tunneling lengths (cm) were averaged across hybrid and planting date and eggs masses across planting date, sampling date and hybrid
Number of live larvae. In 2006, no significant planting date × hybrid interaction occurred at either Hoytville (F = 0.17; df = 6, 27, P = 0.982) or Wooster (F = 1.41; df = 6, 27, P = 0.249). The number of second generation live larvae per stalk recovered after stalk splitting did not vary among planting date either at Hoytville (F = 0.20; df = 2, 6; P = 0.823) or Wooster in 2006 (F = 1.30; df = 2, 6; P = 0.339) (Fig 3.5A and B). The number of larvae per stalk was significantly affected by hybrid at both locations (Hoytville: F = 239.98; df = 3, 27; P <0.0001, Wooster: F = 66.36; df = 3, 27; P <0.0001). Larvae were recovered only from non-Bt hybrids with virtually none from Bt hybrids, which explains a high significant difference between the two treatments at both Hoytville (F = 706.42; df = 1, 27; P <0.0001) and Wooster (F = 195.11; df = 1, 27; P <0.0001). A comparison between short season and full season hybrids showed significantly more live larvae per stalk on short season hybrids planted on 28 April at Hoytville (Fig. 3.5A). In contrast significantly more live larvae were recorded on full season non-Bt hybrid planted on 7 June at Wooster (Fig. 3.5B).

In 2007, a planting date × hybrid interaction was detected at Hoytville (F =3.84; df = 6 22; P = 0.009) but not at Wooster (F = 1.31; df = 6, 35; P = 0.280), indicating that the differences in the number of larvae observed between hybrids were affected by planting date at Hoytville but not at Wooster. More than twice the number of larvae was recovered from stalks of late planted maize than early planted maize at Hoytville (Fig. 3.5C). Unlike at Hoytville, there were significantly more larvae per stalk recovered on middle planting than early and late plantings at Wooster (Fig. 3.5D). The number of larvae per stalk increased more than 100% on middle planting compared with earlier planting at Wooster in 2007. A significant difference in the number of larvae per stalk
was observed between hybrids at both locations (Hoytville: $F = 52.82$; $df = 3, 22$; $P < 0.0001$, Wooster: $F = 28.28$; $df = 3, 35$; $P < 0.0001$). Like in 2006, no larvae were recovered from Bt hybrids; therefore the significant difference was due to high numbers obtained from non-Bt hybrids (Hoytville: $F = 153.88$; $df = 1, 22$; $P < 0.0001$, Wooster: $F = 84.49$; $df = 1, 35$; $P < 0.0001$) (Fig. 5C and D). Hybrid maturity did not significantly affect the number of larvae recovered from stalks at Wooster: $F = 0.23$; $df = 1, 35$; $P = 0.633$). When maize was planted on 7 May at Hoytville significantly higher number of larvae was observed on the short season non-Bt hybrid but when planted on 8 June, the number of larvae was lower than the full season non-Bt hybrid (Fig. 3.5C).

In 2008, significant planting date × hybrid interactions were detected (Hoytville: $F = 4.85$; $df = 3, 16$; $P = 0.014$, Wooster: $F = 3.32$; $df = 6, 27$; $P = 0.014$), revealing that hybrid treatment differences were affected by planting date for the number of larvae per stalk at both locations. The non-Bt hybrids had significantly higher number of live larvae than Bt hybrids at both locations (Hoytville: $F = 25.44$; $df = 1, 16$; $P = 0.0001$, Wooster: $F = 75.34$; $df = 1, 27$; $P < 0.0001$). Multiple comparisons using planting date x hybrid interaction showed that when maize was planted on 22 May at Hoytville significantly higher number of larvae was observed on full season non-Bt hybrid but when planted on 9 June, the number of larvae was lower than the short season non-Bt hybrid (Fig. 3.5E). At Wooster, the short season non-Bt hybrid had significantly more larvae compared to the short season hybrid when maize was planted on 21 May (Fig. 3.5F).
Fig. 3.5. Mean number of live ECB larvae recovered after splitting maize stalks at Hoytville and Wooster from 2006 to 2008. Letters associated with planting date indicate that a significant difference occurred, with hybrid means having the same letter not significantly different (Fisher protected LSD, $\alpha = 0.05$). PD = planting date; PDH = planting date by hybrid interaction; * and ** denote significance at $P \leq 0.05$ and $P \leq 0.01$. S = short season and L = full season.
**Percentage of tunneled stalks.** In 2006, no planting date × hybrid interaction was detected at Hoytville (F = 1.31; df = 6, 27; P = 0.285) but at Wooster the significant interaction (F = 2.78; df = 6, 27; P = 0.025) showed a decrease in percentage tunneling as planting was delayed (Fig. 3.6B). The percentage of stalks tunneled by European corn borer larvae was not significantly influenced by date of planting at Hoytville (F = 1.47; df = 2, 6; P = 0.302). However the percentage of tunneled stalks varied significantly among hybrids at Hoytville (df = 3, 27; F = 125.36; P <0.0001) and Wooster (F = 125.34; df = 3, 27; P<0.0001). At each location, the percentage stalks tunneled in the non-Bt maize hybrids was significantly higher than Bt maize hybrids (Hoytville: F =375.95; df = 1, 27; P < 0.0001, Wooster: F = 375.26; df = 1, 27; P<0.0001). No significant differences occurred in percentage tunneling between full season and short season hybrids at either location (Hoytville: F = 0.11; df = 1, 27; P = 0.745, Wooster: F = 0.70; df = 1, 27; P = 0.407).

In 2007, there was no significant planting date × hybrid interaction effects at Hoytville (df = 6 F = 1.46, P = 0.247), but significance occurred at Wooster (df = 6, F = 4.13, P = 0.003). The percentage of stalks tunneled was not significantly affected by planting date at Hoytville (F = 1.46; df = 2, 6; P =0.304), but the percentages of stalks tunneled at Wooster was 38% lower on early planted maize (Fig. 3.6D). Percentage of stalks tunneled was significantly affected by hybrid at Hoytville (F = 58.72; df = 3, 18; P <0.0001) and also at Wooster (df = 3, 35 = F =216.00, P< 0.0001). Assessment of planting date by hybrid interaction revealed that a significant reduction in percentage of stalks tunneled on non-Bt maize occurred on early planted maize than late plantings (P ≤ 0.05). The non-Bt hybrids had a significantly higher percentage of tunneled stalks than
Bt hybrids at both locations (Hoytville: $F = 176.13; df = 1, 18; P < 0.0001$, Wooster: $F = 647.82; df = 1, 35; P < 0.0001$). At both locations, no significant differences were observed between full season and short season hybrids (Hoytville: $F = 0.01; df = 1, 18; P = 0.9167$, Wooster: $F = 0.64; df = 1, 35; P = 0.477$).
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Table 3.5. Linear correlation coefficients among stalk tunneling, number of tunnels, incidence of broken stalks, infested stalks and number of larvae per stalk in field experiments at Wooster 2006 to 2008. ** and *** denote significance at $P \leq 0.01$ and $P \leq 0.001$ respectively. §Data not collected in 2006.
Fig. 3.6. Mean percentage of infested stalks tunneled by ECB larvae at Hoytville and at Wooster from 2006 to 2008. Means with the same letter within a planting date are not significantly different (Fisher protected LSD (α = 0.05). PD = planting date; PDH = planting date by hybrid interaction; *, ** and *** denote significance at P ≤ 0.05, P ≤ 0.01 and P ≤ 0.001 respectively. S = short season and L = full season.

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In 2008, a significant planting date × hybrid interaction occurred at Wooster (F = 5.25; df = 6, 36; P = 0.001) but not at Hoytville (df = 3, 14; F = 2.72, P = 0.084). The percentage of stalks tunneled increased more than 100% on late planted maize compared to early planting at Wooster (Fig. 3.6F). Although no significant differences were observed at Hoytville (df = 1, 14; F = 0.06; P=0.805) (Fig. 3.6E). The significant effect due to hybrid was detected at Hoytville (df = 3, F = 28.84, P<0.0001) and Wooster (df = 3, 36; F = 118.05; P= 0.005). This difference was due to a significantly higher percentage of tunneled stalks on non-Bt than Bt hybrids at both Hoytville (df = 1, 14; F = 86.53; P<0.0001) and Wooster (F = 351.64; df = 1, 36; P<0.0001). The percentage of stalk tunneled increased as planting was delayed and showed a significantly higher percentage on the short season non hybrid on late planted maize compared to the full season one (Fig. 3.6F). There was no significant difference in percentage of stalk tunneled between short season and full season hybrids at Hoytville (F = 0.00; df = 1, 14; P =1.000).

*Number of tunnels per stalk.* The number of tunnels per stalk was only recorded in 2007 and 2008. There was a significant planting date × hybrid interaction effects on the number of tunnels at both Hoytville (F = 7.81, df = 6, P = 0.0003) and Wooster (F = 3.05; df = 6, 27; P = 0.021). The number of tunnels per stalk on late planted maize increased more than three-fold at Hoytville and by 53% at Wooster when compared to early planting (Fig. 3.7A and B). Hybrid significantly affected the number of tunnels per stalk at Hoytville (F = 104.05, df = 3, P<0.0001) and Wooster (F = 164.29, df = 3, P<0.0001).
Fig. 3.7. Mean number of stalk tunnels per infested stalks at Hoytville and at Wooster from 2006 to 2008. Means with the same letter within a planting date are not significantly different (Fisher protected LSD (α = 0.05). PD = planting date; PDH = planting date by hybrid interaction; *, ** and *** denote significance at P ≤ 0.05, P ≤ 0.01 and P ≤ 0.001 respectively. S = short season and L = full season.
The interaction indicated that a significant reduction number of stalks on non-Bt maize occurred on early planted maize but increased significantly as planting was delayed (Fig. 3.7A and B). Significant differences observed between hybrids were mostly due to higher number of tunnels obtained from non-Bt than Bt hybrids at either location (Hoytville: F = 291.03, df = 1, 27 P<0.0001, Wooster: F = 488.54; df = 1, 36; P<0.0001). However, to a less extent, the difference could also be due to more tunnels observed on short season hybrids at Hoytville (F = 10.56, df = 1, 27; P = 0.0045) (Fig. 3.7A). No significant difference occurred between full season and short season hybrid at Wooster (F = 1.76; df = 1, 27; P = 0.196) (Fig. 3.7B).

In 2008, a significant planting date × hybrid interaction occurred at both locations (Hoytville: F =17.46 df =3, 14, P<0.0001, Wooster: F = 3.98; df = 6, 36; P = 0.004). Like in 2007, planting date x hybrid interaction showed a significant reduction in number of tunnels on non-Bt maize in early planted hybrids but infestation increased significantly as planting was delayed at Wooster (Fig. 3.7D). The number of tunnels on late planted maize at Wooster increased more than 100% when compared to early planting (F = 8.42, df = 2, 36, P = 0.001). The number of tunnels per stalk was significantly affected by hybrid at Hoytville (F =17.46 df =3, 14, P<0.0001) and Wooster (F =52.9; df = 3, 36; P<0.0001). No tunnels were recorded on Bt hybrids at either location, resulting in significantly more tunnels on non-Bt hybrids (Hoytville: F = 52.26 df = 1, 14, P<0.0001, Wooster: F = 158.32; df = 1, 36; P<0.0001). No significant differences were found between short season and full season hybrids at Wooster (F = 0.24; df = 1, 36; P = 0.628) (Fig. 3.7C and D). At Hoytville, the short season non-Bt hybrid had significantly more tunnels compared to the long hybrid when maize was planted in 9 June (Fig. 3.7C).
Percentage of broken stalks. In 2006, planting date × hybrid interaction was not significant at Hoytville (F = 0.78; df = 6, 33; P = 0.593), but significance was observed at Wooster (F = 2.31; df = 6, 36; P = 0.05). The percentage of broken stalks did not vary significantly among planting dates at Hoytville (F = 1.87; df = 2, 33; P = 0.171), but a significant reduction in percentage of broken stalks was observed on late planted maize compared with middle and early planting at Wooster (Fig. 3.8A). The percentage of broken stalks was significantly affected by hybrid at both Hoytville (F = 52.53; df = 3, 33; P < 0.0001) and Wooster (F = 145.02; df = 3, 36; P < 0.0001). A significant reduction (100%) in broken stalks on Bt hybrids compared to no-Bt hybrids was observed at Hoytville (F = 157.57; df = 1, 33; P < 0.0001) as well as Wooster (F = 432.31; df = 1, 36; P < 0.0001) (Fig. 3.8A and B). The interaction showed that percentage broken stalks decreased as planting was delayed (Fig. 3.8B). The percentage of broken stalks was not significantly influenced by hybrid relative maturity at either locations (Hoytville: F = 0.01; df = 1, 33; P = 0.920, Wooster: F = 0.138; df = 1, 36; P = 0.248).

