

PEST MANAGEMENT STUDIES OF EARLY SEASON AND STALK-BORING
INSECTS ON CORN IN VIRGINIA

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ABSTRACT

Separate field studies were started in fall 2005, which continued through fall 2007, to investigate the effect of different levels of European corn borer tunneling on yield in corn grown for grain and to predict spring infestation levels of early season soil insects, specifically white grubs (Coleoptera: Scarabaeidae) and wireworms (Coleoptera: Elateridae) in cornfields.

In the first study, model variables included corn growth stage and larvae per plant. In both years of this study, larvae per plant had a significant effect on grain yield. Grain yield was reduced by 13.1 and 3.65% in plants infested with four larvae per plant in 2006 and 2007, respectively. For 2006, linear regression models provided average percent yield loss per larva per plant at 4.1, 6.8, and 1.8% during late vegetative (V12), early silking (R1), and blister (R2) growth stages, respectively. Economic injury levels (EILs) were calculated based on average percent yield reductions across each growth stage and year.

In the second study, no significant differences were detected in both fall and spring between two sampling methods after correcting for differences in sampling volume. Strong correlations were observed between fall and spring grub densities in both years. In 2006, fields with grub densities above the spring nominal threshold had significantly greater stand and yield in the Poncho 1250 (1.25 mg clothianidin / kernel) treatment when compared to the Poncho 250 (0.25 mg clothianidin / kernel) and untreated plots. This information was used to develop fall EILs and economic thresholds for white grubs.

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LITERATURE REVIEW

European Corn Borer

European corn borer (ECB), *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae), is an introduced pest that was imported via broomcorn in the early 1900s (Mason et al. 1996). Following its introduction, the ECB has dispersed across the United States, west past the Rockies, north into Minnesota and southern Canada, and south through Mississippi and Alabama. For decades the ECB has posed a potential risk to corn growers in the Corn Belt and the eastern U.S., costing growers annually more than one billion dollars in management and damage related costs (Mason et al. 1996). However, because of years of infrequent and sporadic infestations and the cost and effort associated with conventional management, most growers ($\approx 90\%$) have elected not to aggressively control this pest (Calvin 1995). Therefore, because of the importance of this pest in the Midwest and the eastern U.S., research continues to improve management of ECB by resolving the relationship between the life history and biology of ECB and corn growth and development.

Although the ECB has spread throughout the U.S. without much impediment, the recent commercial introduction of Bt (*Bacillus thuringiensis*) corn hybrids now provides farmers with a viable control option. Biological control has been investigated for managing ECB on field corn, but augmentative releases of natural enemies were not considered an effective control option in corn until recently (Kuhar et al. 2004, Youngman and Tiwari 2004). The variation in ECB voltinism across different climates combined with a broad host range compliment this insect's ability to adapt to a wide coverage of climates and habitats. Though optimal development occurs on corn, the ECB

has a host range of more than 200 plant species, and is able to complete more than three generations per year depending on temperature and location (Mason et al. 2006, Youngman and Tiwari 2004). The life history of ECB has been described by several authors (Mason et al. 1996, Calvin and Van Duyn 1999, Youngman and Tiwari 2004). ECB growth and development described by Calvin and Van Duyn (1999) is discussed in the following text.

ECB larvae that reach the fifth instar in late summer to fall will enter diapause and overwinter before reaching the pupal stage in the spring. Pharate pupae overwinter inside weed hosts or in the surface debris on the soil such as corn stubble, stalks, and cobs. A key climatic variable for ECB development is growing degree-days (ECB base threshold 10 °C) (Calvin 1995). When spring temperatures exceed 10°C in the spring, pupal development (7-10 days) will resume, and the adult female will emerge, mate, and lay eggs on appropriate plant hosts, giving rise to the first generation. Although ECB can complete development on plant hosts other than corn, the likelihood of survival is directly related to stem diameter of the host where survival is almost 40-fold greater on corn than host plants with smaller stem diameters (Losey et al. 2002). First generation eggs will hatch within 5-7 days depending on temperature. If the host plant is corn, the young larvae will feed in the whorl and or tunnel into leaf midribs. Mid to late third instars will tunnel into the stalk and pupate (Mason et al. 1996). Upon adult eclosion, first generation moths will reproduce and lay second generation eggs on corn that is commonly in the silking stage or later (Calvin and Van Duyn 1999). Because of pest introduction during critical plant development stages before and after silking, the second generation feeding generally will cause the greatest damage to corn (Mason et al. 1996). Along the Atlantic

coast, the E-multivoltine genetic type is the most prominent with the first flight occurring early in the spring and the second flight occurring in mid-summer (Calvin 1995). The difference between the E and Z-type ECB is the chemical used in sexual communication, where different populations of males respond to different isomers (E or Z) of 11-tetradecenyl acetate that are released by the female (Calvin 1995).

European Corn Borer Management in Corn

It is important to understand ECB life history and biology to establish an effective management program. Conventional management requires consideration of control costs, expected yields, seasonal ECB development patterns, timing of ECB infestation, and regional growing conditions (Calvin et al. 1988, Mason et al. 1996, Hyde et al. 1999). In the Mid-Atlantic, Northeast, and Midwest Corn Belt, ECB generally complete two to three generations per year, with the second generation being of the most concern resulting in both physiological and mechanical yield losses (Lynch 1980, Bode and Calvin 1990, Youngman and Tiwari 2004).

Control methods for ECB consist of aerial or ground application of an insecticide in cornfields identified with above threshold egg mass counts (Mason et al. 1996). Optimal control of ECB requires application at an approximate number of days following hatch of first instar larvae, in which the length of the eclosion period (21 to 50 days) may strongly alter the extent of control (Calvin 1995). The best management of ECB requires that insecticide application timing work in tandem with scouting, while consideration of other variables like expected yield and spraying costs determine whether to treat (Hyde et al. 1999). It must be mentioned that conventional controls (insecticides and Bt sprays) provide 50 to 80% control of ECB typically resulting in a negative net return from the

costs associated with scouting and application (Calvin 1995, Mason et al. 1996). However, since the introduction of Bt corn hybrids in 1996, the convenience of planting hybrids that resist ECB feeding injury has completely changed the pest management scenario for ECB (Pilcher et al. 2002). Seed choice is important since much of Bt efficacy is based on how the Bt is expressed in the plant. Walker et al. (2000) evaluated the effectiveness of genetically altered corn on late-instar ECB survival. They found the level of protection offered from Bt technology varied with different events. Bt events correspond to the difference in toxic core fragments of the Bt gene its interaction with proteases and enzymes in the insect. Events Bt11, MON810, MON802, and CBH351 demonstrated protection against late instar ECB from early vegetative (V8) through physiological maturity (R6); however, event 176 provided excellent control of first-generation ECB through the early to late vegetative stages, where efficacy declined at the onset of silking (Walker et al. 2000). ECB larvae were able to compensate for the sub-lethal effects of event 176, which suggests that Bt hybrids expressing event 176 may increase the incidence of resistance (Zoerb et al. 2003). Therefore, the importance of Bt hybrid selection strongly relies on the probability of infestation by first and or second generation ECB and the level of desired control against each generation.

In a survey of growers in the Corn Belt, Pilcher et al. (2002) observed a four-fold increase in Bt corn acreage over three years following the introduction of commercial Bt hybrids in 1996. This high adoption rate was partly influenced by grower expectations of less insecticide exposure, less insecticide in the environment, and higher yields (Rice and Pilcher 1998, Pilcher et al. 2002). Although planting Bt corn seed is straightforward, it is not possible to predict at the time of planting whether a field will sustain enough ECB

pressure to benefit from the additional cost of \$17-25 per ha for the Bt corn seed (Hyde et al. 1999). In order for growers to break even with current Bt seed premiums in Indiana, Hyde et al. (1999) determined that growers must experience >40% probability of infestation.

Surveys from 1997-02 by Youngman et al. (1998, 1999, 2000), and Youngman and Laub (2002) on second generation ECB damage in conventional (i.e., non-Bt) cornfields in eastern (1997-99) and western Virginia (2000-02) have provided relevant insight on the role for Bt corn in Virginia. Less than 7% of the fields (n = 172) surveyed in eastern Virginia experienced economic damage from ECB (Youngman et al. 1998, 1999, 2000). The western Virginia survey findings, however, revealed a different picture (Youngman and Laub 2002). Of the 126 fields in that survey nearly 25% experienced economic damage. For the fields in these surveys (1997-2002), economic damage was based on a threshold of 1 or more tunnels per stalk with tunnel lengths greater than 2.5 cm. Based on these findings, the ECB may be a higher management priority in western Virginia where most corn is grown for silage. The ECB yield relationship for Virginia-grown silage was reported recently by Tiwari (2007). However, the benefit of Bt hybrids in eastern Virginia may not be realized until years of moderate to high infestation (Hyde et al. 1999, Baute et al. 2002).

European Corn Borer Infestation in Corn Grown for Grain

Most research on the relationship of ECB infestation levels and economic loss in corn has focused on corn grown for grain (Youngman and Tiwari 2004). The relationship between ECB feeding injury and grain yield loss in corn has been reported in numerous studies (Patch et al. 1951, Lynch 1980, Lynch et al. 1980, Calvin et al. 1988, Bode and

Calvin 1990). Patch et al. (1951) and Lynch (1980) concluded that ECB injury induced to the plant resulted in greater yield loss from reduced ear size than from non-harvestable ears occurring because of stalk breakage. Corn growth stages are depicted in Figure 1 (NSW 1998). Studies have shown ECB injury affects grain yield at different growth stages (Lynch 1980, Calvin et al. 1988, Bode and Calvin 1990), where Lynch (1980) found greater physiological yield losses in the late vegetative growth stage (late whorl, V12) than during the early vegetative (early whorl, V8) and blister (R2) growth stage; however, mechanical yield loss from broken stalks and non-harvestable ears was greatest at blister and was comparable to physiological loss. The relationship between corn plant development when ECB larvae initiate tunneling and time remaining to physiological maturity has been investigated by Calvin et al. (1988). In contrast to Lynch (1980), they found that tunneling initiated during the blister stage resulted in greater yield loss than during the stages before and after. In addition, as corn entered the grain fill period, the effects of tunneling decreased as time remaining to physiological decreased. Using a linear regression model, Bode and Calvin (1990) determined that greater yield loss occurred with initiation of ECB tunneling during mid to late vegetative stages (5.9% in 10-leaf; 5.0% in 16-leaf) of development in contrast to tunneling initiated during grain-fill (3.1% in blister; 2.4% in dough). A study from Baute et al. (2002) quantified the impact of ECB by level of tunneling. When tunnel lengths per plant were >6 cm, yield in non-Bt isolines was reduced \approx 5.0% compared to yield in Bt hybrids (Baute et al. 2002).