In 2007, date of planting had a significant influence on hybrid treatment effects, which was confirmed by a significant planting date × hybrid interaction at Hoytville (F = 14.73; df = 6, 22; P < 0.0001) and at Wooster (F = 0.94; df = 6, 27; P = 0.048) (Fig. 3.8C and D). Higher percentage of broken stalks occurred on middle planting than early and late plantings at Hoytville and at Wooster (Fig. 3.8C and D). These differences were mostly due to higher percentage of broken stalks in the non Bt hybrids at Hoytville (F = 791.23; df = 1, 22; P < 0.0001) and also at Wooster (F = 544.39; df = 1, 27; P < 0.0001).
Fig. 3.8. Mean percentage of broken stalks at Hoytville and Wooster from 2006 to 2008. Means with the same letter within a planting date are not significantly different (Fisher protected LSD ($\alpha = 0.05$). PD = planting date; PDH = planting date by hybrid interaction; *, ** and *** denote significance at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$ respectively. S = short season and L = full season.
Multiple comparisons using planting date x hybrid interaction showed that when maize was planted on 7 May at Hoytville significantly higher percentage of broken stalks was observed on full season non-Bt hybrid but when planted on 23 May, the percentage was lower than the short season non-Bt hybrid (Fig. 3.8C). The percentage of broken stalks were numerically higher on full season hybrids compared to short season hybrids at Wooster but not significant (F = 0.51; df = 1, 27; P = 0.483).

In 2008, a planting date × hybrid interaction was significant at Hoytville (F = 3.50; df = 3, 14; P = 0.044), but not at Wooster (F = 1.44; df = 6, 27; P = 0.176). The interaction showed that percentage broken stalks decreased as planting was delayed in the non-Bt hybrids at Hoytville (Fig. 3.8E) but no effects occurred at Wooster (Fig. 3.4F). At each location, percentage of broken stalks varied among hybrids (Hoytville: F = 34.83; df = 3, 14; P < 0.0001, Wooster: F = 60.21; df = 3, 27; P < 0.0001). The difference was due to a significantly higher percentage of broken stalks on non-Bt hybrids than Bt hybrids (Hoytville: F = 104.49; df = 1, 14; P < 0.0001, Wooster: F = 179.15; df = 1, 27; P < 0.0001) (Fig. 8E and F). The percentage of broken stalks were numerically lower on full season hybrids compared to short season hybrids at both locations but not significant (Hoytville: df = 1; 14; F = 0.01, P = 0.933; Wooster: F = 1.44; df = 1, 27; P = 0.240).

**Agronomic Parameters**

*Plant population.* Plant population during the three years of this study was not significantly affected by date of planting except in 2007 at Hoytville (F = 3.68; df = 2, 24; P = 0.0404) and at Wooster (df = 2, 32 F = 4.61 P = 0.0173). Plant population per
hectare ranged from 64,000 – 66,000, 66,000 – 81,000, and 76,000 – 84,000 plants in 2006, 2007, and 2008, respectively. Mean populations of short-season hybrids at Wooster were significantly higher than short season hybrids in 2006 (F = 15.12; df = 1, 33; P = 0.0006) and in 2007 (F = 3.23; df = 1; P = 0.019). No other comparison showed any significant differences in plant population over three years. Harvest moisture for the three years ranged from 17.78 to 27.8 % for the early planting, 20.90 to 29.6 % for the middle planting and 26.3 to 30.02 % for the late planting.

*Grain yield.* In 2006, no significant planting date × hybrid interactions was observed at Hoytville (F = 1.88; df = 6, 27, P = 0.121) nor at Wooster (F = 0.84; df = 2, 27; P = 0.549). Delaying planting until early June resulted in a 26% yield reduction when compared to planting in late April at Hoytville (F = 21.84; df = 2, 27; P<0.0001) (Fig. 3.9A). Multiple comparisons using planting by hybrid interaction showed no significant yield increase or reduction when maize was planted on 24 May at Hoytville compared to early planting (t = 1.71; df = 6; P = 0.1374). At Wooster, the reduction in grain yield caused by late planting (7 June) was 13.5% when compared to early planting (4 May) (F = 10.10; df = 2, 27; P = 0.0005) (Fig. 3.9B). Like at Hoytville, planting maize on 25 May resulted in no significant reduction or increase when compared to early planting at Wooster (t = 1.32; df = 6; P = 0.23). Grain yield did not differ significantly between hybrids at Hoytville (F = 0.89; df = 3, 27; P = 0.457), but significant differences were observed at Wooster (F = 7.46; df = 3, 27; P = 0.0009).

Orthogonal contrasts between hybrid groups showed that the difference was due to higher yields being obtained from full season hybrids compared to short season hybrids (F = 31.82; df = 1, 27; P = <0.0001) at Wooster, although no such differences
were observed at Hoytville (F = 1.52; df = 1, 27, P = 0.228) (Fig. 9A). A multiple comparison of least square means for planting date × hybrid interactions revealed that yield improved when full season hybrids were planted the first week of May. The main effect was due to the full season Bt hybrid DKC 63-81 which significantly out-yielded the short season hybrids DKC 51-46 and DKC 50-20 when planted early May (P ≤ = 0.01) and 22 May ( P = ≤ 0.05), with very little effect when planted early June (Fig. 3.9B). The complete protection of maize from corn borer injury in Bt hybrids did not result in the improvement yields at either locations (Hoytville: F = 0.67; df = 1, 27; P = 0.419, Wooster: F = 1.38; df = 1, 27; P = 0.250) (Fig. 3.9A and B).

Multiple regression analysis showed that the model did not account for any significant variation in yield at Hoytville (F = 2.31; df = 2; r^2 = 0.09; P = 0.111) but a significant difference occurred at Wooster (F = 10.64; df = 2; r^2 = 0.32; P<0.0001). The best predictor model at Hoytville included tunneling length and leaf injury which explained an insignificant 9% of variation in yield. However at Wooster the best predictor model that included tunneling length and growing degree days explained a significant 32% of variation in yield. Growing degree days accounted for the significance in the model at Wooster but not tunneling length (Table 3.6).

In 2007, a planting date × hybrid interaction effect was not detected at Hoytville (F = 1.41; df = 6, 18; P = 0.264), but a significant interaction occurred at Wooster (F = 3.55; df = 2, 27; P = 0.010), indicating that yield differences among hybrids were influenced by date of planting at the latter location. Delayed planting at Hoytville resulted in a significant reduction in grain yield (14%) when compared to middle planting, but not to early planting (F = 16.32; df = 2, 18; P<0.0001) (Fig. 3.9C).
Fig. 3.9. Mean grain yield obtained at Hoytville and Wooster from 2006 to 2008. Letters associated with planting date indicate that a significant difference occurred, with hybrid means having the same letter not significantly different (Fisher protected LSD, $\alpha = 0.05$). PD = planting date; PDH = planting date × hybrid interaction; *, ** and *** denote significance at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$ respectively. S = short season and L = full season.
In contrast, a significant yield reduction (8.4%) at Wooster was associated with maize planted on 25 May (middle planting) \( (F = 6.28; \text{df} = 2, 27; P = 0.006) \) compared to early planting and late planting (Fig. 3.9D). Grain yield differed significantly between hybrids at Hoytville \( (F = 4.34; \text{df} = 3, 18; P = 0.018) \) and at Wooster \( (F = 12.01; \text{df} = 3, 27; P < 0.001) \). Unlike in 2006, protection against second-generation larval injuries by Bt gene translated to statistically significant advantages in yield between hybrid groups tested at Hoytville \( (F = 11.72; \text{df} = 1, 18; P = 0.003) \) and Wooster \( (F = 15.67; \text{df} = 1, 27; P = 0.0005) \).

A comparison of least square means for planting date × hybrid interactions showed that transgenic hybrids improved yields on middle \( (P \leq 0.05) \) and late plantings \( (P \leq 0.05) \) at both locations, but not at the early planting (Fig. 3.9C and D). No significant yield differences were observed between full season and short season hybrids at Hoytville \( (F = 1.12; \text{df} = 1, 18; P = 0.305) \), but significantly higher yields were observed on full season hybrids at Wooster \( (F = 8.13; \text{df} = 1, 27; P = 0.008) \). Similar to 2006, a significant increase in yield from planting full season hybrids occurred when they were planted during early May \( (P \leq 0.05) \) or beginning of last week of May \( (P \leq 0.05) \), but not when planted early June (Fig. 3.9A and B). In 2007 the best fit model at Hoytville included tunneling length and the number of larvae per plant while at Wooster included tunneling length and growing degree days. Statistical analysis showed significance in the models at both Hoytville \( (F = 7.90; \text{df} = 2; r^2 = 0.22; P = 0.014) \) and Wooster \( (F = 13.01 \text{ df} = 2; r^2 = 0.32; P < 0.0001) \). A significant negative linear response in yield was explained only by the number of larvae per stalk at Hoytville while at

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Wooster both growing degree days and tunneling length accounted for the significance (Table 3.6).

In 2008, no significant planting date × hybrid interaction was observed at Hoytville (F = 0.92; df = 3, 12, P = 0.457), but a significant interaction occurred at Wooster (F = 3.33; df = 6, 27; P = 0.014), indicating that hybrid treatment differences were influenced by planting date at Wooster. Grain yield did not differ significantly among planting dates at Hoytville (F = 4.15; df = 1, 12; P = 0.064) (Fig. 3.9E). Similar to 2007, a significant yield reduction (7.5%) at Wooster was associated with maize planted on 21 May (middle planting) compared to early planting, but not to late planting (Fig. 3.9F). Grain yield differed significantly between hybrids at Hoytville (F = 5.70; df = 3, 12; P = 0.012) and Wooster (F = 6.21; df = 3, 27; P = 0.002). The significant yield difference between hybrids was due to higher yields from Bt maize than non-Bt hybrids at both Hoytville (F = 13.38; df = 1, 27; P = 0.003) and Wooster (F = 16.47; df = 1, 36; P = 0.0003).

Multiple comparison within planting dates showed that transgenic maize significantly improved yield at Hoytville (P ≤ = 0.01) when maize was planted on 9 June compared to 22 May (Fig. 3.9E). The same trend was observed at Wooster where late planted transgenic maize significantly (P ≤ = 0.001) out yielded non-Bt corn, but no such differences occurring on earlier planted maize (Fig. 3.9F). No significant difference in grain yield was observed between full season and short season hybrids at either location (Hoytville: F = 1.52; df = 1, 14, P = 0.237, Wooster: F = 1.94; df = 1, 36; P = 0.172 (Fig. 3.9E and F). Reduction in yield at both locations was significantly related to increase in stalk tunnel length in 2008 (Table 3.5). In 2008 the best predictor model at Hoytville
included the number of larvae per plant and percentage of broken stalks. At Wooster the model included tunneling length and percentage of broken stalks. At both locations the model indicated a significant negative linear response of yield to predictor variables (Hoytville: $F = 4.44; r^2 = 0.30; df = 2; P = 0.025$, Wooster: $F = 6.06; df = 2; r^2 = 0.21; P = 0.005$), and explained 30% of the variation in yield at Hoytville and 21.2% at Wooster. A significant reduction in yield was associated with percentage of broken stalks, tunneling length and the number of larvae per plant (Table 3.6).

**Phenology and thermal accumulation time**

The average hybrid GDD values required to reach R1 and R6 during early planting were not consistent with those provided by the seed company (Table 3.7). With exception of short season hybrids grown at Wooster in 2006, measured GDD intervals after planting (AP) to R1 for both maturity groups were greater than the respective company values (Table 3.6). In contrast, measured GDD intervals after planting to R6 were less than those provided by the seed company except the short season hybrids at Wooster in 2006 and at Hoytville in 2007 (Table 3.6). Results of regression analysis of day of planting during the year and growing degree days required for maize to reach physiological maturity (R6) at both locations is shown in Table 3.8. Across the two maturities delayed planting significantly reduced growing degree days at each location.
Table 3.6. Results of multiple-regression showing relationships between grain yield various independent variables associated with variation in yield. ‡Predictor variables not included because of high multicollinearity (>10 VIF values) and/or had higher Cₚ and MSE values with low adjusted r². VIF = variance inflation ratio; MSE = mean square error; Cₚ = Mallow’s Cₚ value. §Grain yields were averaged across hybrid and planting date.