Part of the problem with linking these studies is the regional variation and intensity of ECB infestation from differences in voltinism, pest-plant synchrony, and climatic conditions (Calvin et al. 1988). In some areas, particularly in the central Corn

Belt, first generation ECB caused greater yield loss than second generation (Chiang et al. (1954) in Minnesota, Everett et al. (1958) in Iowa and Minnesota, Kwolek and Brindley (1959) in Iowa, Jarvis et al. (1961) in Iowa). In contrast, yield loss from second generation ECB has been more of a concern in the western Corn Belt and in the Northeast (Deay et al. (1949) and Patch et al. (1951) in Indiana, Calvin et al. (1988) in Kansas, Bode and Calvin (1990) in Pennsylvania, Dillehay et al. (2005) in Maryland and Pennsylvania). Dillehay et al. (2005) used the yield loss relationship described by Bode and Calvin (1990) as a model to predict yield loss on a regional scale for climatic zones from 1100-1700 degree-days (model base threshold of 12.5°C). Their model predicted \approx 2.69% yield loss from second generation ECB in non-Bt hybrids. As potential yield and corn market value increased in their model, the value of Bt corn hybrids increased (Dillehay et al. 2005).

Economic Injury Level for European Corn Borer

With the commercial introduction of Bt corn hybrids, the yield loss associated with ECB infestation has become clearer (Hyde et al. 1999, Pilcher et al. 2002). When the probability of infestation or resultant populations are moderate to high, growers are able to perceive the influence of Bt hybrids on yield (Baute et al. 2002, Pilcher et al. 2002). Likewise, when populations are low, generally no yield benefit is observed when costs of scouting are less than cost associated with Bt seed premiums (Calvin 1995). As a result of different management scenarios between Bt and non-Bt corn hybrids, economic analyses have been performed to determine the applicability of planting Bt corn for control of ECB (Bode and Calvin 1990, Hyde et al. 1999, Baute et al. 2002, Dillehay et al. 2005). The calculation of economic injury levels (EIL) is a tool that was

developed as a measure of insect pest density with respect to level of pest significance on its associated crop (Pedigo et al. 1986). Stern et al. (1959) defined the EIL as “the lowest pest population density that will cause economic damage.” An important parameter of the EIL is the economic threshold, which typically is set lower than the EIL to allow time for treatment that will prevent an economically damaging population (Luckmann and Metcalf 1982).

Several studies have applied the EIL concept to ECB on field corn (Calvin 1985, Bode and Calvin 1990, Mason et al. 1996, Myers and Wedburg 1999, Tiwari 2007). Several studies have reflected yield reduction by the number and length of stalk tunnels (Everett et al. 1958, Kwolek and Brindley 1959, Baute et al. 2002), while other studies have focused on the number of egg masses and or larvae per plant (Lynch 1980, Calvin et al. 1988, Bode and Calvin 1990, Tiwari 2007),

Equations presented by Calvin (1985) and Bode and Calvin (1990) examine the calculation of EILs at varying control costs and crop values for control of ECB. The EIL was calculated using the following formula from Bode and Calvin (1990) with modifications by Tiwari (2007): $NL = TC / CV \times PL \times PC$ (EIL = NL, number of larvae per plant; TC = total control cost of planting Bt corn seed (dollars/ha); CV = MV \times EY, CV = crop value, MV = expected market value (dollars/kg), EY = expected yield (kg/ha); PL = expected average proportional yield reduction per larva per plant from regression equations developed for each growth stage; PC = expected proportional control from planting Bt corn, where PC = 1 assumes 100% control.

White Grubs and Wireworms

Several soil insects are considered sporadic pests of germinating corn seed and may cause significant reductions in plant stands and yield (Youngman et al. 1993). These insects include wireworms (Coleoptera: Elateridae) and annual white grubs (Coleoptera: Scarabaeidae). Numerous species of wireworms are found in Virginia, predominantly in genera *Melanotus*, *Conoderus*, *Aeolus*, *Hemicrepidius*, and *Limonius* (Briggs 1980, Youngman et al. 1993). Annual white grubs have a 1-year life cycle in contrast to true white grubs that complete their life cycle in 2-3 years (McLeod et al. 1999). Annual white grubs commonly found in Virginia include *Popillia japonica* Newman, the Japanese beetle, *Maladera castanea* (Arrow), the Asiatic garden beetle, *Cyclocephala* spp., the northern and southern masked chafers, and *Cotinus nitida* (Linnaeus), the green June beetle. True white grubs are predominantly in the genus *Phyllophaga* (Briggs 1980). True white grubs cause significant economic damage in the Midwest Corn Belt (McLeod et al. 1999), though cause less injury than annual white grubs in the Mid-Atlantic region (Youngman and Tiwari 2004). Other major soil insects that negatively impact corn are seedcorn maggot, *Delia platura* (Meigen) (Diptera: Anthomyiidae), and corn rootworms, *Diabrotica* spp. (Coleoptera: Chrysomelidae) (Youngman and Tiwari 2004).

Annual white grubs and wireworms are the most important insect pests attacking corn seeds immediately following planting in Virginia (Briggs 1980, Youngman et al. 1993). Because of their particular life cycles and seasonal development, white grubs and wireworms usually cease to cause economic damage after the seedling stage. However, because they occur in the soil, their presence in fields and subsequent damage often go

unnoticed until too late. Thus, a sampling procedure that effectively predicts infestation of each group of insects is necessary for an effective pest management strategy.

Wireworm Sampling Methods

Soil sampling. Wireworm sampling methods have traditionally focused on analysis of soil samples. This labor intensive method was employed in many studies (Jones 1937, Ladell 1938, Finney 1946, Onsager 1969, Onsager and Day 1975). Soil sampling by excavation is time consuming because a large number of soil samples are required to reduce sampling error (Yates and Finney 1942). Mechanical innovations have been employed to reduce the amount of effort spent in taking and sorting through soil samples. Sieving of soil samples was investigated by Ladell (1936) and flotation devices were used to ensure all life stages of wireworms were collected (Ladell 1936, Salt and Hollick 1944, Cockbill et al. 1945). A device to aid in separation of turf from soil samples was employed by LaFrance and Tremblay (1964). Smith et al. (1981) used a self-propelled soil sampler that effectively extracted soil core samples. Sampling by core extraction was employed in many studies (Salt and Hollick 1944, Doane 1977, Lefko et al. 1998, Simmons et al. 1998), and sampling by square grids was employed in others (Jones 1937, Yates and Finney 1942, Onsager and Day 1975).

Soil sample extraction methods vary by surface area and depth of sample. Jones (1937) evaluated sample sizes of 9.6-cm square, 15-cm square, and 30-cm square to a depth of 30-cm, and found that 30-cm square samples gave better precision in estimating the number of wireworms per unit area. This is further broken down to show that twenty-five 30-cm square samples is equivalent to fifty 15-cm square samples and one hundred 9.6-cm square samples (Jones 1937, Fenton 1947).

Bait sampling. An alternative sampling method incorporates attractant baits into bait stations. Baits in combination with toxic compounds were used in studies as a means of wireworm control (Apablaza et al. 1977). Different mixtures of attractant baits elicit preference responses by wireworms. Doane et al. (1975) measured the response of *Ctenicera destructor* Brown to CO₂ gradients produced from germinating wheat seeds. Apablaza et al. (1977) studied various bait mixtures and concluded that wireworm species of the genus *Melanotus* in the Midwest were most attracted to wheat seeds or a corn-wheat mixture. Many studies justify the use of a corn-wheat mixture (Toba and Turner 1983, Parker et al. 1994, Simmons et al. 1998). Ward and Keaster (1977) used a mixture of corn and wheat seed to study different baiting techniques for spring sampling and found that bait stations with a heat trapping mechanism performed best in early spring. A standard bait station consists of a 1:1 corn-wheat seed mixture buried 10 to 15-cm deep and covered by black plastic used to aid in germination of the seed mixture (Youngman et al. 1993).

Youngman et al. (1993) developed the baited wire trap method for spring sampling of multiple soil-borne insect pests. Essentially, the baited wire trap method is unique because it relies on direct feeding injury as an indicator of the potential for subsequent stand loss in fields at risk to seed-feeding pest damage to germinating kernels. However, because of low pest densities across most of the fields in their study, Youngman et al. (1993) were not able to develop predictive models further suggesting that these insects are sporadic pests of corn in Virginia.

Bait sampling is a relative sampling method that uses no particular unit for an estimation of population size, although the samples may be used to make density

estimates (Southwood 1978). Relative sampling techniques are less labor intensive; however, in contrast to a one-time visit with soil sampling, bait stations require two visits for installation and evaluation. Finney (1946) criticized population estimates from bait stations, stating that it is hard to estimate from what area wireworms were attracted and if all wireworms in a given area were attracted to the bait.

Wireworm infestations are often associated with corn planted in fields coming out of sod, pastures, small grains, and forage crops (Simmons et al. 1998, Keaster and Riley 1999). The most common wireworms associated with corn in Virginia collected in a study by Briggs (1980) using both soil sampling and bait stations were *Melanotus* Eschscholtz, *Conoderus* Eschscholtz, and *Aeolus* (Say).

Youngman (2007) lists two pre-plant baiting methods for detection of wireworm infestation levels. The bait station method is two paired bait stations per 2.4 hectares containing a 1:1 corn/ wheat ratio, and soil insecticide application is recommended if 1 or more wireworms per bait station are collected (Munson et al. 1986, Lefko et al. 1998, Youngman 2007). With the baited wire trap method, one trap is installed for every acre of corn to be planted by burying 20 untreated corn seeds, and control is recommended if 10 percent or more of seeds show damage (Youngman et al. 1993, Youngman 2007).

White Grub Sampling Methods

Numerous phytophagous grubs in the family Scarabaeidae attack germinating corn seed in Virginia, though the most commonly occurring species from most common to least common are of the genera *Popillia*, *Phyllophaga*, and *Cyclocephala* (Briggs 1980). Annual white grubs, like *P. japonica*, are voracious feeders on germinating corn seed and roots of corn seedlings (McLeod et al. 1999). The Japanese beetle accounted

for 94.1% of white grubs in a study of white grubs associated with corn in Virginia (Briggs 1980). From a later study in Virginia, Youngman et al. (1993) found 73% of white grubs were Japanese beetle while no true white grubs were collected.

Soil sampling. Sampling for white grubs generally is done by soil extraction methods, although Youngman et al. (1993) used the baited wire trap to detect damage to germinating corn seed from an assemblage of soil insects including white grubs.