<table>
<thead>
<tr>
<th>Location, year</th>
<th>Predictor variable‡</th>
<th>Coefficient ± SE</th>
<th>t</th>
<th>P</th>
<th>MSE</th>
<th>Adj. r²</th>
<th>Cₚ</th>
<th>VIF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hoytville, 2006</td>
<td>Intercept</td>
<td>11.22 ± 0.28</td>
<td>40.44</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tunnel length</td>
<td>-0.95 ± 0.51</td>
<td>-1.88</td>
<td>0.067</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Foliar damage</td>
<td>1.778 ± 0.927</td>
<td>1.91</td>
<td>0.062</td>
<td>1.35</td>
<td>0.05</td>
<td>3.30</td>
<td>1.45</td>
</tr>
<tr>
<td>Hoytville, 2007</td>
<td>Intercept</td>
<td>12.17 ± 0.28</td>
<td>44.13</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tunnel length</td>
<td>0.13 ± 0.09</td>
<td>1.44</td>
<td>0.159</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>No of larvae</td>
<td>-2.67 ± 1.05</td>
<td>-2.54</td>
<td>0.016</td>
<td>1.28</td>
<td>0.17</td>
<td>0.60</td>
<td>3.23</td>
</tr>
<tr>
<td>Hoytville, 2008</td>
<td>Intercept</td>
<td>7.37 ± 0.18</td>
<td>41.27</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tunnel length</td>
<td>-0.40 ± 0.14</td>
<td>-2.90</td>
<td>0.009</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>% Broken stalks</td>
<td>0.05 ± 0.02</td>
<td>2.09</td>
<td>0.049</td>
<td>0.68</td>
<td>0.23</td>
<td>3.40</td>
<td>3.53</td>
</tr>
<tr>
<td>Wooster, 2006</td>
<td>Intercept</td>
<td>2.76 ± 4.51</td>
<td>0.61</td>
<td>0.545</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tunnel length</td>
<td>-0.52 ± 0.42</td>
<td>-1.22</td>
<td>0.230</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>GDDS</td>
<td>0.004± 0.002</td>
<td>1.88</td>
<td>0.047</td>
<td>1.33</td>
<td>0.08</td>
<td>2.83</td>
<td>1.02</td>
</tr>
<tr>
<td>Wooster, 2007</td>
<td>Intercept</td>
<td>-0.91 ± 4.99</td>
<td>-0.18</td>
<td>0.856</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Tunnel length</td>
<td>-1.20 ± 0.31</td>
<td>-3.98</td>
<td>&lt;0.0001</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>GDDS</td>
<td>0.01 ± 0.002</td>
<td>2.62</td>
<td>0.012</td>
<td>1.03</td>
<td>0.38</td>
<td>2.9</td>
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<tr>
<td>Wooster, 2008</td>
<td>Intercept</td>
<td>11.53 ± 0.23</td>
<td>52.63</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>No of larvae</td>
<td>-0.83 ± 0.42</td>
<td>-1.99</td>
<td>0.05</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>% Broken stalks</td>
<td>-0.02 ± 0.01</td>
<td>-2.03</td>
<td>0.049</td>
<td>1.13</td>
<td>0.16</td>
<td>2.2</td>
<td>1.12</td>
</tr>
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</table>
Table 3.7. Effect of planting date on thermal time phenological events within frosted† and unfrosted environments.

DAP = Days after planting to R1 (silking) and R6 (black layer or physiological maturity).

‡GDD values in parenthesis are those stated by seed supplier. np = Not planted due to continuous high soil moisture during late April until mid May 2008.

†The GDDs values represent means across two hybrids for each short and full season relative maturity ratings (see Table 2).

†Full season hybrids failed to reach physiological maturity (R6) before the first occurrence of frost (-2.2°C or less) when planted early June over three years. Frost dates: 13 October 2006, 13 October 2007 and 19 October 2008. Short = 101 to 102 relative maturity days. Full = 113 relative maturity days.

<table>
<thead>
<tr>
<th>Environment</th>
<th>Cumulative GDDs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GDDAP- R1 Early</td>
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<tr>
<td>Hoytville</td>
<td></td>
</tr>
<tr>
<td>2006</td>
<td>Full</td>
</tr>
<tr>
<td></td>
<td>Short</td>
</tr>
<tr>
<td>2007</td>
<td>Full</td>
</tr>
<tr>
<td></td>
<td>Short</td>
</tr>
<tr>
<td>2008</td>
<td>Full</td>
</tr>
<tr>
<td></td>
<td>Short</td>
</tr>
<tr>
<td>Wooster</td>
<td></td>
</tr>
<tr>
<td>2006</td>
<td>Full</td>
</tr>
<tr>
<td></td>
<td>Short</td>
</tr>
<tr>
<td>2007</td>
<td>Full</td>
</tr>
<tr>
<td></td>
<td>Short</td>
</tr>
<tr>
<td>2008</td>
<td>Full</td>
</tr>
<tr>
<td></td>
<td>Short</td>
</tr>
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</table>

Table 3.7. Effect of planting date on thermal time phenological events within frosted† and unfrosted environments.
<table>
<thead>
<tr>
<th>location</th>
<th>year</th>
<th>Maturity¥</th>
<th>Regression†</th>
<th>$r^2$</th>
<th>Significance‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wooster</td>
<td>2006</td>
<td>Short</td>
<td>$Y = -6.20x + 3246.83$</td>
<td>0.93</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Full</td>
<td>$Y = -6.61x + 3389.13$</td>
<td>0.86</td>
<td>**</td>
</tr>
<tr>
<td></td>
<td>2007</td>
<td>Short</td>
<td>$Y = -2.99x + 2903.04$</td>
<td>0.99</td>
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<td>Full</td>
<td>$Y = -5.91x + 3420.75$</td>
<td>0.99</td>
<td>***</td>
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<td></td>
<td>2008</td>
<td>Short</td>
<td>$Y = -65x + 2421.05$</td>
<td>0.96</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Full</td>
<td>$Y = -94.45x + 2548.82$</td>
<td>0.98</td>
<td>***</td>
</tr>
<tr>
<td>Hoytville</td>
<td>2006</td>
<td>Short</td>
<td>$Y = -4.51x + 3009.98$</td>
<td>0.99</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Full</td>
<td>$Y = -4.60x + 3112.57$</td>
<td>0.98</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td>2007</td>
<td>Short</td>
<td>$Y = -4.42x + 3141.58$</td>
<td>0.91</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Full</td>
<td>$Y = -3.11x + 3054.32$</td>
<td>0.76</td>
<td>**</td>
</tr>
</tbody>
</table>

Table 3.8. Effects of delayed planting on thermal intervals [(growing degree days (GDDs) from planting to kernel black layer. *** Significant at $P \leq 0.001$ and ** significance at $P \leq 0.01$

† $Y =$ GDD from planting to R6; $x =$ day of year
‡ Significance of linear regression models
¥Data from each maturity group was combined and average used. Short = 101 to 102 relative maturity days. Full = 113 relative maturity days.
3.5 DISCUSSION

The results demonstrated that maize planted early May recruited significantly more egg masses from first generation moths during June and early July than later planted corn. Conversely, later planted maize recruited more eggs masses from second generation than early planted corn. We observed a gradual increase in egg masses per plant as new leaves appeared and a sharp increase when maize plants reached reproductive stages (Fig. 3.1). These results are in agreement with previous reports that European corn borer attack earlier planted fields or tallest maize during the June flight of the first generation, while the second generation attacks the later planted fields with the youngest maize plants (Spangler and Calvin 2000, Pilcher and Rice 2001). This finding was also supported by occurrence of higher leaf damage from first generation larvae on early planted maize than late plantings (Fig. 3.3). On the contrary more stalk tunneling injury generally occurred on later planted maize caused by second generation European corn borer larval feeding (Fig. 3.4).

Egg mass density did not vary significantly between Bt and non-Bt maize indicating that ovipositional preference was not influenced by the Cry1Ab crystal protein from *B. thuringiensis* in transgenic corn. Orr and Landis (1997) concluded that Bt had no antixenosis effects because of lack significant differences in the number of egg masses on Bt and non-Bt corn. However, planting date consistently affected ovipositional preference by European corn borer moths. With exception of Wooster in 2006 where slightly more eggs masses were found on early plantings, significantly higher eggs mass densities were found on late planted corn. In three out of six comparisons (2 locations over three years), fewer eggs masses were laid on the middle planting compared to early and late plantings.
Similar results were obtained by Pilcher and Rice (2001) who suggested that recruiting a majority of the egg masses into early and late plantings would decrease the egg mass density in a middle planting. Regression and correlation analysis showed a higher inconsistency in translation of egg mass densities to larval stalk injury by European corn borer (Table 3.4 and 3.5).

The results showed that in most cases recruitment of egg masses away from middle planting to early and late plantings did not translate into reduction in stalk damage in the middle planted corn. Actually, stalk tunneling, number of tunnels, larval infestation and broken stalks were generally more on middle than early planting (Fig. 3.4-3.8), reflective of a higher population of the second generation. Egg masses laid by second generation moths have been shown to account for 70% of the variation in stalk cavity numbers (Sorenson et al. 1993). There could be other factors like natural enemies and abiotic factors that played a role in reducing the number of larvae that ultimately hatched and successfully tunneled maize stalks. In some years of this study, planting date significantly affected stalk tunneling, percentage of infested plants, number of tunnels and percentage of broken stalks.

In 2006, a significant increase in the number of egg masses on early planted maize at Wooster and late planting at Hoytville did not significantly affect stalk damage. This might partially be due to fewer adult moths in 2006 that laid fewer egg masses and as a result inadequate number of larvae to cause significant differences in stalk injury among planting dates. It could also be due to density-dependent or density-independent mortality factors that regulate European corn borer population such as natural enemies and adverse weather conditions which were not measured in this study.
In 2007, maize planted late suffered significantly longer stalk tunneling than early planting at each location. Females from the overwintering populations prefer to lay their eggs in the tallest, earliest-planted maize in the area (Ritchie et al. 1993). The larvae that hatch generally cause damage to maize on whorl-stage and less tunneling on later planted maize (Pilcher and Rice 2001). Earlier planted maize had significantly shorter tunneling because second generation moths that emerged from first brood preferred laying eggs on late planted maize that was succulent and physiologically less mature (R1 or silking stages) than earlier plantings. Consequently an increase in egg mass density from the second generation resulted in an increase in stalk tunneling length in years when European corn borer populations are high. However, tunneling length in 2008 at Hoytville was not affected by the two planting dates, perhaps because of the lack of early planting that could have recruited part of egg laying away from middle planting (Pilcher and Rice 2001, Spangler and Calvin 2000). At Wooster, an increase in egg mass density as planting was delayed translated into significantly higher tunneling length in 2008. This was further demonstrated by positive regression and correlation between egg mass densities and both tunneling and number of live larvae per stalk.

The Cry1Ab crystal protein from *B. thuringiensis* in transgenic maize provided complete protection against European corn borer injury to leaves and stalks throughout this study (Figs. 3-8). Leaf and stalk damage from first and second generation European corn borer was practically nil. Similar results were obtained by Barry et al. (2000) working with Bt hybrids containing Cry1Ab crystal protein gene. In our study, when populations of European corn borer were relatively low, complete protection against larval injury by Bt gene did not translate into statistically significant advantages in yield.
when compared to non-Bt hybrids. Where no variation occurred in stalk tunneling among planting dates like in 2006 at both locations and in 2008 at Hoytville (Fig. 4), the benefit of using Bt hybrids was insignificant. However, in 2007 at each location and 2008 at Wooster, the use of transgenic hybrids was more beneficial because of increased stalk tunneling that also differed among planting dates. The benefit of Bt maize hybrids was more pronounced on late planted than early planted maize when European corn borer damage was high.

At least three out six comparisons showed that the differences in hybrid effects on corn borer damage and infestation was influenced by crop phenology as shown by planting date × hybrid interactions (Fig. 3.3- 3.8). The number of larvae per stalk, tunneling length, percentage infestations, and number of tunnels and percentage of broken stalks were generally higher in late planted maize than early plantings for the non-Bt hybrids, while they were always very low for the Bt hybrids across all planting dates. It has been proposed that Bt maize could be used as a trap crop to recruit egg masses and reduce the amount of stalk tunneling damage that occurs in non-Bt maize planted during certain times in the spring planting sequence (Pilcher and Rice 2001). In our study, significant effects of planting date on stalk tunneling occurred when populations of European corn borer were high, in most cases occurring on late planted maize that commonly had higher numbers of eggs from second generation moths. Tunneling length, percentage of plants infested, number of tunnels and percentage of broken stalks were lower when maize was planted early May and increased with delayed planting for the non-Bt corn. Delayed planting often subjects maize plants to heavier infestation of pests and diseases which can result in yield loss (Wiatrak et al. 2005). The most important
factor to consider therefore is managing European corn borer when anticipating delayed planting or replanting.