Absolute sampling methods are used to determine population densities where many samples of soil of specific surface area and depth are extracted and examined for white grubs (Southwood 1978). Fleming and Baker (1936) developed a sampling method for estimating Japanese beetle larval densities in the fall and spring by taking 30-cm square samples to a depth of 30-cm. This study further determined that a 30-cm square sample was more accurate in estimating grub populations, stating that sampling error grew larger as the sampling unit size was increased (Fleming and Baker 1936). However, even samples of this size are labor intensive.

White grubs typically exhibit a patchy distribution (Dalthorp et al. 1999). Studying the spatial distribution of the annual white grub *P. japonica* in turf grass, Dalthorp et al. (2000) found that high and low density patches of grubs tended to recur year after year in the same locations and noted that high density patches occurred with high local adult activity. Growers may then expect recurring white grub problems from historically active fields. Adult sampling is also used to monitor populations of insects and predict future infestation areas (Potter and Held 2002).

Acoustic technology has been used in recently developed sampling methods to identify soil insect infestation with acoustic sensors that detect the movement and feeding

noises of subterranean individuals. Mankin et al. (2000) made real-time visual and sound observations in the lab confirming sound pulses were produced by feeding and movement of soil insects. Sound pulses were analyzed, and interference was removed to separate insect-generated signals from non-insect sounds. One study showed 100 percent reliability of acoustic identification of active insects within about a 30-cm surface area diameter in soil in the laboratory (Mankin et al. 2000). Brandhorst-Hubbard et al. (2001) established a positive correlation between acoustical sampling and traditional soil sampling methods. Zhang et al. (2003) used acoustic identification to detect changing behavioral patterns of *Phyllophaga* and *Cyclocephala* grub species. This method is considered a nondestructive sampling method, unlike soil excavation. However, reported difficulties with detecting soil borne insects in the field included background noise, temperatures below 9° C, and inactive insects that reduced the reliability of results (Mankin et al. 2000, Brandhorst-Hubbard et al. 2001, Zhang et al. 2003).

Bait sampling and sampling recommendations. Similarly for wireworms, the baited wire trap can be used to detect fields at risk to white grub damage (Youngman et al. 1993). Based on the same wire trap method, if an average of 5% of seeds are damaged from evident white grub feeding, then a control measure is recommended (Youngman 2007). An alternative white grub sampling method for growers includes taking ten 30-cm square by 10-cm deep soil samples per 4.1-hectare field and justifies treatment if an average of 2 or more white grubs per soil sample is obtained (Youngman 2007).

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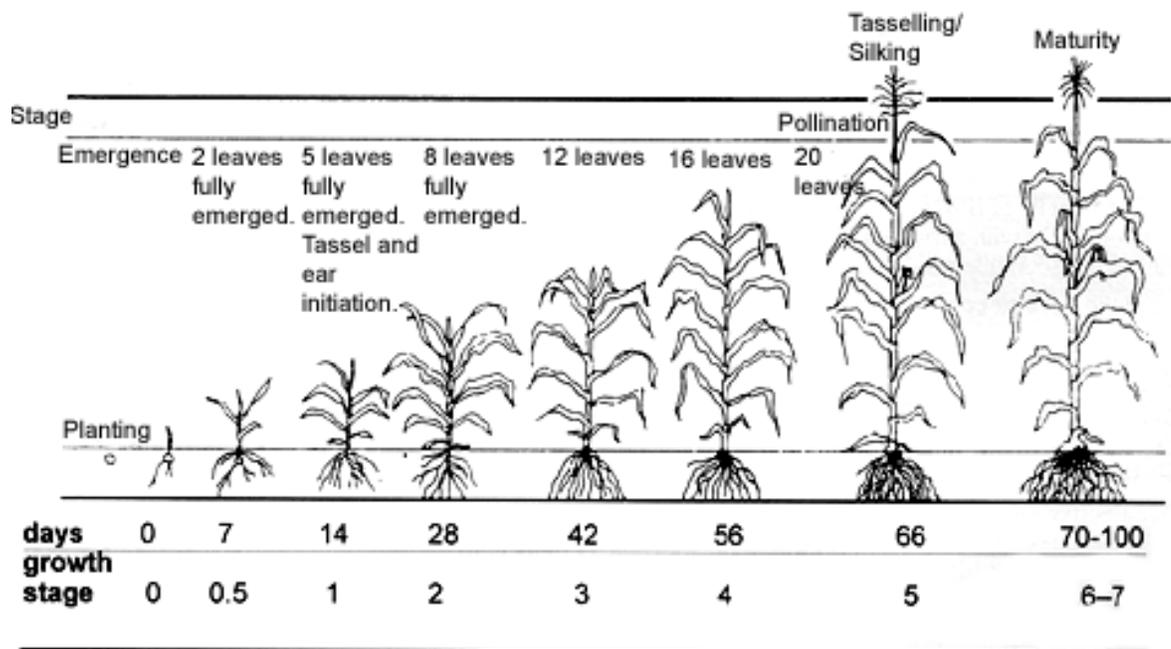


Figure 1. General depiction of corn growth stages from the time of planting to physiological maturity. Blister (R2) growth stage not shown; however, the stage follows directly after silking (R1).

CHAPTER 1

INTRODUCTION

Corn, *Zea mays* L., is an important agricultural commodity in the U.S. and is harvested for a variety of purposes including grain, silage, and seed production. Field corn is planted on more acreage in the Mid-Atlantic than any other crop, and corn grown for grain is the second most valuable crop in Virginia (NASS 2007, VASS 2008). In 2007, more than 218 thousand hectares of corn were planted in Virginia, the most area planted for grain since the late 1980s (NASS 2007). With increasing corn acreage in the Mid-Atlantic, effective management information is needed now more than ever for determining the roles of Bt corn hybrids for use against ECB and seed-applied insecticides for soil insects like white grubs and wireworms.

Pest management is a key issue for corn grown for both grain and silage purposes since the commodity is impacted by a range of insect pests of minor to major importance (Luckmann and Metcalf 1982). Growers must be well equipped with knowledge of the pests attacking their crops by employing life history-specific scouting techniques, from which they can make the most informed decision concerning a pest management strategy. Preventive application of pesticides (pretreated seeds, Bt corn hybrids) is often used as the first line of defense against some pest insects. However, alternative control tactics available with cultural control, biological control, and genetically modified organisms like Bt corn, enable a grower to integrate several control techniques into one comprehensive management plan.

There is continuing interest among growers in Virginia and other Mid-Atlantic states concerning the efficacy of new insecticidal seed treatments against early season corn pests, such as wireworms, seedcorn maggot, and annual white grubs. Traditionally, soil sampling for early season corn pests has been performed in the spring. However, an important consideration when purchasing treated corn seed is that it must be ordered months prior to planting time, typically in the fall or winter. If a fall sampling method could adequately predict spring infestation levels of early season corn pests, growers would know which fields were at risk to damage prior to when they need to order treated seed, and thus could purchase the precise amount more efficiently. Current sampling methods for these soil pests include some form of baiting or soil sampling method performed in the spring before planting (McLeod et al. 1999, Youngman and Tiwari 2004).

The leading early season soil pests of corn in Virginia include annual white grubs (Coleoptera: Scarabaeidae) and wireworms (Coleoptera: Elateridae). Numerous species of wireworms found in Virginia are predominantly in genera *Melanotus*, *Conoderus*, *Aeolus*, *Hemicrepidius*, and *Limonius* (Briggs 1980, Youngman et al. 1993). Wireworms tend to be more problematic in areas where corn is planted following sod or pasture; while the incidence of white grubs potentially can be a problem following sod, soybean, and corn rotations (Luckmann and Metcalf 1982). Annual white grubs have a 1 year life cycle in contrast to true white grubs that complete their life cycle in 2-3 years (McLeod et al. 1999). True white grubs predominantly of the genus *Phyllophaga* are found in Virginia (Briggs 1980) but historically cause less disturbance in the Mid-Atlantic region (Youngman and Tiwari 2004) than in the Midwest Corn Belt (McLeod et al. 1999).

Common annual white grubs found in Virginia include Japanese beetle, *Popillia japonica* Newman; Asiatic garden beetle, *Maladera castanea* (Arrow); grubs in the genus *Cyclocephala*, northern and southern masked chafers; and *Cotinus nitida* (Linnaeus), the green June beetle. In order to develop an effective sampling method for these soil pests in the fall, the relationship between spring and fall pest densities of white grubs and wireworms must be understood.

European corn borer (ECB) has posed a potential risk to corn growers in the Mid-Atlantic and southeastern U.S. for decades (Mason et al. 1996). Therefore, it is important that research be done to determine the level at which ECB causes economic damage to corn grown for grain and can justify the planting of Bt seeds. Occasional infestations in the southeastern U.S. and the Mid-Atlantic region do not warrant the cost of planting Bt corn on a yearly basis. Regions of Virginia rarely experience economic damage from ECB. Over three years, sporadic infestations resulted in economic loss in less than 7% of 179 fields of corn surveyed in eastern Virginia (Youngman et al. 1998, 1999, 2000). In western Virginia, however, economic loss occurred in almost 20% of fields surveyed from 2000-2002 (Youngman and Laub 2002). In these studies by Youngman et al. (1998, 1999, 2000, 2002), economic loss occurred when 1 or more tunnels per plant > 2.5 cm in length were observed. Based on these findings, the ECB may be a higher management priority in western Virginia where most corn is grown for silage. The ECB yield relationship for Virginia-grown silage was reported recently by Tiwari (2007). However, the benefit of Bt hybrids for grain in eastern Virginia may not be realized until years of moderate to high infestation, or when the probability of infestation reaches > 0.40 (Hyde et al. 1999, Baute et al. 2002).

I have two research objectives: 1) to develop economic injury levels for corn grown for grain in Virginia by determining the effect of European corn borer infestation on corn grain yield and 2) to develop economic injury levels for white grubs and wireworms using a fall soil sampling method that adequately predicts spring infestation and subsequent yield response. Findings from these studies should be immediately applicable to corn growers throughout much of the Mid-Atlantic and southeastern U.S.

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CHAPTER 2

EFFECT OF EUROPEAN CORN BORER, *Ostrinia nubilalis* (Hübner) (LEPIDOPTERA: CRAMBIDAE) ON FIELD CORN DURING LATE VEGETATIVE, REPRODUCTIVE, AND GRAIN-FILL GROWTH STAGES

Abstract

Field studies were performed in 2006 and 2007 to investigate the effect of different levels of European corn borer (ECB) tunneling on yield in corn grown for grain. Model variables were corn growth stage with two classifications of infestation level (larvae per plant, tunnel length per plant). In both years of this study, both classifications of infestation level had a significant effect on grain yield. The main effect of year was highly significant. The main effect of growth stage was significant in all cases, except in 2006 with infestation level by larvae per plant. The interaction between growth stage and larvae per plant was significant in 2006, and the interaction between growth stage and tunnel length per plant was significant in 2007. Grain yield was reduced by 13.1 and 3.65% in plants infested with four larvae per plant in 2006 and 2007, respectively. Similarly, there was a significant yield response to tunnel length in both years of this study. In 2006, low (≤ 2 cm) and high (> 8 cm) infestations reduced yield 2.5 and 9.2%, respectively. Alternatively, in 2007, only high infestations ($> 6 - 8$ cm and > 8 cm) reduced yield 4.6 and 7.1%. For 2006, linear regression models provided average percent yield loss per larva per plant at 4.1, 6.8, and 1.8% for each growth stage, respectively. In 2007, significant regression relationships were observed in V12 (late vegetative) and R2 (blister) with 1.4 and 1.5% yield loss, respectively. Economic injury levels (EILs) for

different growth stages were calculated based on average percent yield reductions across each growth stage and year. The initiation of tunneling appeared to have a greater effect on yield during late vegetative (V12) and the onset of reproductive (R1), which resulted in lower EILs across both stages than compared to EILs calculated for blister (R2).

Introduction

The European corn borer (ECB), *Ostrinia nubilalis* (Hübner), is a primary pest of corn throughout the corn growing regions in the U.S. (Calvin and Van Duyn 1999, Youngman and Tiwari 2004, Dillehay et al. 2005). As a result, more than one billion dollars of annual damage results from ECB management costs and yield reduction in corn (Mason et al. 1996). This insect pest must be managed regionally due to differences in agronomic practices, climate, pest voltinism, and host-plant synchrony. Field corn is planted on more hectares in the Mid-Atlantic than any other crop, and corn grown for grain is the second most planted crop in Virginia with 218,000 ha of corn, the most area planted for grain since the late 1980s (NASS 2007). With increasing corn hectares being planted in the Mid-Atlantic, effective management information is needed now more than ever for justifying the use of Bt corn hybrids against ECB.

Conventional treatment for ECB usually consists of aerial application of insecticides in fields where above-threshold egg mass counts were observed. However, the availability of Bt corn hybrids has changed the pest management environment for ECB. Bt corn typically costs the grower approximately \$17-\$25/ ha when compared to conventional insecticide treatments that are more expensive and provide a lower level of control (Hyde et al. 1999). Lower control costs of Bt corn typically result in lower treatment thresholds. Bt technology provides a high level of protection against moderate to high levels of infestation (Baute et al. 2002). In years of low infestation, and when traditional hybrids can sustain minimal feeding damage, farmers still may elect to purchase Bt corn for no additional yield benefit. Therefore, effective management of

ECB needs to include regional information regarding the impact of low to high infestations at different growth stages on non-Bt corn.

Because economic infestations from ECB have been historically rare in eastern Virginia grain corn, the cost of planting Bt corn on a yearly basis is not warranted. Over three years, sporadic infestations resulted in economic loss in less than 7% of 179 fields of corn surveyed in eastern Virginia (Youngman et al. 1998, 1999, 2000). In western Virginia, however, economic loss was expected to occur in almost 25% of fields surveyed from 2000-2002 (Youngman and Laub 2002). In these studies by Youngman et al. (1998, 1999, 2000, 2002), economic loss occurred when 1 or more tunnels > 2.5 cm in length per plant were observed. Based on these findings, ECB may be a higher management priority in western Virginia where most corn is grown for silage. The ECB yield relationship for Virginia-grown silage was reported recently by Tiwari (2007). However, the benefit of Bt hybrids in eastern Virginia may not be realized given the lack of region-wide infestation from year to year until years of moderate to high infestation.

Many studies have quantified the effect of ECB infestation level on yield in corn. Patch et al. (1951) estimated an average 3.0% yield loss per larva per plant. In Pennsylvania grain corn, the greatest yield loss from ECB was observed during the late stages of vegetative development resulting in 6.0% and 5.0% yield loss during the 10-leaf and 16-leaf life stages, respectively (Bode and Calvin 1990). An estimated 6.5% annual yield loss results from combined first and second generation ECB infestation in field corn in the Northeast (Calvin 1995). In addition to number of larvae per plant, extent of tunneling has also been used as a measure of yield loss. Baute et al. (2002) observed an

average 5.0% yield loss from tunneling below the main ear in a non-Bt isoline compared to its Bt counterpart. Bt corn may yield better than its non-Bt counterpart during years of low infestation (Rice and Pilcher 1998), but the advantage of Bt corn generally will be realized by growers when ECB populations are moderate to high (Hyde et al. 1999, Baute et al. 2002).

Even during years of low infestation, corn growers in Virginia and perhaps other regions of the Mid-Atlantic are faced with the decision to control this sporadic pest. Therefore, it is important to justify the planting of Bt corn seeds by determining the level at which ECB causes economic damage to corn in Virginia. This study evaluated the relationship of yield loss to two classifications of infestation level (larvae per plant; tunnel length) at three corn development stages.

Methods & Materials

Field studies were completed in 2006 and 2007 at the Virginia Tech Kentland Research Farm in Montgomery Co., Virginia, to determine the relationship between ECB infestation level and corn grain yield. Corn was planted on 1 May 2006 and 9 May 2007 using the commercial, non-Bt hybrid Trisler TR5244 (Augusta Seeds Corp., Staunton, Virginia) with 0.762 m row spacing at about 64,493 seeds per hectare. To deter unwanted pests, seed was treated with Kernel Guard (captan, diazinon, lindane at 56.7 g/bushel corn seed) prior to planting, and Force 3G granular insecticide (tefluthrin at 113.4 g/305 row-m) was applied at planting. The experiments were designed in a split-plot randomized complete block with blocks replicated eight times. Each block consisted of main plots of three plant growth stages (V12: late vegetative, R1: early silking, and

R2: blister), and each main plot consisted of subplots representing six infestation levels of ECB larvae per plant (0, 1, 2, 3, 4, and 5). Subplots were four rows wide by 9.14 m long, and successive blocks were separated by 4.57 m alleyways. For subplots with specified infestation levels of \leq three larvae per plant, at least 16 plants were selected for infestation from the middle two rows, and for levels four and five larvae per plant at least 20 plants were selected. Plants were selected on the basis of uniformity in size and spacing. In 2006 and 2007, a low percentage ($< 1.4\%$ and $< 0.3\%$) of plants in the control (zero larvae per plant) were found to be naturally infested by ECB 1.3% (five plants) and 0.3% (one plant) of plants in the control (zero larvae per plant) were found to be naturally infested by ECB for 2006 and 2007, and were not used for artificial infestation or yield analysis.

Artificial infestation. For each infestation period, laboratory-reared late third to early fourth instars of ECB were purchased from French Agricultural Research, Inc. (Lamberton, Minnesota). Larvae were hand infested on corn stalks using the wire-nut method (Tiware 2007). The wire-nut method comprised of modified WingGard™ (Gardner Bender, Milwaukee, Wisconsin) plastic wire connectors ('wire nuts', size 10-086) that were affixed to cornstalks with rubber bands. A single larva was placed into each wire-nut, which was then capped with a cork and stored above a foam barrier over crushed ice in an ice cooler. In 2006, all larvae were loaded into wire-nuts and transported to the field on the day of infestation. However, in 2007 larvae were held overnight in an ice cooler and then transported to the field the following day for infestation.

To facilitate larval penetration the outer epidermal layers were removed from the stalk with a 1-2 mm deep hole using a 5 mm diameter biopsy punch (Biopunch, Fray Products Corp. Buffalo, New York). In 2006, shallow holes (1-2 mm) were punched only removing the outer epidermal layers. In 2007, the punch depth (1-4 mm) was modified to access the pith of the stalk after observing poor tunnel initiation in 2006. Larvae were attached to the stalk at the rate of one larva per internode. All infested plants were marked with non-toxic landscape paint at the base of the stalk. All wire-nuts were checked after 24 hours, and any dead or missing larvae were replaced with live larvae.

In the V12 stage, all larvae were attached to elongated internodes below the whorl at one larva per internode. In the R1 and R2 stages, half of the larvae were placed just above the primary ear with the remaining half just below the primary ear. With infestation levels (three and five larvae per plant), more larvae were infested below the main ear where tunneling was observed to be most consistent. At the infestation level of one larva per plant, the larva was placed onto the elongated internode above the main ear.

Harvest. Harvest data were collected after the majority of plants reached physiological maturity (Richie and Hanway 1993), and all infested plants were cut 5 cm from the ground when the corn grain had dried to 15% moisture. At harvest, all infested stalks were split lengthwise to record the number of tunnels per plant as well as tunnel lengths. Data were not recorded for plants with tunneling observed outside of the infested internodes. In the field, ears were shelled individually with a manual corn ear sheller (ALMACO: FHS, Nevada, Iowa) and respective grain weights were recorded (g/plant) on a digital scale (AND: HL-400, Toshima-ku, Tokyo). Grain moisture was

obtained from an average of three grain moisture samples per subplot to determine grain dry weight.

Economic injury level. The economic injury level (EIL) was calculated for each growth stage assuming different levels of crop value and costs of control. The EILs were calculated using the following formula from Bode and Calvin (1990) with modifications by Tiwari (2007): $NL = TC / CV \times PL \times PC$; NL, number of larvae per plant; TC, total control cost of planting Bt corn seed (dollars/ha); $CV = MV \times EY$, CV, crop value, MV, expected market value (dollars/kg), EY, expected yield (kg/ha); PL, expected average proportional yield reduction per larva per plant from regression equations developed for each growth stage, PC, expected proportional control from planting Bt corn; and where $PC = 1$, 100% control is assumed.

Statistics. Within each year, variation of the response variable yield was analyzed by the main effects of growth stage and infestation levels. Data were analyzed using two-way analysis of variance for a split-plot randomized complete block design (ANOVA; PROC GLM, general linear models) (SAS Institute 2001). An alpha of 0.05 was used unless specified otherwise. ANOVA and Fisher's Protected LSD mean separation tests were performed following significant main effects (SAS Institute 2001). Linear regression models were developed for each growth stage by regressing yield against number of larvae per plant. Weighted means analyses were performed to adjust for variation of treatment means across blocks and between infestation levels. Similarly, weighted means were used to adjust for model outliers that were not biologically sound. For example, with the highest infestation level of five larvae per plant in V12 and R2 in 2007, mean grain yield was not different from the control. Several yield data

transformations $[(y + 0.5)^{1/2}, \log_{10}(y), \ln(y)]$ were performed to examine the best relationship between infestation level and yield, though original values were not improved by any of the transformations.