Various growth stages of maize greatly influence plant response to European corn borer infestation and subsequent yield losses (Lynch 1980, Bode and Calvin 1990). Planting dates significantly affect field maize yield performance because they impact phenological growth and development and the ability to cope with environmental stresses. Factors such as weather conditions (e.g. excessive soil moisture) and equipment breakdowns which can push planting beyond the optimum dates of late April to early May in the Eastern USA Corn Belt (Nielsen et al., 2002, Jarvis 1986). For this reason our study, unlike previous ones (Barry et al., 2000, Catangui and Berg 2002, Picher and Rice 2001), included short season hybrids. We showed that when planting was delayed full season hybrids did not produce higher yields than short season hybrids, and in some cases such as experienced at Wooster, full season hybrids fail to reach maturity before first frost occurred. Yield advantage therefore occurs when planting full season hybrids early and short season hybrids when planting is delayed.

In 2006, the interaction between maize phenology and hybrid did not affect stalk tunneling, and stalk tunneling was not significantly associated with yield reduction in late planted maize (Table 3.5). Yield reduction due to delayed planting would therefore be due to decreased growing degree days that were required for maize hybrid to reach physiological maturity. This was supported by the significant relationship between thermal intervals and yield across planting date and location. We also showed that delayed planting was significantly associated with reduced growing degree days at Wooster and Hoytville over three years of this study. In 2007, yields were higher on
middle planted maize than early and late planting at Hoytville. No significant yield differences were observed between full season and short season hybrids. Higher yields were therefore possibly due to protection of maize by Bt gene because the highest yields were obtained from Bt hybrids (Fig. 3.9C). Since we did not use isolines to compare with their Bt counterparts, it is probable that the Bt hybrids out yielded non-Bt ones by virtue of being genotypically higher yielding hybrids, and not necessarily because of European corn borer damage.

Comparison of Bt and non-Bt hybrids using non-isolines have been reported (Wiatrack et al. 2004, Bruns and Abbas 2005, Bruns and Abbas 2006). Bruns and Abbas (2006) observed higher yield from Bt hybrid compared to non-Bt genotypes, but could not attribute the improvement in yield to insect damage or yield potential of Bt hybrids because they did not collect data on insect injuries. Wiatrack et al. (2004) obtained negative correlation between yield and insect damage to corn, and attributed increased yield among Bt hybrids to reduced ear infestation by fall armyworm and earworm. In this study, multiple regression analysis showed a significant negative linear yield response to European corn borer injuries, indicating the potential for yield loss if pest management tactics like use of Bt are not applied during years when higher populations of European corn borer occur.

On late planted corn, significantly higher yields were obtained from Bt than non-Bt maize hybrids. Unlike at Hoytville, yield was lower on middle planted maize at Wooster. The significantly lower yield obtained from middle planting compared to early planting at Wooster can be attributed to the effect of maize relative maturity and the Bt gene. Early planted full season hybrids significantly out yielded short season hybrids.
with no significant Bt effect. When planted on 23 May, mean yield from full season hybrids was higher than short season hybrids while yield from non-Bt was lower than Bt hybrids.

It is probable that differences in hybrid yield response were caused by planting different short season hybrids in 2006 compared to both 2007 and 2008. Despite the use of different hybrids between the seasons, we observed consistently higher yields on full season hybrids than short season hybrids at Wooster in 2006 and 2007 on early planted corn, although no such differences occurred on later plantings. This is in line with our hypothesis that short season hybrid would yield comparably with full season hybrids especially when planting is delayed. Hybrid selections were based on seed availability and matching of hybrids for both long and short season hybrids as close as possible to allow them similar exposure to growing degree days. In 2006 and 2007, regression model showed a significant relationship between yield and growing degree days at Wooster. That probably contributed to significant differences observed among short season and full season hybrids at Wooster for those years.

During late planting, numerically higher yields, though not significant, were obtained from Bt hybrids compared to non-Bt hybrids. Earlier planted, full season hybrids used full season growing degree days exposed to relatively lower injury while later planted Bt hybrids maize got protection from Bt gene against increased larval feeding associated with delayed planting. Lower yields obtained from middle planting at Wooster in 2008 were also associated with crop relative maturity and the Bt gene effect. Significantly higher yields were obtained from early planted maize which used full growing season with significantly lower stalk injury than middle planting (Fig. 3.9F). In
contrast, middle planted non-Bt maize on average suffered significantly more stalk injury and subsequent reduction in yield compared with early planted corn. No significant differences were observed between late and middle planting with respect to stalk tunneling and yield. Within late planted corn, significantly higher yields were obtained from Bt than non-Bt hybrids, clearly showing the advantage of the Bt gene when planting is delayed.

This study has shown that manipulation of planting date affected phenological growth stages of maize which significantly influenced oviposition preferences by European corn borer. However, there was a higher variation in subsequent damage associated with egg mass populations. When the population of European corn borer was higher as planting was delayed, there was an increase in tunneling length. It was during higher European corn borer populations that the use of transgenic Bt hybrids became beneficial. The benefit was more significant when maize planting was delayed due to the impact of increased tunneling associated with late planting. Throughout the study non-Bt hybrids of both maturities incurred the same amount of damage by European corn borer. When damage was severe and planting delayed, yields from short season hybrids protected by Bt gene were comparable with their full season counterparts. The short season hybrids also matured early before first frost kill in areas where frost is early. When circumstances allow, growers need to follow the optimal planting of late April to early May time frame in Eastern Corn Belt. Planting within this time frame provide maize plants with full season maximum growing degree days and reduce pest pressure associated with late planting. When planting is delayed due to heavy moisture or unforeseen circumstances and European corn borer population is known to cause
economic damage in an area, selection of short season hybrids with Bt gene could be beneficial.

3.6 REFERENCES


CHAPTER 4

THE EFFECTS OF PLANTING DATE IN COMBINATION WITH TRANSGENIC MAIZE HYBRIDS ON STALK AND EAR ROTS FOLLOWING EUROPEAN CORN BORER INFESTATION

4.1 ABSTRACT

Field studies were carried out at Hoytville and Wooster, Ohio from 2006 to 2008 to determine the influence of planting date and transgenic Bt maize on incidence and severity of stalk and ear rots of corn. Transgenic and non-transgenic Bt maize hybrids with different maturity ratings (short vs. full season) were planted on three planting dates (early, middle, and late), targeting late April/early May, mid-May and early June respectively. The incidence and severity of the maize stalk rot complex and ear rots caused by Fusarium and Gibberella fungal species were compared among planting dates and between Bt and non-Bt genotypes of different relative maturity ratings. The significant effect of planting date on levels of both maize stalk and ear rots was variable during the three years of the study. Significant linear correlations between European corn borer injury to stalk and stalk rot symptoms were observed in 2006, and 2007, but not in 2008.
Apart from 2008 at Hoytville, we observed significant reduction in stalk discoloration and incidence of maize stalk rot on Bt hybrids compared with non-Bt genotypes. Significant linear correlations were obtained between European corn borer ear damage and both Fusarium and Gibberella ear rot. The use of Bt maize also reduced the levels of European corn borer damage to ears, and incidence and severity of both Fusarium and Gibberella ear rots compared with non-Bt hybrids. Compared with short season hybrids, significant reduction in both maize stalk and ear rots occurred on full season hybrids. Where the interaction between planting date and hybrid treatment was significant, the benefit of using Bt hybrids to reduce stalk and ear rots was more evident as planting was delayed than early planting.

4.2 INTRODUCTION

Stalk and ear rots are significant problems on maize (Zea mays L.) in many parts of the world (Nordby et al., 2006, Dorrance et al., 1998, Gatch et al., 2002), with stalk rots often resulting in annual losses estimated at 5 to 10 percent (OSU Extension 1998). Yield losses also occur due to reduced grain fill caused by pathogens invading maize stalks prior to physiological maturity (Koehler 1960). Destruction of the pith tissue also predisposes the plants to lodging. The most common fungal pathogens that cause stalk rot in the United States are Gibberella zeae (Schwein.) Petch (Anamorph: Fusarium graminearum (Schwabe), Colletotrichum graminicola (Ces.) G. W. Wils., Stenocarpella maydis (Berk.) Sutton, and other members of the genus Fusarium, including F.

Stalk rot fungi are aided in the colonization and decay of maize stalks by environmental stresses that heighten the plant’s susceptibility to infection, particularly following anthesis when carbohydrate shortages can occur (Dodd 1980). Injury due to feeding by European corn borer is one stress that can promote the progression of stalk rot (White 1999), contributing to the development of stalk rot when larvae tunnel into stalks and create points for fungal invasion. European corn borer can also serve as a vector of fungal pathogens. Borer feeding also causes physiological stress that predisposes the maize to stalk rot development (Bergstrom and Nicholson 1999, Jarvis et al. 1984).

The most common ear rots of maize are Gibberella ear rot, caused by G. zeae, Fusarium ear rot caused by F. moniliforme (J. Sheldon), and Diplodia ear rot caused by Stenocarpella maydis (Berk.) Sutton. Gibberella ear rot is often the most important rot in many areas where maize is grown (Nordby et al. 2006.). Gibberella zeae produces a pink- to reddish-colored mold on kernels that usually spreads from the tip of the ear downward or outward from an insect wound. Fusarium moniliforme produces a whitish-colored mold growth that tends to be scattered on the ear (Reid et al., 1999). Kernel infection by Fusarium species and G. zeae can reduce yield and quality, and result in the accumulation of mycotoxin in grain (Headrick and Pataky 1991).

These different pathogens can enter the maize ears through wounds created by insect feeding (Attwater, and Busch 1983) or by the growth of mycelium from spores germinating on the silks (Hesseltine, and Bothast 1977). Injuries to plants caused by
insects such as the European corn borer (*Ostrinia nubilalis*) are often the initial infection sites for *Fusarium* species (Jarvis, et al. 1984, Chiang, and Wilcoxson 1961). European corn borer larvae can also act as vectors of ear rot fungi by carrying the fungus from the plant surface into the maize ears (Munkvold, et al. 1997). Yield loss attributed to European corn borer is often due in part to subsequent fungal decay of tissue injured by larvae (Keller et al. 1986). Thus, the relationship between European corn borer (ECB) injury and stalk and ear rots is an important factor in management of these diseases.

European corn borer infestations were reported to increase symptoms of *Fusarium* ear rot, as well as increase symptomless kernel infection (Sobek and Munkvold 1999). Control of ECB using transgenic maize has been shown to reduce the amount of injury to stalks and ears, and subsequently lower the severity and incidence of stalk ear rots (Munkvold et al., 1997, Gatch et al., 2002). These studies were carried out under normal planting conditions using full season hybrids.

Often, the planting of field maize is delayed beyond the optimum late April to early May time frame by excessive soil moisture (Nielsen et al. 2002, Jarvis et al. 1986). Occasionally, cornfields that were planted during the optimum time frame will require replanting at later dates after weather stress or other pests cause excessive plant mortality. One of the more important factors growers need to consider if planting late is hybrid maturity. Short season hybrids suitable for a shortened growing season have been developed and can be planted during late planting conditions. Short season hybrids may facilitate earlier harvest and reduce frost risk and minimize drying costs when planted late.
Late planting subjects maize plants to pest and disease pressure (Wiatrak et al. 2005). Date of planting is an important factor in developing disease management strategies because it influences occurrence of diseases (Paul & Munkvold 2004, Wiatrak et al. 2005). In this part of the study we investigated the influence of planting date in combination with transgenic maize on stalk and ear rots following ECB injury. We had the following questions in mind; a) does planting transgenic hybrids resistant to European corn borer significantly reduce incidences of stalk and ear rots in late planted crop? b) Does hybrid maturity influences severity and incidences of these diseases? c) Is there an interaction between planting date and Bt maize on incidence and severity of stalk and ear rots?