Results

The 2006 and 2007 results from SAS GLM (general linear model) procedure for a split-plot randomized complete block design (SAS Institute 2001) are shown in Tables 2.1 and 2.2. An overall ANOVA revealed that the main effect of year was highly significant with mean grain yields (g/plant) of 201.2 ± 0.95 and 140.0 ± 0.66 in 2006 and 2007, respectively. In 2006, 27% of the larvae produced tunnels; whereas 75% of the larvae produced tunnels in 2007. Provided the low level of overall tunneling (1787 of 6576) in 2006, standard error values were similar for infestation levels up to three larvae per plant, except where fewer than 30 plants were collected in higher infestation levels. Few plants ($n = 4$) were collected experiment wide for the highest infestation level of five larvae per plant. In contrast, the overall level of tunneling (4932 of 6576) in 2007 was much higher than 2006 and contributed to consistent standard errors across infestation levels.

In 2006, few infested stalks were broken above or below the ear; whereas more than 6% of infested stalks were broken above or below the ear in 2007. Greater than 99% of stalks were broken above the ear. Grain yield was not significantly different for stalks broken above or below the ear. For stalks broken above and below the ear, yield (g/plant) averaged 122.00 ± 3.17 and 88.50 ± 13.74 , respectively. Slightly less than 40 and 60% of broken stalks were observed in the R1 and R2 growth stages, respectively.

Information regarding corn relative maturity by accumulated growing degree-days for both years is shown in Table 2.3. Less than 300 GDD accumulated in May 2006, while almost 450 GDD accumulated in May 2007. Warm daily temperatures (average high of 22.9°C over the 15 days following planting) contributed to high vegetative growth for corn planted 9 May 2007, enabling the corn to reach VE (emergence) as early as corn planted 1 May 2006. Following the lag in development from cooler temperatures in May 2006, physiological maturity was delayed \approx 14 days later than corn grown in 2007. However, corn planted in 2007 experienced 11.5 cm less rainfall than corn planted in 2006 through the months of May, June, and July. Although the 2007 plot was irrigated with >7 cm water over two applications, rapid corn development combined with drought stress may have lowered yield and potentially masked the effects of infestation.

2006 experiment. In 2006, there was a significant effect on grain yield from block, block by growth stage, larvae per plant, and the interaction between growth stage and larvae per plant (Table 2.1). Because of the significant growth stage by larvae per plant interaction, the effect of number of larvae per plant on grain yield within growth stages was analyzed (Table 2.4). Across all growth stages, mean grain yield (\pm SE) was significantly greater in the non-infested control plants (206.0 ± 1.7 g/plant) than in plants infested with 4 (179.1 ± 7.6 g/plant) and 5 (154.7 ± 21.2 g/plant) larvae per plant, resulting in a 13.1% and 24.9% yield loss, respectively (Table 2.5). Though yields were not different among growth stages, there was a trend for decreasing yield loss when tunneling was initiated at V12 compared to the R1 and R2 stages.

Mean total tunnel length per plant significantly increased for each additional larva per plant ($F = 628.94$; $df = 5, 23$; $P < 0.0001$). Likewise, mean tunnel length per larva

was significantly greater for all number of larvae per plant when compared to the control (Figure 2.1). A significant effect on mean total tunnel length by plant growth stage was observed ($F = 29.30$; $df = 5, 23$; $P < 0.0001$). Mean total tunnel length (\pm SE) per plant was the highest in the V12 stage (3.50 ± 0.14 cm), followed by the R1 (3.26 ± 0.17 cm) and R2 stages (2.83 ± 0.14 cm). When tunneling was grouped into categories of ≤ 2 cm, $> 2-4$ cm, $> 4-6$ cm, $> 6-8$ cm, and > 8 cm, a significant effect on yield was detected (Table 2.2). When compared to the control for categories of ≤ 2 cm, $> 2-4$ cm, $> 4-6$ cm, and $> 6-8$ cm, yield was significantly reduced by 2.48, 3.50, 2.09, and 1.80, respectively (Table 2.5). Only the highest level of tunneling, with the exception of $> 2-4$ cm, resulted in significantly lower yield from all other categories of tunneling with 9.22% yield loss and an average tunnel length per larva of 4.28 ± 0.18 cm. No significant interaction between growth stage and tunnel length per plant was detected (Table 2.2), therefore the effect of tunnel length per plant on grain yield was analyzed across all growth stages (Table 2.5).

Significant linear regression relationships were observed between grain yield and larvae per plant for the V12 ($P = 0.0232$), R1 ($P = 0.0327$), and R2 ($P = 0.0512$) stages (Table 2.6). Similarly, significant quadratic and cubic relationships were found between grain yield and larvae per plant for each plant growth stage. Average yield reduction per larva per plant was calculated using the linear regression equations in Table 2.6. Each larva per plant reduced yield in the V12, R1, and R2 stages by 4.05, 6.81, and 1.82%, respectively (Table 2.7).

Economic injury levels (EIL) were calculated from average percent grain yield reduction per larva per plant for each growth stage and were assessed by a range of

control costs and crop values (Table 2.8). EILs were calculated on the assumption that Bt hybrids, or insecticide applications, provided 100% control of ECB. Proportion control can be changed assuming varying levels of protection. The EIL decreases as yield reduction per larva increases. In my study, as percent yield reduction per larva per plant increased from 4.05 to 6.81% for V12 and R1 growth stages, respectively, the EIL at low control costs and crop value decreased from 1.98 to 1.17 larvae per plant. In addition, the EIL decreases as control costs decrease, but increases as crop value decreases. In my study, when control costs and crop values are low, the EIL at the V12 stage is 1.98 larvae per plant; conversely, when control costs and crop values are high the EIL at the V12 stage is 1.65 larvae per plant. When control costs are high and crop value is low, the EIL at the V12 stage is 4.94 larvae per plant, which is substantially higher than the EIL of 0.66 larvae per plant at low control costs and high crop value.

2007 experiment. In 2007, all main effects and interactions had a significant effect on yield except the interaction of growth stage by larvae per plant (Table 2.1). Similar to 2006, mean total tunnel length per plant increased significantly by larvae per plant ($F = 1373.22$; $df = 5, 24$; $P < 0.0001$). Mean total tunnel length was significantly different across plant growth stages ($F = 24.26$; $df = 5, 24$; $P < 0.0001$) (Table 2.7). Mean total tunnel length per plant (\pm SE) was not different between the R1 (4.17 ± 0.12 cm) and R2 (4.13 ± 0.13 cm) growth stages; however, mean total tunnel length in the V12 (2.45 ± 0.09 cm) growth stage was significantly less than mean total lengths in the R1 and R2 growth stages. When tunneling was grouped into categories of ≤ 2 cm, $> 2-4$ cm, $> 4-6$ cm, $> 6-8$ cm, and > 8 cm, a significant effect on yield was detected (Table 2.2). Tunneling < 6 cm did not result in significant yield losses when compared to the

control. However, tunneling > 6 cm resulted in 4.50% yield loss with average tunnel lengths of 2.07 ± 0.04 cm per larva (Figure 2.2). Tunneling > 6 cm was not different from tunneling > 8 cm, though tunneling > 8 cm that averaged > 2.5 cm per larva resulted in a 7.09% yield loss when compared to the control. A significant interaction between growth stage and tunnel length per plant was detected, therefore the effect of tunneling on grain yield was analyzed separately within each growth stage (Table 2.5).

Because of regional drought stress, overall mean grain yield (139.9 ± 0.7 g/plant) was significantly lower than in 2006. The effects of growth stage, block, growth stage by block, and larvae per plant significantly affected yield (Table 2.3). Tunneling initiated during R1 resulted in significantly lower average yield (130.5 ± 1.19 g/plant) than in V12 (148.6 ± 1.16 g/plant) and R2 (140.7 ± 1.02 g/plant) growth stages. Although no significant interaction between growth stage and larvae per plant was observed, each growth stage was analyzed separately to determine the effect of larvae per plant on grain yield (Table 2.5).

Across all growth stages, mean grain yield (\pm SE) was significantly greater in the non-infested control plants than in plants infested with four larvae per plant (137.2 ± 1.7 g/plant), but not with five larvae per plant (138.5 ± 2.3 g/plant), resulting in 3.65% and 2.74% yield loss for four and five larvae per plant, respectively (Table 2.4). However, the yield loss at five larvae per plant was not different from the non-infested control.

When regressing grain yield against larvae per plant, significant linear relationships were observed for the V12 and R2 growth stages (Table 2.6). Each larva per plant significantly reduced yield in the V12 and R2 stages by 1.37 and 1.54%,

respectively (Table 2.7). Mean yield reduction per larva per plant was not significantly different from zero for the R1 growth stage.

The relationship between percent yield loss per larva per plant and accumulated corn growing degree-days for infestation events was developed using a second degree polynomial equation:

$$\text{PYL} = -0.6476 + (0.0062 \cdot \text{GDD}) - (0.000002 \cdot \text{GDD}^2) \quad R^2 = 0.4316$$

where PYL is percent yield loss per larva per plant and GDD are the accumulated growing degree days by infestation events. Parameter estimates of the upper and lower 95% confidence intervals for GDD and GDD^2 were -0.042 to 0.055 and -0.00001538 to 0.00001056, respectively. Although this relationship was not significant, the maximum yield loss per larva was 4.16%, which occurred ≈ 1550.0 GDD. Physiological maturity was predicted to occur at ≈ 2488 GDD.

Discussion

Temperature and precipitation are common factors that affect larval survival (Mason et al. 1996). The influence of precipitation on tunneling was observed following infestation in each year of study. Following infestations in 2006, approximately 2.51, 1.01, and 0.96 cm precipitation fell within 48 hours of V12, R1, and R2 infestation, respectively. In contrast in 2007, only 2.21 cm precipitation fell within 48 hours of R1 infestation. In both years of study, however, drainage holes were made in the sheath below the infestation hole to drain excess water when precipitation events were expected. While environmental factors during and after infestation may have inhibited tunneling in 2006, the impact of rain following R2 in 2007 had no observable effect on larval

mortality. The best explanation for improved tunneling rates in 2007 relative to the previous year was the change in infestation method. Average depth of the original hole made to ease larval penetration was increased from 1.5 mm in 2006 to 2.5 mm in 2007. Though the change of infestation method in 2007 may have eased larval penetration, average tunnel length (\pm SE) per larva per plant in 2006 (1.91 ± 0.04) was greater than in 2007 (1.35 ± 0.02). In 2007, the relationship between drought stress and lower yield may have contributed to thrifty tunneling. This is in contrast to studies by Patch et al. (1942), Lynch (1980), and Youngman and Laub (2002) who reported that drought effects tended to intensify infestation effects.

While ear size and kernels per ear are determined in the mid to late vegetative plant stages, the four weeks that encompass R1 (silking) stage are the most important in determining grain yield since nutrient and water stress from tunneling will negatively influence pollination and seed set (Ritchie and Hanway 1993). After R1, during the linear grain-fill period (R2 to physiological maturity), ECB tunneling can influence the movement of photosynthates from vegetative growth to the reproductive center which ultimately affects dry weight accumulation in the kernel (Ritchie and Hanway 1993). In my study, percent yield loss was highest in V12 and R1 when compared to R2. In 2006, yield loss was highest when tunneling was initiated in R1. However, in 2007, when lack of moisture was potentially a factor of plant stress, no clear relationship existed between level of infestation and yield in R1, even though the initiation of tunneling in R1 resulted in significantly lower yield across growth stages.

The timing of ECB infestation, as a function of time remaining to corn physiological maturity, results in greater yield loss during mid to late vegetative stages

than infestations initiated during the grain-fill stages (Lynch 1980, Calvin et al. 1988, Bode and Calvin 1990). In this study, maximum yield loss (4.16% per larva per plant) was predicted to occur at ≈ 1550.0 GDD (37.7% of GDD remaining to physiological maturity). According to Ritchie and Hanway (1993), the grain-fill period begins when $\approx 39.0\%$ of the GDD remain until physiological maturity. For corn development in my study, 37.7% corresponded to late R1 stage for maximum yield loss. Therefore, the timing of infestation and corresponding yield loss in my study agree with results from previous studies (Lynch 1980, Calvin et al. 1988, Bode and Calvin 1990), such that yield loss from infestation increased through the late vegetative stages of development but decreased as grain-fill was initiated.

Physiological yield loss from ECB feeding is generally greater than mechanical loss up to the grain-fill period, where feeding during the blister plant stage causes comparable mechanical and physiological yield loss (Patch et al. 1951, Lynch 1980). In my study, the incidence of stalk breakage was greatest in R2, though the yield response was primarily physiological because almost all stalks were broken above the main ear. Yield loss was greater for stalks broken below the ear than for those broken above; however, broken stalks below the main ear usually result in non-harvestable ears, especially when ears fall to the ground.

Based on yield results from 2006 and 2007, grain yield decreased significantly with infestations $> one$ and $> two$ larvae per plant, with each larva per plant averaging >2.5 and >1.7 cm, respectively. The economic injury levels for the 2006 experiment are presented in Table 2.8, which provide estimates of larval populations per plant necessary to break even at varying control costs and crop values. In contrast to Bode and Calvin

(1990) percent yield reductions per larva across growth stages in my study were highest in R1. Similarly, for my study this resulted in a proportional increase of EILs in contrast to those observed by Bode and Calvin (1990). However, EILs in my study were higher in V12 and R2 since percent yield reductions were less than those reported by Bode and Calvin (1990) during the late vegetative and grain-fill periods. Since infestation levels greater than two to three larvae per plant were necessary to reduce yield in V12 and R1 growth stages, no benefit from control would be realized unless control costs were low and crop values were high.

Alternatively, by quantifying the yield loss relationship by tunnel length per plant, the results from both years of my study demonstrated that infestations >8 cm per plant with average tunnel lengths per larva greater than 4.3 and 2.5 cm were needed to significantly reduce yield ≈ 9.02 and 7.09% , respectively. Baute et al. (2002) quantified the impact of ECB infestation at varying levels of tunneling. In their study, the yield benefit of Bt hybrids was only realized during high infestations with total tunnel lengths per plant >6 cm, which resulted in $\approx 5\%$ yield loss (Baute et al. 2002). In my study, tunneling ≤ 2 cm, 2-6 cm, and > 6 cm significantly reduced yield in 2006. In 2007, only tunneling > 6 cm significantly reduced yield.

Baute et al. (2002) observed high infestations in less than 35% of locations sampled in Ontario, Canada. In contrast, low tunneling was observed over three years of surveys in eastern Virginia that revealed only 7% of fields were infested with over threshold tunneling (≥ 1 tunnel per plant with tunnels >2.5 cm) (Youngman et al. 1998, 1999, 2000) versus moderate infestation (25% over threshold) in western Virginia (Youngman and Laub 2002). According to the 1998-2000 average in eastern Virginia,

economic infestations would occur every 1 in 14 years, or 1 in 7 years for areas of greatest pressure where >15% of fields exceeded threshold (9 of 58 in 1998). Pest pressure was highest in 1998 suggesting that some of the farmers would have benefited from Bt corn.

Regional differences of plant-pest synchrony for ECB on corn and corn crop variation between eastern and western Virginia suggest that a second generation infestation may impact corn in western Virginia more than corn grown in the east (Youngman et al. 1998, 1999, 2000, Youngman and Laub 2002). The relative maturity of corn across the state decreases from east to west, where eastern Virginia corn is planted \approx 1 month before fields in the west. However, in western Virginia where silage corn is the primary corn crop, harvest occurs before physiological maturity. In contrast, grain corn is harvested following maturity.

Regional phenology of ECB was not monitored during this study; however, stalk tunneling by late third to early fourth instar larvae generally occurs \approx 85 days following the first moth flight (Mason et al. 1996). In eastern Virginia, moths will lay eggs in the youngest and latest planted corn, allowing in a higher percentage of second generation larvae that are able to overwinter for the following year versus those that develop on mature corn (grain-fill to physiological maturity). Similarly, in western Virginia, the later-developed corn has higher pest-plant synchrony resulting in ECB infestation. Therefore, corn grown in western Virginia might expect infestation from both first and second generation borers. While during most years in eastern Virginia, the lack of plant-pest synchrony may limit damage from either generation.

In contrast to years with high infestation, several studies have reported no yield benefit from Bt hybrids compared to non-Bt hybrids when ECB infestation was low or absent (Pilcher and Rice 1998, Myers and Wedberg 1999, Youngman et al. 1998, 1999, 2000, Baute et al. 2002). Bt corn may yield better than its non-Bt counterpart during years of low infestation (Rice and Pilcher 1998), but the advantage of Bt corn generally will be realized by growers when ECB populations are moderate to high (Hyde et al. 1999, Baute et al. 2002). Planting non-Bt corn during years of low infestation will limit the need maintain non-Bt refugia as well as delay resistance of ECB to Bt (Youngman and Tiwari 2004). Furthermore, most farmers throughout Virginia would not benefit from Bt hybrids because infestation rarely exceeds 40% (Hyde et al. 1999). This is the case for corn planted early or within the normal range of planting dates. However, late planted corn is typically more susceptible to infestation from ECB, since egg-laying moths prefer the youngest corn in early to late vegetative growth stages (Mason et al. 1996). Growers that reportedly experience ECB infestations in eastern Virginia have found that only late planted corn is at risk to ECB infestation (Youngman, personal communication). The economic injury levels reported in this study and results of surveys in Virginia suggest that management decisions should be oriented towards the late planted corn, which is most susceptible during late vegetative and early reproductive stages. If corn must be planted late, then using a Bt hybrid may be a viable management option for ECB (Pilcher and Rice 2001). Otherwise, the best management options for ECB control in Virginia should include consideration of planting dates and appropriate hybrids, knowledge of historic local and regional pest incidence, and or use of the best adapted non-Bt corn hybrid for the area to be planted. While Bt hybrids may be

considered as an insurance option for late planted corn, future research should be aimed at the phenology of ECB in different corn growing regions throughout Virginia to better understand the pest-plant synchrony for which corn grown for grain is the most susceptible.

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Table 2.1. Two-way split-plot ANOVA of 2006 and 2007 yield data for main effects of growth stage and larvae per plant.

2006			
Source of variability ¹	df	<i>F</i> value	<i>P</i> > <i>F</i>
Growth stage (GS)	2	1.70	0.1835
Block (B)	7	5.29	< 0.0001
GS•B	14	4.29	< 0.0001
Larva / plant (LP)	5	6.73	< 0.0001
GS•LP	9	2.95	0.0018
2007			
Source of variability ¹	df	<i>F</i> value	<i>P</i> > <i>F</i>
Growth stage (GS)	2	39.93	< 0.0001
Block (B)	7	43.62	< 0.0001
GS•B	14	29.11	< 0.0001
Larva / plant (LP)	5	1.97	0.0795
GS•LP	10	1.53	0.1209

¹SAS PROC GLM (general linear models) for a split-plot randomized complete block design.

Table 2.2. Two-way split-plot ANOVA of 2006 and 2007 yield data for main effects of growth stage and total tunnel length per plant.

2006			
Source of variability ¹	df	<i>F</i> value	<i>P</i> > <i>F</i>
Growth stage (GS)	2	4.08	0.0171
Block (B)	7	5.39	< 0.0001
GS•B	14	4.17	< 0.0001
Tunnel length (TL)	5	4.76	0.0003
GS•TL	10	1.06	0.3881
2007			
Source of variability ¹	df	<i>F</i> value	<i>P</i> > <i>F</i>
Growth stage (GS)	2	5.27	< 0.0001
Block (B)	7	1.70	0.1836
GS•B	5	6.75	< 0.0001
Tunnel length (TL)	14	4.29	< 0.0001
GS•TL	10	2.95	0.0018

¹SAS PROC GLM (general linear models) for a split-plot randomized complete block design.

Table 2.3. Relative plant growth days after planting (DAP) and associated accumulated growing degree-days (GDD) in 2006 and 2007.

2006		
DAP	Plant growth stage	GDD
0	Corn planted	11
6	VE (emergence)	129
27	V5 (5-leaf)	483
60	V10 (10-leaf)	1055
65	V12 (12-leaf)	1167
77	R1 (silking)	1493
85	R2 (blister)	1799
121	Physiological maturity	2488
2007		
DAP	Plant growth stage	GDD
0	Corn planted	13
15	VE (emergence)	110
38	V5 (5-leaf)	457
67	V10 (10-leaf)	1137
73	V12 (12-leaf)	1229
87	R1 (silking)	1456
99	R2 (blister)	1625
135	Physiological maturity	2488

Table 2.4. Mean grain yield at different levels of European corn borer (ECB) larvae per plant at three growth stages (V12, R1, R2) in 2006 and 2007.