4.3 MATERIALS AND METHODS

The experiment was carried out at the Northwest Agricultural Research Station near Hoytville (41° 12´ N, 83° 45´ W) and at the Wooster Campus (40° 46´N, 81° 55´W) of The Ohio State University Ohio Agricultural Research and Development Center in Ohio from 2006 - 2008. The land was prepared by plowing in the spring followed by disc harrowing to break up the sod. The field was previous planted with a crop of soybean. Individual plots were 10 m long and 12 rows wide. Corn, planted with a 4-row planter, had row spacing of 0.76 m and a seeding rate of 79 000 seeds ha⁻¹. The maize seeds planted were hybrids commonly used by farmers that had comparable agronomic characteristics. Experimental design was a 3 x 4 treatment factorial replicated 4 times in a randomized complete block arranged in split plot layout. The first factor (main plot)
was planting date, randomized within each replication. There were three planting dates targeting normal/early, mid and late; late April/early May, late May and early June each year (Table 4.1). The second factor (subplot) was four maize hybrids (Table 4.2) assigned randomly within the planting date. The hybrids represented two maturity group, a short and a full season hybrids. Each maturity group was then represented by two Bt hybrids containing Bt endotoxin gene confers resistance against European corn borer and two non Bt hybrid. All seeds were obtained from Dekalb, and were treated with clothianidin (Poncho 250™) (Gustafson LLC, Dallas TX) at rate of 0.25 mg a.i. per seed to control secondary soil pests.
<table>
<thead>
<tr>
<th>Location</th>
<th>Planting dates</th>
<th>Harvest dates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Year</td>
<td>Early</td>
</tr>
<tr>
<td>Wooster</td>
<td>2006</td>
<td>4 May</td>
</tr>
<tr>
<td></td>
<td>2007</td>
<td>7 May</td>
</tr>
<tr>
<td></td>
<td>2008</td>
<td>1 May</td>
</tr>
<tr>
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<td>2006</td>
<td>28 April</td>
</tr>
<tr>
<td></td>
<td>2007</td>
<td>7 May</td>
</tr>
<tr>
<td></td>
<td>2008</td>
<td>np</td>
</tr>
</tbody>
</table>

Table 4.1. Planting and harvest dates at Wooster and Hoytville, Ohio from 2006 to 2008. np =Not planted due to continuous high soil moisture during late April until mid May 2008.
<table>
<thead>
<tr>
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<th>Maturity days</th>
<th>Maturity GDDs</th>
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</thead>
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<td>101</td>
<td>2528</td>
</tr>
<tr>
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<td>Non Bt</td>
<td>101</td>
<td>2530</td>
</tr>
<tr>
<td>Dekalb DKC63-81</td>
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<tr>
<td>Dekalb DKC63-80</td>
<td>Non Bt</td>
<td>113</td>
<td>2790</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>2007 &amp; 2008</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dekalb DKC52-63</td>
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<tr>
<td>Dekalb DKC52-62</td>
</tr>
<tr>
<td>Dekalb DKC63-81</td>
</tr>
<tr>
<td>Dekalb DKC63-80</td>
</tr>
</tbody>
</table>

Table 4. 2. Maize hybrids planted at Wooster and Hoytville, Ohio from 2006 to 2008
Stalk rot sampling

Stalks were sampled at physiological maturity (R6) as indicated by formation of a black layer on the kernel. A total of ten stalks were sampled from rows three and ten to avoid border effect which left the middle rows for yield collection. The stalks were split longitudinally and evaluated for the length (cm) of stalk discoloration. The incidence (presence or absence) of pith discoloration was determined by counting the number of stalks infected which was then expressed as the percentage of total stalks sampled. The isolation of fungal pathogens was done by cutting four pieces from one side of the stalk and then surface sterilized for two minutes with 10% sodium hypochlorite (NaOCl) solution. The stalk pieces were then rinsed with deionized water and excess water removed by squeezing them between double paper towels. The four pieces from each stalk were then placed in water agar and incubated in the dark at 25°C until recognizable fungal colonies were formed after approximately three days. The colonies were transferred to Komada and fresh potato dextrose agar (Difco, Becton Dickinson and Co., Sparks, MD). The identification of fungal pathogens to the genus level was based on morphological characteristics.

Ear rots sampling

When maize had reached physiological maturity, individual ears were collected from 10 randomly selected plants (five each from row 3 and 10), husked, and visually evaluated for ear damage by European corn borer, and fungal ear rot pathogens. The number of ears damaged by European corn borer was expressed as percentage of the ten ears sampled per plot. Samples of kernels were cultured in appropriate media and
pathogens isolated to confirm the symptoms. The isolation of pathogens was done by randomly collecting twenty five kernels with ear rot symptoms and surface-sterilized for 3 min in a 10% solution of sodium hypochlorite. After removing excess water, kernels were then cultured on Komada and fresh potato dextrose agar, incubated for 5 to 8 days, and then observed for the presence of fungal pathogens. Fungal colonies were identified by conidial morphology to genus level.

Data were recorded as the number of kernels displaying symptoms on each ear. Ear rot incidence was calculated as the percentage of plants per plot with symptoms of Fusarium and Gibberella ear rots. Ear rot severity was calculated as the mean number of kernels with symptoms in each ear for each disease (Munkvold et al. 1997).

Statistical analysis

Data on stalk rots, ear rots and ECB injury were analyzed using mixed model analysis (PROC MIXED) (SAS Institute, 2003). Planting dates and hybrids were considered fixed variables, while replications and interactions were assumed to be random effects. The interaction between planting date and hybrids was tested. To compare disease severity, incidence and ECB damage between long vs. short season and transgenic vs. non transgenic hybrids, planned orthogonal contrasts were performed using CONTRAST option within PROC MIXED. Multiple comparisons were performed on least square means of the fixed effects using the PDIF option of the LSMEANS statement in SAS. All the comparisons were based on Fisher’s protected least significant difference and considered significant at P = 0.05. Correlation analysis (PROC CORR) was performed to determine the relationship between European corn borer damage and
stalk rot symptoms. The linear correlation analysis was also conducted on incidence of
ear rot, severity of ear rot and ECB damage on ears. Percentages were arcsine
transformed to stabilize variance and length of discoloration was transformed to log
(x+1).

4.4 RESULTS

Stalk rot

Stalk discoloration. Length of stalk discoloration and incidence of stalk rot
(percentage of infected stalks) were affected by hybrid treatments and planting date, but
these effects varied with year and location (Fig. 4.1 and 4.2). Stalk discoloration and
incidence of stalk rot were significantly correlated to stalk tunneling, percentage of stalk
injured, and number of ECB larvae per plant (see Chapter 3); however, they were not
consistent from year to year (Table 3.3). In 2006, a significant planting date × hybrid
interaction was observed at each location (Hoytville: $F_{6,27} = 3.22$; $P = 0.016$, Wooster: $F_{6,
27} = 2.61$; $P = 0.033$). Hybrid treatment significantly affected the length of stalk
discoloration at both locations (Hoytville: $F_{3,27} = 66.47$; $P <.0001$, Wooster: $F_{3,27} =
13.31$; $P <.0001$). A significant reduction in stalk discoloration occurred on Bt hybrids
compared with non-Bt hybrids at Hoytville ($F_{1,27} = 50.86$; $P <.0001$) and Wooster ($F_{1,27}
= 15.12$; $P = 0.0004$). Multiple comparisons showed significantly longer discoloration on
the short season non-Bt hybrid compared to the full season non-Bt hybrid when maize
was planted in middle and late plantings at Hoytville (Fig. 4.1A). At Wooster, significant
increase in stalk discoloration was observed on the short season non-Bt hybrid than full season non-Bt hybrid only when maize was planted on 24 May (Fig. 4.1B).

In 2007, significant planting date × hybrid interaction effects were observed at Hoytville ($F_{6,35} = 4.71; P = 0.001$) and Wooster ($F_{6,22} = 9.38; P <.0001$). Significantly less stalk discoloration was observed on early planted maize compared to late plantings at Hoytville and at Wooster (Fig. 4.1C and D). The length of stalk discoloration was affected by hybrid treatments at each location (Hoytville: $F_{3, 22} = 26.09; P <.0001$, Wooster: $F_{3, 35} = 11.16; P <.0001$). A significant decrease in stalk discoloration was observed on Bt maize than from non-Bt hybrids at Hoytville ($F_{1, 22} = 44.68; P = <.0001$) and at Wooster ($F_{1, 35} = 27.68; P <.0001$). Multiple comparisons of least square means indicated no significant differences among hybrids on early planted maize at both locations (Fig. 4.1B). When maize was planted in middle and late plantings, the short season non-Bt hybrid showed a significant increase in stalk discoloration compared to full season non-Bt hybrid at Hoytville and Wooster (Fig. 4.1C and D).
Fig. 4.1. Mean length of discoloration per stalk among hybrids planted on different dates in 2006, 2007 and 2008 at Hoytville and Wooster. Letters associated with planting date indicate that a significant difference occurred, with hybrid means having the same letter not significantly different (Fisher protected LSD, $\alpha = 0.05$). PD = planting date; PDH = planting date x hybrid interaction;*, ** and *** denote significance at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$ respectively. SS = short season, LS= full season.
In 2008, a significant planting date × hybrid interaction was not observed at Hoytville (F3,12 = 1.14 P = 0.3708), but was at Wooster (F6, 27 = 2.59; P = 0.041). Delaying planting resulted in significant reduction in stalk discoloration on maize planted on 9 June at both Hoytville and at Wooster (Fig.4.1E and F). Stalk discoloration varied among hybrid treatments at Hoytville (F3,12 = 11.93; P = 0.0007) and at Wooster (F3,27 = 45.25; P <.0001). Stalk discoloration did not differ between Bt and non-Bt hybrids at Hoytville (F1,12 = 2.95; P = 0.112), but a significant decrease was observed on Bt hybrids at Wooster (F1,27 = 17.69 P = 0.0003). At both locations, a significant reduction in stalk discoloration was observed on full season hybrids compared to short season ones (Hoytville: F1,12 = 32.34 P = 0.0001, Wooster: F1,27 = 99.12 P <.0001). A significant increase in stalk discoloration on short season hybrid DKC 52-62 was consistent among the three planting dates at Wooster (Fig. 4.1F).

Incidence of stalk rot. In 2006, no planting date × hybrid interaction was observed at either location (Hoytville: F6,33 = 2.16; P = 0.072; Wooster: F6,36 = 1.91; P = 0.1057). Incidence of stalk rot (% of infected plants) was significantly higher on earlier planted maize than late planted maize at Hoytville (F2,33 = 10.67; P = 0.0003) and also at Wooster in 2006 (F2,36 = 7.49; P = 0.002) (Fig. 4.2A and B). The incidence varied among hybrid treatments at each location (Hoytville: F3,33 = 92.80; P <0.0001; Wooster: F3,36 = 11.76 P<.0001). This difference was due to a significant reduction in stalk rot incidence found on Bt hybrid compared with non-Bt hybrids at Hoytville (F1,33 = 16.60 P = 0.0003 ) and Wooster (F1,36 = 12.17; P = 0.001). The incidence was also significantly reduced on full season hybrids compared with short season hybrids at Hoytville (F1,33 = 259.85 P <.0001) and at Wooster (F1,36 = 22.54; P <.0001).
In 2007, significant planting date × hybrid interaction effects were observed at both locations (Hoytville: F6, 18 = 6.81; P = 0.0007; Wooster: F6, 35 = 4.39; P = 0.0021). The interactions showed that significantly less stalk rot incidence was obtained on early planted maize than middle and late plantings at both locations (Fig. 4.2C and D). Hybrid treatment significantly affected the incidence of stalk rot at both locations (Hoytville: F3, 18 = 16.83; P <.0001; Wooster: F3, 35 = 11.09; P <.0001). Planting Bt hybrids significantly reduced incidence of stalk rot compared with non-Bt hybrids at Hoytville (F1, 18 = 32.64; P <.0001) and at Wooster (F1, 35 = 24.94 P <.0001). At both locations, significant planting date × hybrid interaction effects were observed (Hoytville: F6, 18 = 6.81; P = 0.0007; Wooster: F6, 35 = 4.39; P = 0.0021). Examination of planting date × hybrid interaction showed that the short season non-Bt hybrid had significantly longer discoloration compared to full season non-Bt hybrid on later planted maize than early planted maize at both locations (Fig. 4C and D).

In 2008, planting date × hybrid interaction was not significant at Hoytville (F3, 12 = 1.92; P = 0.1799), but significant effects occurred at Wooster (F6, 33 = 3.52 P = 0.0084). Significantly lower percentages of infected stalks were obtained on late planted maize than earlier plantings at both Hoytville (F1, 4 = 10.80; P = 0.030) and Wooster (Fig. 4.2 E and F). Stalk rot incidence varied among hybrids at Hoytville (F3, 12 = 12.37; P = 0.0006) and at Wooster (F3, 33 = 29.97; P <.0001). There was no significant difference in stalk rot incidence between Bt and non-Bt hybrids at Hoytville (F1, 12 = 3.24; = 0.0973), but a significant reduction was observed on Bt hybrids at Wooster (F1, 33 = 13.48; P = 0.0008).
<table>
<thead>
<tr>
<th>location</th>
<th>year</th>
<th>Variable ‡</th>
<th>Stalk discoloration (cm)</th>
<th>% stalk discoloration</th>
</tr>
</thead>
<tbody>
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<td>Tunneling</td>
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<td>0.21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>% tunneled stalk</td>
<td>0.42**</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No. larvae</td>
<td>0.47**</td>
<td>0.30*</td>
</tr>
<tr>
<td></td>
<td>2007</td>
<td>Tunneling</td>
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<td>0.72**</td>
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<td></td>
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<td>No. larvae</td>
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<td>0.50**</td>
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<td>0.70***</td>
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<td></td>
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<td>Tunneling</td>
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<td>0.18</td>
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<td></td>
<td></td>
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<td>0.13</td>
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<td>Tunneling</td>
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<td>0.33*</td>
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<td></td>
<td></td>
<td>% tunneled stalk</td>
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<td></td>
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<td>No. larvae</td>
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<td>No tunnels</td>
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</table>

Table 4.3. Linear correlation coefficients between European corn borer injury and maize stalk rot symptoms at Hoytville and Wooster in 2006, 2007 and 2008. *\*, ** and *** denote significance at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$ respectively. §Data not collected in 2006; ‡ European corn borer data not presented here (see chapter 3).
Fig. 4.2. Mean percentage of stalks with discoloration symptoms from hybrids planted on different dates in 2006, 2007 and 2008 at Hoytville and Wooster. Letters associated with planting date indicate that a significant difference occurred, with hybrid means having the same letter not significantly different (Fisher protected LSD, $\alpha = 0.05$). PD = planting date; PDH = planting date x hybrid interaction; *, ** and *** denote significance at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$ respectively. SS = short season, LS = full season.
Compared with short season hybrids, full season hybrids showed significant reduction in stalk rot incidence at both locations (Hoytville: $F_{1,12} = 33.86; P < .0001$; Wooster: $F_{1,33} = 62.96 P < .0001$). Full season hybrids consistently showed low incidence among the three planting dates (Fig. 4.2E and F).