Growth stages ¹	V12		R1		R2		Mean across all growth stages ²
	No. of larva per plant	Yield g/plant (\pm SE) ²	Plants with no. larvae per plant	Yield g/plant (\pm SE) ²	Plants with no. larvae per plant	Yield g/plant (\pm SE) ²	
2006							
0	199.5 \pm 2.4a	123	206.7 \pm 3.2a	125	211.7 \pm 3.1a	125	206.0 \pm 1.7a
1	198.4 \pm 2.8a	183	205.3 \pm 2.2a	182	202.8 \pm 2.6b	195	202.2 \pm 1.5ab
2	197.4 \pm 3.3a	165	207.6 \pm 3.3a	102	191.1 \pm 3.3c	84	198.9 \pm 2.0b
3	193.9 \pm 4.8a	66	197.5 \pm 9.3a	30	197.0 \pm 5.2abc	19	195.4 \pm 3.7b
4	185.2 \pm 8.7ab	27	136.7 \pm 22.0b	7	201.1 \pm 3.3abc	6	179.1 \pm 7.6c
5	145.5 \pm 27.1b	3	--	0	182.2	1	154.7 \pm 21.2c
2007							
0	149.2 \pm 2.3	249	127.1 \pm 3.1	153	145.7 \pm 2.3	192	142.4 \pm 1.5a
1	148.0 \pm 2.5	180	128.1 \pm 2.4	173	141.8 \pm 2.4	144	139.3 \pm 1.5ab
2	150.0 \pm 2.6	154	135.0 \pm 2.5	147	140.1 \pm 2.3	125	142.0 \pm 1.5a
3	148.3 \pm 2.7	145	127.4 \pm 2.8	135	137.6 \pm 2.6	127	138.1 \pm 1.6b
4	145.7 \pm 3.2	81	134.1 \pm 2.9	132	135.3 \pm 2.6	150	137.2 \pm 1.7b
5	147.4 \pm 5.7	11	132.8 \pm 4.0	82	142.7 \pm 2.7	89	138.5 \pm 2.3b

¹ Corn growth stage at time of ECB infestation, where V12: late vegetative, R1: early silking, R2: blister.

² Values within a column followed by the same letter are not significantly different (Fisher's protected LSD, $P > 0.05$).

Table 2.5. Mean grain yield at different levels of European corn borer (ECB) total tunnel length per plant from at three growth stages (V12, R1, R2) in 2006 and 2007.

Growth stages ¹	V12		R1		R2		Mean across all growth stages ²
Level of tunneling (cm per stalk)	Yield g/plant (\pm SE) ²	Plants with level of tunneling	Yield g/plant (\pm SE) ²	Plants with level of tunneling	Yield g/plant (\pm SE) ²	Plants with level of tunneling	
2006							
0	199.5 \pm 2.4	123	206.7 \pm 3.2	125	211.7 \pm 3.1	125	206.0 \pm 1.7a
\leq 2	197.7 \pm 3.1	148	202.7 \pm 2.6	107	203.2 \pm 3.3	107	200.9 \pm 1.8b
>2 to 4	193.5 \pm 3.6	121	207.9 \pm 3.3	95	196.5 \pm 3.6	95	198.8 \pm 2.1bc
>4 to 6	199.9 \pm 4.6	80	208.4 \pm 4.9	56	196.7 \pm 4.6	56	201.7 \pm 2.8b
>6 to 8	201.8 \pm 4.9	56	205.4 \pm 7.4	28	199.7 \pm 5.9	28	202.3 \pm 3.4b
>8	183.4 \pm 8.2	38	187.2 \pm 9.6	35	193.1 \pm 4.6	35	187.0 \pm 5.0c
2007							
0	149.2 \pm 2.3a	249	127.1 \pm 3.2a	154	145.7 \pm 2.3a	192	142.4 \pm 1.5a
\leq 2	149.1 \pm 2.3a	207	128.0 \pm 2.5a	144	141.7 \pm 2.3ab	124	140.8 \pm 1.5a
>2 to 4	148.7 \pm 2.5a	175	130.8 \pm 2.5a	159	145.2 \pm 2.5ab	146	141.7 \pm 1.5a
>4 to 6	148.1 \pm 3.0a	117	133.8 \pm 2.8a	137	138.6 \pm 2.4bc	115	139.8 \pm 1.6ab
>6 to 8	145.1 \pm 3.4a	54	132.9 \pm 3.4a	110	134.1 \pm 2.9c	112	135.8 \pm 1.9bc
>8	145.9 \pm 5.9a	18	131.4 \pm 3.1a	119	131.0 \pm 3.1c	93	132.3 \pm 2.1c

¹ Corn growth stage at time of ECB infestation, where V12: late vegetative, R1: early silking, R2: blister.

² Values within a column followed by the same letter are not significantly different (Fisher's protected LSD, $P > 0.05$).

Table 2.6. Linear regression equations relating grain yield (g/plant) to larvae per plant of European corn borer (ECB) at three growth stages (V12, R1, R2) in 2006 and 2007.

Plant growth stage ¹	Regression equation	SE		r^2	$P > F$
		Slope	Intercept		
2006					
V12	$Y = 208.6 - 8.5X$	2.36	7.15	0.7621	0.0232
R1	$Y = 215.1 - 14.7X$	3.89	9.97	0.8256	0.0327
R2	$Y = 209.8 - 3.8X$	1.21	2.57	0.7681	0.0512
2007					
V12	$Y = 151.3 - 2.1X$	0.66	1.61	0.7127	0.0345
R1	$Y = 129.0 + 0.6X$	1.01	3.04	0.0906	0.5622
R2	$Y = 145.2 - 2.2X$	0.24	0.59	0.9555	0.0007

¹ Corn growth stage at time of ECB infestation, where V12: late vegetative, R1: early silking, R2: blister, ALL: analysis across growth stages.

Table 2.7. Mean grain yield \pm SE (g/plant), mean tunnel length per larva per plant \pm SE (cm), and mean percent yield loss per larva per plant for each of three growth stages in 2006 and 2007.

Plant growth stage ¹	2006	2007
	Mean grain yield (g / plant) \pm SE ²	
V12	196.9 \pm 1.6b	148.6 \pm 1.2a
R1	204.6 \pm 1.7a	130.5 \pm 1.2c
R2	202.8 \pm 1.7a	140.5 \pm 1.1b
	Mean tunnel length (cm) per larva / plant \pm SE ²	
V12	3.49 \pm 0.14	1.06 \pm 0.03c
R1	3.26 \pm 0.17	1.54 \pm 0.03a
R2	2.83 \pm 0.14	1.43 \pm 0.04b
	Ave. % yield loss (larva / plant) *	
V12	4.05 *	1.37 *
R1	6.81 *	NS
R2	1.82 *	1.54 *
ALL	4.40 *	0.74 *

¹ Corn growth stage at time of ECB infestation, where V12: late vegetative, R1: early silking, R2: blister.

² Values within a column followed by the same letters are not significantly different (Fisher's protected LSD, $P > 0.05$).

* Values followed by an asterisk are significantly different from zero ($P < 0.1$)

Table 2.8. Economic injury level values for European corn borer larval populations at various control costs and crop values on grain yield, for growth stages with significant regression relationship between grain yield and infestation levels in 2006 (PC = 1, assuming 100% control).

Crop value (dollars/ha)	Control cost (dollars/ha)						
	20	25	30	35	40	45	50
V12 stage ¹							
250	1.98	2.47	2.96	3.46	3.95	4.44	4.94
375	1.32	1.65	1.98	2.30	2.63	2.96	3.29
500	0.99	1.23	1.48	1.73	1.98	2.22	2.47
625	0.79	0.99	1.19	1.38	1.58	1.78	1.98
750	0.66	0.82	0.99	1.15	1.32	1.48	1.65
R1 stage ¹							
250	1.17	1.47	1.76	2.06	2.35	2.64	2.94
375	0.78	0.98	1.17	1.37	1.57	1.76	1.96
500	0.59	0.73	0.88	1.03	1.17	1.32	1.47
625	0.47	0.59	0.70	0.82	0.94	1.06	1.17
750	0.39	0.49	0.59	0.69	0.78	0.88	0.98
R2 stage ¹							
250	4.40	5.49	6.59	7.69	8.79	9.89	10.99
375	2.93	3.66	4.40	5.13	5.86	6.59	7.33
500	2.20	2.75	3.30	3.85	4.40	4.95	5.49
625	1.76	2.20	2.64	3.08	3.52	3.96	4.40
750	1.47	1.83	2.20	2.56	2.93	3.30	3.66

¹ Corn growth stage at time of ECB infestation, where V12: late vegetative, R1: early silking, R2: blister.

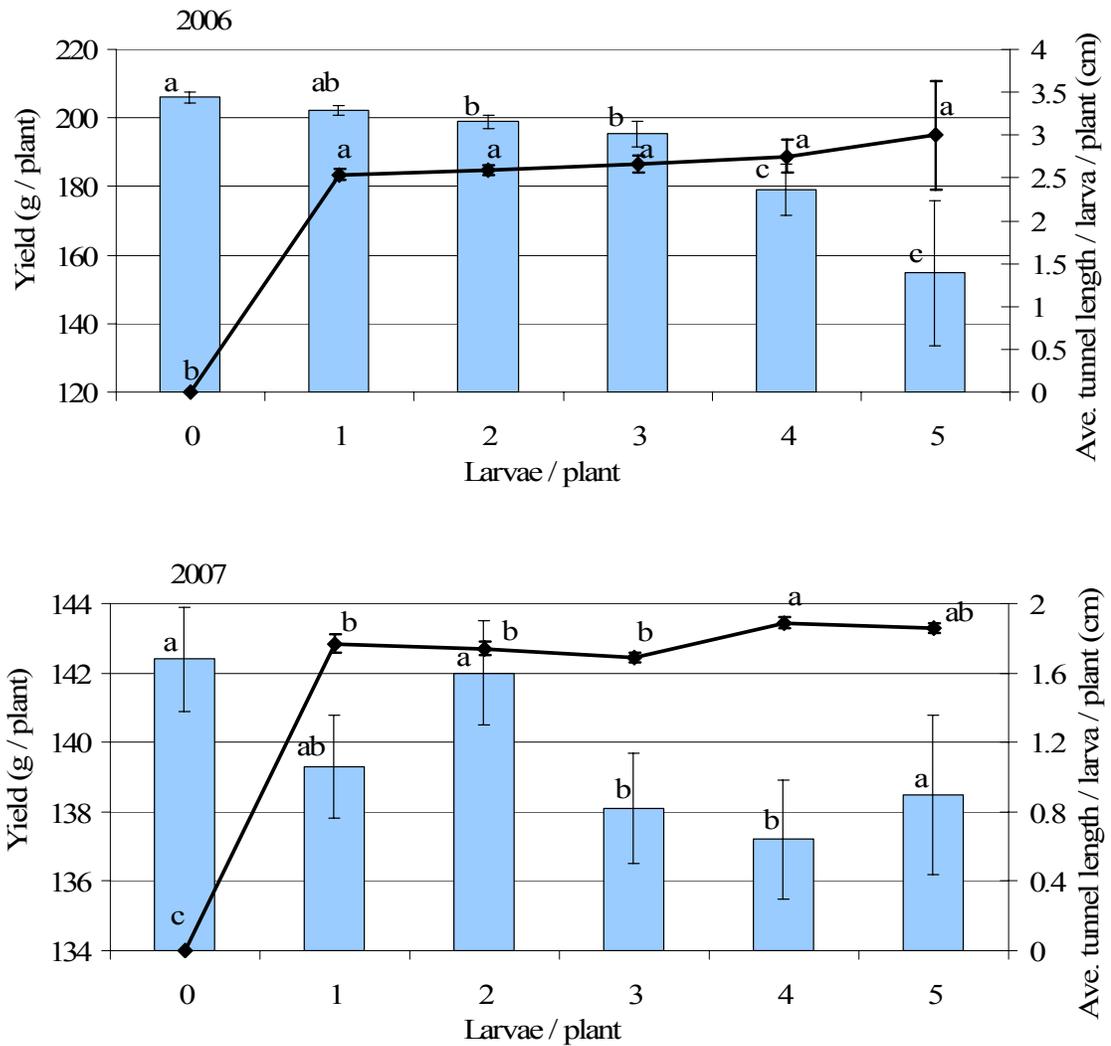


Figure 2.1. Mean grain yield \pm SE (g/plant) and average tunnel length per larva per plant \pm SE (cm) at different levels of European corn borer larvae per plant in 2006 and 2007.