**Ear Rots**

The ear rot diseases that occurred in significant proportions and severity in this study were Fusarium and Gibberella ear rots. Incidences of Aspergillus and Diplodia ear rots were negligible throughout this study (data not reported). There was a closer relationship between incidence and severity of both Gibberella and Fusarium ear rots (Table 4.4). Significant linear correlation coefficients were observed between severity, incidence of ear rots, and European corn borer damage to maize ears in most comparisons (Table 4.4).

*Percentage of damaged ears.* In 2006, planting date × hybrid interaction was detected at Hoytville ($F_{6,33} = 2.59; 0.0364$) but not at Wooster ($F_{6,36} = 0.48 P = 0.821$). Percentage of damaged ears by European corn borer larvae was significantly lower on late planted maize than earlier plantings at Hoytville (Fig. 4.3A), but no such significance occurred at Wooster ($F_{2,36} = 1.09; P = 0.347$ (Fig. 4.3A and B). Percentage of damaged ears varied significantly among hybrids at Hoytville ($F_{3,33} = 61.88; P < 0.0001$) and at Wooster ($F_{3,36} = 59.53; P < 0.0001$). At each location, the percentage of damaged ears in Bt maize hybrids was significantly lower than non-Bt maize hybrids (Hoytville: $F_{1,33} = 183.60; P < 0.0001$; Wooster: $F_{1,36} = 159.00; P<0.0001$). Significantly higher percentage of damaged ears occurred on the short season non-Bt hybrid compared to full season
hybrid when maize was planted on 7 June at Hoytville (Fig. 4.3A). Significant increase in percentage of damaged ears were obtained on the short season non-Bt hybrids at Wooster ($F_{1,36} = 9.04 \ P = 0.005$) among the three planting dates (Fig. 4.3B).

In 2007, there was significant planting date × hybrid interaction effects at Hoytville ($F_{6,24} = 8.69; \ P < 0.0001$) and also at Wooster ($F_{6,27} = 6.52; \ P = 0.0001$). Assessment of planting date × hybrid interaction revealed that an increase in percentage of damaged ears increased as planting was delayed (Fig. 4.3C and D). The percentage of damaged ears was significantly higher on late planted maize at both Hoytville ($F_{2,24} = 12.82; \ P = 0.002$) and Wooster ($F_{2,27} = 16.07; \ P < 0.0001$) (Fig. 4.3C and D). Percentage of damaged ears was significantly affected by hybrid at Hoytville ($F_{3,24} = 126.30; \ P < 0.0001$) and at Wooster ($F_{3,27} = 200.47; \ P < 0.0001$). The non-Bt hybrids had significantly higher percentage of damaged ears than Bt hybrids at both locations (Hoytville: $F_{1,24} = 374.02; \ P < 0.0001$; Wooster: $F_{1,27} = 586.20; \ P < 0.0001$).
<table>
<thead>
<tr>
<th>Year</th>
<th>Location</th>
<th>Variable</th>
<th>Severity Fusarium</th>
<th>Severity Gibberella</th>
<th>ECB Ear damage</th>
</tr>
</thead>
<tbody>
<tr>
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<td>Incidence Fusarium</td>
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<td></td>
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<tr>
<td></td>
<td></td>
<td>Incidence Gibberella</td>
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<td>2.90*</td>
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<td></td>
<td>Severity Gibberella</td>
<td></td>
<td>0.30*</td>
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<tr>
<td>2007</td>
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<td>Incidence Fusarium</td>
<td>0.95***</td>
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<td>Severity Gibberella</td>
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<td>0.74***</td>
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<td>2008</td>
<td>Hoytville</td>
<td>Incidence Fusarium</td>
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<td>0.37*</td>
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<td>Severity Fusarium</td>
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<td>Incidence Gibberella</td>
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<td>0.78***</td>
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<td>Severity Gibberella</td>
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<tr>
<td>2006</td>
<td>Wooster</td>
<td>Incidence Fusarium</td>
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<td>Severity Fusarium</td>
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<td></td>
<td>Incidence Gibberella</td>
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<td>Severity Gibberella</td>
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<td>2007</td>
<td>Wooster</td>
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<tr>
<td>2008</td>
<td>Wooster</td>
<td>Incidence Fusarium</td>
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<td></td>
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<td>Severity Fusarium</td>
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<td>Severity Gibberella</td>
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Table 4.4. Linear correlation coefficients between incidence and severity of Fusarium ear rots, Gibberella ear rot and European corn borer damage to maize ears at Hoytville and Wooster, OH, in 2006, 2007, and 2008. *, ** and *** denote significance at $P \leq 0.01$, $P \leq 0.01$ and $P \leq 0.001$ respectively.
Fig. 4.3. Mean percentage of ears per plant damaged by European corn borer on hybrids planted on different dates in 2006, 2007, and 2008 at Hoytville and Wooster. Means followed by the same letter within a planting date are not significantly different (Fisher protected LSD, α = 0.05). PD = planting date; PDH = planting date x hybrid interaction; *, **, and *** denote significance at P ≤ 0.05, P ≤ 0.01, and P ≤ 0.001 respectively. SS = short season, LS = full season.
At Hoytville, significant increase in ear damage occurred on short season non-Bt hybrid compared to full season non-Bt hybrid when maize was planted on early and middle plantings (Fig. 4.3B). At Wooster the planting date by hybrid interaction revealed that a significant reduction in percentage of damaged ears on full season non-Bt maize occurred on early planted maize than late plantings compared to the short season non-Bt hybrid (Fig. 4.3D).

In 2008, planting date × hybrid interaction was not significant at either location (Hoytville: F3, 12 = 0.11; P = 0.950; Wooster: F6, 33 = 0.93; P = 0.486). Planting date did not significantly affect the percentage of damaged ears at either location (Hoytville: F1, 2 = 0.31; P = 0.635; Wooster: F2, 33 = 0.55 P = 0.582) (Fig. 4.3E and F). A significant effect due to hybrid was detected at Hoytville (F3, 12 = 99.77; P<0.0001) and Wooster (F3, 33 = 59.46; P <0.0001). This difference was due to a significantly higher percentage of damaged ears on non-Bt than Bt hybrids at both Hoytville (F1, 12 = 288.34; P<0.0001) and Wooster (F1, 33 = 158.74; P<0.0001). There was a significant reduction in the percentage of damaged ears on full season hybrid compared with short season ones at Hoytville (F1, 12 = 5.49; P = 0.037) and at Wooster (F1, 33 = 9.81; P = 0.0036) (Fig. 4.3E and F).

*Incidence of Fusarium ear rot.* In 2006, no planting date × hybrid interaction effects were observed at either location (Hoytville: F6, 27 = 0.45 P = 0.841, Wooster: F6, 27 = 1.16; P = 0.357). The incidence of Fusarium ear rot was not significantly affected by date of planting at either location (Hoytville: F2, 6 = 0.93; P = 0.446; Wooster: F2, 9 = 0.14; P = 0.869). The incidence differed significantly among hybrid treatments at Hoytville (F3, 27 = 4.24 P = 0.0140), but not at Wooster (F3, 27 = 0.46; P = 0.710). A significant reduction in Fusarium incidence was observed on Bt hybrids at Hoytville (F1,
27 = 7.14; P = 0.013), but no such differences occurred at Wooster (F1, 27 = 0.29 P = 0.596). A significantly lower incidence of Fusarium ear rot was observed on full season hybrids compared with short season hybrids at Hoytville (F1, 27 = 4.62 = 0.041), but no significant differences occurred at Wooster (F1, 27 = 0.82; P = 0.374) (Fig. 4.4A and B).

In 2007, incidence of Fusarium ear rot was not affected by planting date at either location (Hoytville: F2, 22 = 1.44; P = 0.259, Wooster: F2, 33 = 0.95; P = 0.396), it was affected by hybrid treatment (Hoytville: F3, 22 = 3.69; P = 0.027, Wooster: F3, 33 = 2.75; P = 0.050) (Fig. 4.4C and D). Compared with non-Bt hybrids, Fusarium ear rot incidence was numerically lower on Bt hybrids at Hoytville but it was not significant (F1, 22 = 3.60; P = 0.071), while a significant reduction occurred at Wooster (F1, 33 = 8.08; = 0.008). A significant decrease in Fusarium ear rot incidence was observed on full season hybrids compared with short season hybrids at Hoytville (F1, 22 = 5.64; P = 0.027), but no significant differences occurred at Wooster (F1, 33 = 0.02; P = 0.893) (Fig. 4.4C and D). Planting date × hybrid interaction had no significant effects at either location (Hoytville: F6, 22 = 0.51; = P = 0.796, Wooster: F6, 33, = 1.05; P = 0.411).

In 2008, there was no significant planting date × hybrids interaction effect at either location (Hoytville: F3, 12 = 2.91; P = 0.078, Wooster: F6, 27 = 1.79; P = 0.139). The incidence of Fusarium ear rot did not vary significantly among planting dates (Hoytville: F1, 2 = 0.19 P = 0.705, Wooster: F2, 9 = 0.52; P = 0.611) or hybrid treatments (Hoytville: F3, 12 = 2.07; P = 0.156, Wooster: F3, 27 = 0.52; 0.675).
Fig. 4.4. Mean percentage of plants with Fusarium ear rot symptoms on hybrids planted on different dates in 2006, 2007, and 2008 at Hoytville and Wooster. Letters associated with planting date indicate that a significant difference occurred, with hybrid means having the same letter not significantly different (Fisher protected LSD, $\alpha = 0.05$). SS = short season, LS = full season.
However, multiple comparisons of least square means within planting dates showed a significant reduction in Fusarium ear rot on Bt hybrids planted on 22 May and 9 June at Hoytville (Fig.4.4E). At Wooster, a significant reduction in Fusarium incidence occurred on Bt hybrids planted on 9 June only (Fig. 4.4F).

**Fusarium ear rot severity.** In 2006, no planting date × hybrid interaction effects were observed at either location (Hoytville: $F_{6, 27} = 0.47; P = 0.821$, Wooster: $F_{6, 27} = 1.39; P = 0.256$). Severity of Fusarium ear rot was not significantly affected by planting date at either location (Hoytville: $F_{2, 9} = 0.78; P = 0.486$, Wooster: $F_{2, 6} = 0.07; P = 0.932$). Significant hybrid treatment effects occurred at Hoytville ($F_{3, 27} = 3.56; P = 0.027$), but not at Wooster ($F_{3, 27} = 1.62; P = 0.209$). Ear rot severity was significantly lower on Bt hybrids than non-Bt hybrids at Hoytville ($F_{1, 27} = 7.26; P = 0.012$), but no such significant differences occurred at Wooster ($F_{1, 27} = 2.09; P = 0.160$). Hybrid relative maturity did not affect severity of ear rot at either location (Hoytville: $F_{1, 27} = 3.13; P = 0.088$, Wooster: $F_{1, 27} = 1.22; P = 0.280$). At both locations, short season non-Bt hybrid DKC 51-45 showed a significant increase in ear rot severity, particularly on late planting (Fig. 4.5A and B).

In 2007, there were no planting date × hybrid interaction effects observed at Hoytville ($F_{6, 24} = 0.34; P = 0.910$) and at Wooster ($F_{6, 33} = 1.38; P = 0.252$). Fusarium ear rot severity did not vary among planting date at either Hoytville ($F_{2, 24} = 0.60; P = 0.559$) or Wooster ($F_{2, 33} = 0.50; P = 0.609$). Fusarium ear rot severity was significantly affected by hybrids at Hoytville ($F_{3, 24} = 3.89; P = 0.021$), but not at Wooster ($F_{3, 33} = 2.67; P = 0.063$). Contrast analysis showed a significant reduction in ear rot severity on Bt Hybrids compared to non-Bt hybrids at both Hoytville ($F_1, 24 = 5.04; P = 0.034$) and
Wooster ($F_{1,33} = 5.77; P = 0.022$). A significant reduction in Fusarium ear rot severity occurred on full season non-Bt hybrid compared to short season non-Bt hybrid at Hoytville on middle and late plantings (Fig. 4.5C). Like in 2006, the increase in Fusarium ear rot severity was more associated with the short season non-Bt hybrid DKC 51-45 on later planted maize at both locations (Fig. 4.5C and D).
Fig. 4.5. Fusarium ear rot severity (mean number of infected kernels/ear) recorded on hybrids planted on different dates in 2006, 2007 and 2008 at Hoytville and Wooster. Letters associated with planting date indicate that a significant difference occurred, with hybrid means having the same letter not significantly different (Fisher protected LSD, $\alpha = 0.05$). PD = planting date; PDH = planting date x hybrid interaction; *, and ** denote significance at $P \leq 0.05$ and $P \leq 0.01$ respectively. SS = short season, LS = full season.
In 2008, there was no significant planting date × hybrid interaction effects at Hoytville (F3, 12 = 1.68; P = 0.225), but a significant interaction occurred at Wooster (F6, 27 = 3.42; P = 0.012). No significant differences among planting dates occurred at Hoytville (F1, 4 = 0.24; P = 0.652), but a significant reduction in ear rot severity occurred on earlier planted maize compared with late planting at Wooster (Fig. 4.5F). Fusarium ear rot severity did not vary among hybrid treatment at Hoytville (F3, 12 = 1.21; P = 0.346), but did at Wooster (F3, 27, 6.19; P = 0.002). There was no significant differences between Bt and non-Bt hybrids at Hoytville (F1, 12 = 4.08; P = 0.066), but a significant reduction in Fusarium ear rot severity occurred on Bt hybrids at Wooster (F1, 27 = 16.12; P = 0.0004). Hybrid relative maturity did not affect the severity of Fusarium ear rot at Hoytville (F1, 12 = 0.48; P = 0.500; Wooster: F1, 27 = 0.34; P = 0.563). At both locations, within planting date comparisons indicated that a significant reduction in ear rot severity associated with Bt maize occurred on late planted maize (Fig. 4.5E and F).

Incidence of Gibberella ear rot. In 2006, no significant planting date × hybrid interaction occurred at either Hoytville (F6, 33 = 1.14; P = 0.363) or Wooster (F6, 33; P = 0.31 P = 0.929). The incidence of Gibberella ear rot was not affected by planting date in 2006 (Hoytville: F2, 33 = 0.27; P = 0.766; Wooster: F2, 33 = 0.17; P = 0.844), but hybrid treatment did have an effect (Hoytville: F3, 33 = 6.95; P = 0.0009; Wooster: F3, 33 = 10.46; P<.0001) (Fig. 4.6A and B). A significant reduction in incidence was obtained from Bt hybrids compared with non-Bt hybrids at both Hoytville (F1, 33 = 16.13; P = 0.0003) and Wooster (F1, 33 = 30.16 P <.0001). At either location, hybrid maturity did not have significant effects on incidence of Gibberella ear rot (Hoytville: F1, 33 = 3.06; P = 0.089; Wooster: F1, 33; = 0.31 P = 0.583).
In 2007, planting date × hybrid interaction occurred at both Hoytville ($F_{6, 22} = 4.04; P = 0.0071$) and Wooster ($F_{6, 35} = 2.78; P = 0.026$). The interactions showed an increase Gibberella ear rot incidence as planting was delayed at both Hoytville and Wooster (Fig. 4.6C and D). Hybrid treatment had significant effects on incidence at both locations (Hoytville: $F_{3, 22} = 11.72; P < 0.0001$; Wooster: $F_{3, 35} = 16.93; P < 0.0001$). This difference was mainly due to a significant reduction in ear rot incidence obtained from Bt hybrids compared to non-Bt hybrids at both Hoytville ($F_{1, 22} = 34.18; P < 0.0001$) and Wooster ($F_{1, 35} = 50.29; P < 0.0001$). There was no significant difference in ear rot incidence between full season and short season at either location (Hoytville: $F_{1, 22} = 0.97; P = 0.335$, Wooster: $F_{1, 35} = 0.06; P = 0.809$).

In 2008, significant differences were not observed among planting date × hybrid interaction (Hoytville: $F_{6, 36} = 1.44; P = 0.228$; Wooster: $F_{6, 36} = 1.44; P = 0.228$), planting dates (Hoytville: $F_{2, 36} = 0.10; P = 0.9059$; Wooster: $F_{2, 36} = 0.10; P = 0.906$), or hybrids (Hoytville: $F_{3, 36} = 0.42; P = 0.737$; Wooster: $F_{3, 36} = 0.42; P = 0.737$). Multiple comparisons using planting date x hybrid interaction revealed a significantly lower incidence of ear rot on Bt maize planted on 22 May and 9 June at Hoytville and 1 May at Wooster (Fig. 4.6E and F).

Gibberella ear rot severity. In 2006, planting date × hybrid interaction was not significant at either Hoytville ($F_{6,33} = 1.02; P = 0.432$) or Wooster ($F_{6,33} = 0.15; P = 0.989$). Severity of Gibberella ear rot was not affected by planting date at either location (Hoytville: $F_{2, 33} = 0.07; P = 0.9315$, Wooster: $F_{2, 33} = 0.02; P = 0.977$) (Fig 4.7A and B). However, severity varied significantly between hybrid treatment at Hoytville ($F_{3, 33} = 6.03; P = 0.002$) and at Wooster ($F_{3, 33} = 9.75; P < 0.0001$). A significant reduction in ear
rot severity was obtained on Bt hybrids compared with non-Bt hybrids at both Hoytville ($F_{1,33} = 11.97; P = 0.002$) and Wooster ($F_{1,33} = 29.06; P <0.0001$). Compared with short season hybrids, full season hybrids had significantly lower ear rot severity at Hoytville ($F_{1,33} = 4.65 P = 0.039$), but no significant differences occurred at Wooster ($F_{1,33} = 0.19; P = 0.669$).

In 2007, a significant planting date × hybrid interaction occurred at both Hoytville ($F_{6,18} = 4.80; P = 0.004$) and Wooster ($F_{6,27} = 6.89; P = 0.0002$). Severity of Gibberella ear rot significant decreased on early planted maize compared with late planting at both locations (Fig. 4.7C and D). At both locations, severity of ear rot differed between hybrid treatments (Hoytville $F_{3,18} = 5.47; P = 0.008$, Wooster: $F_{3,27} = 19.90; P <0.0001$). Severity of Gibberella ear rot was significantly lower on Bt hybrids than non-Bt hybrids at both Hoytville ($F_{1,18} = 48.26; P<.0001$) and Wooster ($F_{1,27} = 58.90; P<0.0001$). An increase in ear rot severity occurred on full season hybrids planted on early June at both locations (Fig. 4.7C and D). Examination of the planting date by hybrid interaction showed that at both locations, ear rot severity increased as planting was delayed, but was reduced on Bt hybrids (Fig. 4.7C and D).
Fig. 4.6. Mean percentage of plants with Gibberella ear rot symptoms recorded on hybrids planted on different dates in 2006, 2007 and 2008 at Hoytville and Wooster. Letters associated with planting date indicate that a significant difference occurred, with hybrid means having the same letter not significantly different (Fisher protected LSD, $\alpha = 0.05$). PD = planting date; PDH = planting date x hybrid interaction; *, and ** denote significance at $P \leq 0.05$ and $P \leq 0.01$ respectively. SS = short season, LS = full season.
In 2008, no planting date × hybrid interaction occurred at either location (Hoytville: F3, 12 = 2.18; P = 0.1437 Wooster: F6, 36 = 1.75; P = 0.137). The severity of Gibberella ear rot did not vary among planting dates at either Hoytville (F1, 2 = 1.42; P = 0.356) and Wooster (F2, 36 = 0.46; P = 0.633). Significant hybrid effects on severity of ear rot were observed at Hoytville (F3, 12 = 10.75 P = 0.001), but not at Wooster (F3, 36, = 1.08; P = 0.369). Severity of Gibberella ear rot was significantly lower on Bt hybrids compared to non Bt hybrids at Hoytville (F1, 12 P = 31.08; P = 0.0001), but no such difference was observed at Wooster (F1, 36 = 2.72 P = 0.108). However, multiple comparisons indicated a significantly lower ear rot severity on maize planted on 1 May and 9 June at Wooster (Fig. 4.7F). No significant effects were observed among hybrid maturities (Hoytville: F1, 12 = 1.17; P = 0.301, Wooster:, F1, 36 = 0.02 P = 0.879).

4.5 DISCUSSION

Stalk rots

The influence of planting date on stalk rot varied among years and locations during this study. In 2007, stalk rot incidence and severity significantly increased as planting was delayed at both Hoytville and Wooster (Fig. 4.1C and D). In three out of six comparisons, an increase in stalk rot was observed on middle planted maize but significantly declined on late planted maize (Fig. 4.1B, E and F). A similar trend was observed on the percentage of plants that were infected with stalk rot pathogens (Fig. 4.2). Variability in the effects of planting date on stalk rots between years and locations in this study illustrate the difficulties of explaining or predicting the distribution of stalk
rot of maize across location and time (Dodd 1980). While late planting is known to subject maize plants to pest and disease pressure (Wiatrak et al. 2005, Jarvis et al. 1986), delayed planting has also been reported to decrease the incidence of stalk rot (Pappelis and Boone 1966, Whitney and Mortimore 1957). The stalk rot incidence is mostly related to the growth stage of the maize plant (Whitney and Mortimore 1957) and the interactions of biotic and abiotic stresses that predispose the plants to infection, particularly following anthesis when carbohydrate shortages can occur (Dodd 1980, Smith and White 1988, Sobek and Munkvold 1999).
Fig. 4.7. Gibberella ear rot severity (mean number of infected kernels/ear) recorded on hybrids planted on different dates in 2006, 2007, and 2008 at Hoytville and Wooster. Letters associated with planting date indicate that a significant difference occurred, with hybrid means having the same letter not significantly different (Fisher protected LSD, $\alpha = 0.05$). PD = planting date; PDH = planting date x hybrid interaction; **, and *** denote significance at $P \leq 0.01$ and $P \leq 0.001$ respectively. SS = short season, LS = full season.
One of the most important biotic factors that predispose maize plants to infection by stalk rot pathogens is the infestation by European corn borer larvae (Chiang, and Wilcoxson 1961). Because of the association between European corn borer injury and fungal stalk rot, management of this insect is recommended as one component of an integrated stalk rot management strategy (Munkvold 1996). Transgenic field maize has been genetically engineered to express an insecticidal protein from *Bacillus thuringiensis* (Bt) to prevent damage by stalk-boring pests, in particular European corn borer, southwestern corn borer, and southern cornstalk borer (Koziel et al. 1993). Gatch et al. (2002) reported a significant reduction in stalk rot on Bt hybrids compared to their non Bt isolines, but the overall effect of Bt maize on stalk rot occurrence was variable among Bt hybrid types. In our study, we included three sequential plantings of short and full season hybrids, each maturity represented by a Bt and non-Bt hybrid. Significant correlations between European corn borer injury and stalk rot symptoms occurred, indicating that European corn borer significantly contributed to occurrence of the disease observed. When stalk rots were higher on late planted maize (in 2007) at both locations, the correlation coefficients were higher because tunneling also increased on late planted maize in that year.

Apart from 2008 at Hoytville, we observed a significant reduction in stalk discoloration and incidence of maize stalk on Bt hybrids compared with non-Bt genotypes (Fig. 4.1 and 4.2). We also observed a significant reduction in stalk rot on full season hybrids compared with short season hybrids. Earlier studies by Whitney and Mortimore (1957) reported that early maturity hybrids of maize became infested before late maturing ones. They suggested that although root and stalk rot pathogens may be
present in roots, stalk rotting does not occur until the host reaches physiological maturity. The mechanism to explain the differences in stalk rot between full season and short season hybrids is beyond the scope of this research because apart from injury by European corn borer, we did not quantify other stress factors that predispose plants to stalk rot pathogens. Stalk injury by European corn borer did not show any significant reduction on full season hybrids compared with short season hybrids (Chapter 3), and therefore, is unlikely to have significantly contributed to the differences in stalk rot between hybrid maturity groups.

The significant reduction in stalk rot on Bt hybrids could be caused by factors other than the Bt gene. The correlations between ECB tunneling and stalk rot symptoms were significantly positive in two out of three years, and in some cases low (Table 4.1). The mostly likely factor that could influence the results of this study would be the complex interaction between stalk rot and the environment (Dodd 1980). In the absence of insect damage, there are still many plant stresses that contribute to stalk rot development, and they vary unpredictable between years and locations (Gatch et al. 2002). In 2006, many areas in Ohio experienced an extended period of dry weather during the grain fill period (Paul et al. 2006) (Appendix A), therefore predisposing plants to increase infection by stalk rot pathogens compared with most years of the study (Fig. 4.1A and B). Albeit these are complicating factors, our results showed that Bt maize hybrids have the potential to contribute to stalk rot management especially when European corn borer tunneling is higher and planting is delayed.
Ear rots

The significant effect of planting date on both Fusarium and Gibberella ear rot levels was variable during the three years of the study. In 2007, the planting date × hybrid interaction showed a significant increase in severity of Fusarium ear rot as planting was delayed at Hoytville in 2007 and at Wooster in 2008 (Fig. 4.5C and D). A significant increase in severity of Gibberella ear rot also increased as planting was delayed in 2007 at both Hoytville and Wooster (Fig. 4.7C and D). Most researchers recommend planting maize early in the growing season to avoid or reduce factors like drought stress and insect injury that create environments conducive for maize ear infection by ear rots pathogens (Jones et al. 1981, Wiatrack 2005, Blandino et al. 2008). The variation of the effect of planting date on incidence of ear damage by European corn borer and levels of ear rot in our study and previous ones (Alma et al. 2005, Blandino et al. 2008) suggest that the key might be to time the onset of reproductive growth in maize to environmental conditions that are less stressful to the plant and less favorable to fungal development regardless of the time of the year (Bruns 2003).

Significant correlations observed between European corn borer ear damage and maize ear rots in this study have also been reported by other researchers (Munkvold 1997, Sobek and Munkvold 1999). Management of an insect pest like European corn borer using insecticides has been reported to reduce Fusarium ear rot and fumonisin contamination (Dowd 1999). In our study, we used maize hybrids expressing the Cry1Ab Bt protein to control European corn borer and associated maize ear rots. A significant reduction in the levels of European corn borer damage to ears and incidence and severity of both Fusarium and Gibberella ear rots occurred on Bt maize compared
with non-Bt hybrids. Nevertheless, it is probable that the significant reduction in ear rot on Bt hybrids could be caused by other factors related to hybrid genetic makeup or the prevailing weather conditions during the growth of the plants and not just due the Bt gene.

Unlike in a previous study by Munkvold et al. (1997), we compared hybrids from two maturity groups and found significant reductions in ear damage by European corn borer and severity of both Fusarium and Gibberella ear rot on full season hybrids compared with short season ones (Fig. 4.4–4.6). Given that significant correlations occurred among European corn borer ear injury and maize ear rots, it is probable that the reduction in ear rots on full season hybrids is associated with the reduced ear injury from corn borers observed on these hybrids. However, solid possible explanation relating to hybrid genetic ability to reduce incidence and severity of ear rots need further assessment.

This research has shown that transgenic maize with the Bt gene consistently reduced incidence and infection of kernels and stalks by fungal pathogens associated with stalk and ear rots of maize compared with non-Bt hybrids. Transgenic Bt hybrids significantly reduced stalk and kernel injury by European corn borer, thus hindering an important pathway for infection by fungal pathogen complex associated with stalk and ear rots. When the interaction between planting date and hybrid treatment was significant, the benefit of using Bt hybrids to reduce both stalk and ear rots was more evident as planting was delayed. Reduction in stalk rot on Bt maize is important because yield losses are known to occur due to reduced grain fill caused by pathogens invading maize stalks prior to physiological maturity (Koehler 1960). Lower incidences and
infections of kernels by fungal pathogens in transgenic hybrids can result in an increase in grain quality, with less molds and reduction of potential development of mycotoxins associated with kernel diseases.

4.6 REFERENCES


The Ohio State University Extension Bulletin # 631-98. Field Crop Disease Management. (http://ohioline.osu.edu/b631/index.html). The Ohio State University Columbus Ohio.


CHAPTER 5

CONCLUSIONS

5.1 Synthesis

The central goal of this thesis was to address pest problems within a maize cropping system that are unpredictably influenced by circumstances that occasionally lead to planting field maize beyond the normal optimum planting time frame. The results of the study supported our hypothesis that manipulating planting date would facilitate the evasion of pests by the maize crop, and that any increase in pests associated with crop phenology can be reduced by intervention with pest management tactics, including the use of transgenic maize and seed treatments. The results also supported the hypothesis that yield from short season hybrids would be equal to or higher than full season hybrids when planted late and European corn borer populations are high.

Delaying planting until early June revealed the paradox of dealing with a pest complex associated with individual crop species like corn. When planting was delayed, the maize crop benefited from reduced larval feeding by corn rootworm injury and injury from other early season pests (Chapter 2). Late planted maize also had reduced foliar injury caused by first generation European corn borer larval feeding compared with
earlier planted maize (Chapter 3). The benefit of reduced root injury associated with late planting was compromised in some instances when adult corn rootworms feeding on late planted maize caused increased silk clipping and resulting in poor seed set and reduced yields (Chapter 2). Unlike with root injury due to corn rootworm larval feeding, European corn borer damage to maize stalk and related diseases (ear and stalk rots) increased on late planted maize compared to early planted maize (Chapter 3 and 4). The increase in stalk tunneling and ear damage by second generation larvae and consequent stalk and ear rots on late planted maize was associated with increased egg masses laid preferentially on late planted maize by moths from first generation brood.

In a farming situation, this pest complex will not be in different experimental plots but on the same field of corn. The goal of farmers is to obtain the longest period of plant protection against pests using management tactics applied in a timely manner, not only to reduce yield loss but also to reduce grain quality loss related to ear rot pathogens. The study showed that when injury caused by corn rootworm was high and planting was early, the use of transgenic Bt maize and seed treatment translated into significant yield advantage.

Planting maize beyond the optimum late April to early May time frame in Eastern USA Corn Belt is not a normal cropping system, but is often caused by unforeseen weather conditions like excessive soil moisture (Nielsen et al., 2002, Jarvis 1986). The results from this thesis do not support the recommendation that growers choose planting late regardless of the prevailing circumstances in their area in order to reduce root injury by corn rootworm. Yield potential of the maize crop is also related to growing degree days available to maize plants during a particular season. In European corn borer and
disease management systems, the use of transgenic maize translated into a yield advantage when populations were high and planting was delayed (Chapter 3 and 4). The results suggest that when both European corn borer and corn rootworm populations are prevalently high enough to cause economic damage and planting is delayed until early June, the benefit of pest management would be significant in controlling European corn borer but not in controlling corn rootworm larval injury to roots.

Economic analysis of using transgenic Bt maize to manage corn rootworm and European corn borer had been done by other authors (Pilcher and Rice 2003, Baute et al. 2002, Alston et al. 2002). However, the significant reduction in yield was related to injury by European corn borer when populations were high and injury increased.

European corn borer populations fluctuated from year to year between locations during the three years of this study. Therefore, economic benefit of using Bt technology would be gained by growers located in regions where its populations are consistently high each year, otherwise the return on investment would be insignificant if its density was below an economic threshold (Baute et al. 2002). As transgenic Bt maize hectarage increases, populations of European corn borer are predicted to decline in succeeding years (Storer 2003, Storer 2008, Hutchison 2008), thereby reducing the expectation of high infestations. The decrease in the occurrence of high European corn borer infestations will decrease the opportunity for economic return on investment if the fixed seed premiums stay the same and the value of maize remains stable or declines.

Transgenic rootworm maize (event MON 863) is reported to provide a significant yield advantage relative to no insecticide over typical ranges of corn rootworm populations (Mitchell, 2002). In the current study, the use of transgenic Bt hybrids and
seed treatment to protect maize roots from injury by corn rootworm larvae translated into yield advantage only in 2007 at Wooster when higher root injury ratings occurred. While the two experimental locations (South Charleston and Wooster) cannot represent the entire State of Ohio or the Corn Belt, the results suggest that the use of Bt maize and seed treatment is not based on an economic threshold, and economic injury levels would be uneconomic and ecologically unsound in most years. The use of sampling and economic thresholds has been shown to slow the evolution of resistance to transgenic insecticidal crops compared with strategies of planting transgenic maize every season (Crowder et al. 2006). There has been an escalating demand for triple-stacked maize hybrids even in areas where corn rootworm and European corn borer do not present a persistent economic threat (Gray and Onstad 2008). The increase in prophylactic use of Bt hybrids is possibly related to ease of using the technology, and therefore, farmers might be inclined to plant transgenic crops every season rather than implementing sampling protocols that are deemed costly and time-consuming. The question is how long the principles of integrated pest management can be overlooked at the expense on using a single technology before reverting to another costly ecological backlash similar to that associated with pesticide misuse.

Planting date significantly influenced the development of pest thus an important interaction might also exist between the planting date and agronomic practices, such as plant density, soil fertility and maturity group of the hybrids, which could influence the vegetative growth of the plant and the length of the maturation period. The performance of short season and full season hybrids varied in this study and the differences were often influenced by date of planting. When planted earlier in the season, full season hybrids
yielded more than short season hybrids, but in late planting environments, no differences occurred and in some instances, higher yield were obtained from short season hybrids. The growing degree days for maize during the study declined significantly as planting was delayed, thus shortening the growing season for full season hybrids which require full season for maximum yield. In one out of five years, a significant number of maize hectares are planted late in Ohio, usually as the result of excessive rainfall (Peter R. Thomison et al. unpublished data). In 2008, we could not implement early planting treatment at Hoytville due to heavy moisture during early May. When these circumstances occur and planting is delayed in an area with high populations of European corn borer, choosing short season hybrids with Bt gene may be more advisable because they mature early, facilitating field drying and escaping frost like at Wooster during this study.

5.2 Possible future research directions

The study could not address some existing questions that rose during the current research because of resource and time limitations. The questions had to do with both economic, agronomic, and pest issues. On the pest aspect, this research showed empirically that ear damage by European corn borer, and both stalk and ear rots tended to be lower on full season hybrids compared with short season hybrids. However, the mechanisms that cause these are not clear and raise some questions. Is the reduction in stalk rot on full season hybrids related to their genetic ability to resist environmental stress associated with stalk rot epidemics? Because short season hybrids mature earlier, did they have more exposure to adverse weather conditions during anthesis than full
season hybrids? Are the short season hybrids used in the study just genetically susceptible to stalk rots? The causes of reduction in incidence of ear damage by European corn borer and subsequent ear rots on full season hybrids need to be investigated as well. The incidence and severity of ear rots were correlated with European corn borer ear damage. Do full season hybrids display any antixenosis against European corn borer which resulted in fewer infested ears and consequently less ear rot? Is reduction in ear rots just due to genotypic abilities of full season hybrids and has nothing to do with ear damage by European corn borer? Are the differences between the two hybrids maturity groups with respect to both stalk and ear rots just due to sheer experimental error?

On agronomic issues, the study showed that short season hybrids yielded comparably with full season hybrids. It would informative to address the economic advantage of choosing short season hybrids over full season hybrids, and more importantly, the switch by farmers from maize to soybean production during late planting environments. Such research needs a systems approach that includes a multi disciplinary team that can address detailed issues related to maize production and pests (including weeds) in late planting situations. Development of an enterprise budget will help to determine the economic viability of late planting of maize relative to soybean. It is important to continue to determine the economic and sustainability of prophylactic planting of Bt hybrids without considering the principles of integrated pest management. Or are we saying that IPM has become irrelevant to maize production system in the biotechnology era?


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http://www.ianr.unl.edu/pubs/insects/ec1563.htm

Appendix A: Tables of temperature and rainfall data
<table>
<thead>
<tr>
<th>Environment location</th>
<th>Year</th>
<th>May</th>
<th>June</th>
<th>July</th>
<th>Aug.</th>
<th>Sept.</th>
<th>Total</th>
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<tr>
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<td>148.2</td>
<td>103.6</td>
<td>164.8</td>
<td>32.1</td>
<td>72.8</td>
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<tr>
<td></td>
<td>2007</td>
<td>67.5</td>
<td>50.3</td>
<td>147.7</td>
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<td>109.2</td>
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<td>100.8</td>
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<td></td>
<td>2007</td>
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<td>102.1</td>
<td>63.1</td>
<td>36.4</td>
<td>478.2</td>
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Table 1. Monthly rainfall totals (mm) from May to September at three locations from 2006 to 2008
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<th>Environment location</th>
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<th>June</th>
<th>July</th>
<th>Aug.</th>
<th>Sept</th>
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</tbody>
</table>

Table 2. Monthly average air temperatures (Celsius) from May to September at three locations from 2006 to 2008