Values followed by the same letter are not significantly different (Fisher's protected LSD, $P > 0.05$).

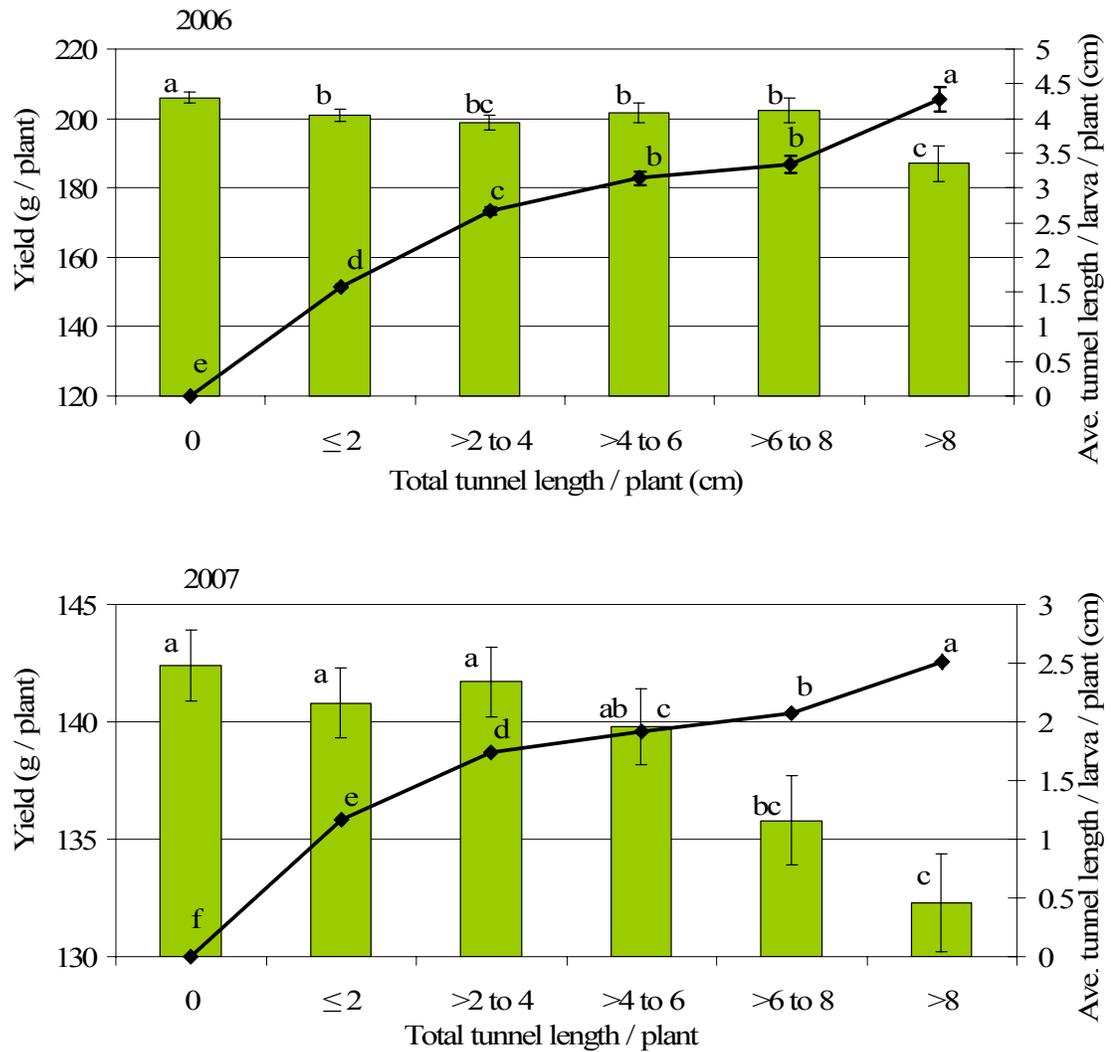


Figure 2.2. Mean grain yield \pm SE (g/plant) and average tunnel length per larva per plant \pm SE (cm) at different levels of European corn borer tunneling per plant in 2006 and 2007. Values followed by the same letter are not significantly different (Fisher's protected LSD, $P > 0.05$).

CHAPTER 3

FALL SOIL SAMPLING FOR PREDICTING SPRING INFESTATION OF WHITE GRUBS (COLEOPTERA: SCARABAEIDAE) IN CORN

Abstract

A field study was started in fall 2005, which continued through spring 2007, to predict spring infestation levels of early season soil pests, specifically white grubs (Coleoptera: Scarabaeidae) and wireworms (Coleoptera: Elateridae) in cornfields. Thirty-four post-harvest soybean fields were sampled in fall and spring in several eastern Virginia counties over two years. Current sampling procedures for early season soil pests are performed in the spring prior to planting by visually inspecting a 30-cm square by 15-cm deep (standard method) volume of soil for annual white grubs or by using some form of baiting method for wireworms and annual white grubs. A 20.3-cm square by 15-cm deep sample (compact method) was evaluated for its potential to correlate to the standard method after a 2.25 weighting factor (the standard method samples 2.25 x more volume of soil than the compact method). Because white grubs were abundant and wireworms scarce in comparison, only white grub data were used for analysis. No significant differences were detected in both fall and spring between the two methods after correcting for differences in sampling volume. Strong correlations were observed between fall and spring pest densities in both years ($r = 0.91$, $P < 0.0001$; $r = 0.88$, $P < 0.0001$). In 2006, fields with ≥ 0.9 white grubs per compact method in the spring had significantly greater stand and yield in the Poncho 1250 (1.25 mg clothianidin / kernel) treatment when compared to the Poncho 250 (0.25 mg clothianidin / kernel) and

untreated control treatments. Based on strong correlations between fall and spring, the fall economic injury level (EIL) was $\geq 1.6 (\pm 0.17)$ white grubs per compact method, which corresponded to an economic threshold (75% of the EIL) of 1.2 white grubs per compact method. Four and 10 compact samples per field (95% confidence) in the fall were needed to be within 25 and 15% of the actual mean.

Introduction

Early season soil insects are considered sporadic pests of seeds and seedlings that may cause significant reductions in plant stand and yield (Youngman et al. 1993). The leading early season soil pests of corn in Virginia include annual white grubs (Coleoptera: Scarabaeidae) and wireworms (Coleoptera: Elateridae). Numerous species of wireworms found in Virginia are predominantly in the genera *Melanotus*, *Conoderus*, *Aeolus*, *Hemicrepidius*, and *Limonius* (Briggs 1980, Youngman et al. 1993). Annual white grubs have a 1 year life cycle in contrast to true white grubs that complete their life cycle in 2-3 years (McLeod et al. 1999). True white grubs predominantly of the genus *Phyllophaga* are found in Virginia (Briggs 1980), but historically cause less injury in the Mid-Atlantic region (Youngman and Tiwari 2004) than in the Midwest Corn Belt (McLeod et al. 1999). Common annual white grubs found in Virginia include Japanese beetle, *Popillia japonica* Newman; Asiatic garden beetle, *Maladera castanea* (Arrow); grubs in the genus *Cyclocephala*, northern and southern masked chafers; and *Cotinus nitida* (Linnaeus), the green June beetle. Other soil insects that negatively impact early-season corn are seedcorn maggot, *Delia platura* (Meigen) (Diptera: Anthomyiidae), and a primary pest, corn rootworm, *Diabrotica* spp. (Coleoptera: Chrysomelidae) (Youngman and Tiwari 2004). Many farmers practice crop rotation as a means of pest management and pest exclusion from crops. The incidence of corn rootworms and wireworms generally is lower in common corn-soybean crop rotations, though damage from white grubs may increase in corn (Luckmann and Metcalf 1982).

Annual white grubs and wireworms are early-season pests attacking corn seeds and seedlings immediately following planting in Virginia (Briggs 1980, Youngman et al.

1993). Because grubs and wireworms occur in the soil, their presence in fields and subsequent damage may go unnoticed until too late. In order to relate relative soil pest densities prior to planting to corn plant stand and yield loss, Youngman et al. (1993) developed the baited wire trap to attract soil insects to germinating seeds. However, because of low pest densities across most of the fields in their study, they were not able to develop predictive models, suggesting that these insects are sporadic pests of corn in Virginia (Youngman et al. 1993). The particular life cycles and seasonal development of white grubs and wireworms limit their economic damage to corn after the seedling stage (McLeod et al. 1999). However, damage can occur when high soil pest infestation and cool wet weather follow planting.

White grubs typically exhibit a patchy distribution (Dalthorp et al. 2000, Potter and Held 2002). High and low density patches of grubs tend to recur year after year in the same locations including high density patches that typically follow high local adult activity (Dalthorp et al. 1999). Growers may then expect recurring white grub problems from historically active fields, where suitable soil type and moisture are prime for oviposition (Fleming 1972). Although adult sampling has potential for predicting future infestation areas (Potter and Held 2002), larval soil sampling provides an adequate measure of population densities in sample areas at any given time (Fleming and Baker 1936, Yates and Finney 1942).

A sampling procedure that effectively predicts infestation of each group of insects is necessary for an effective preventative pest management strategy. Current sampling methods for these soil pests include some form of baiting or soil sampling method performed in the spring before planting (McLeod et al. 1999, Youngman and Tiwari

2004). Potentially damaging soil pest populations usually result in the application of soil insecticides applied in-furrow, as a band application over-furrow, and/ or insecticide treated seeds at planting in the spring. Presently, pretreated seeds can be ordered with select rates of seed-applied insecticide. A sampling method performed in the fall that can adequately predict spring soil pest densities will be important to growers planning to use insecticidal seed treatments because they must order seed treatments in winter prior to the time of traditional spring soil sampling. In addition to improved farmer safety, seed treatments are an efficient use of pesticides provided by minimal dosage applications and fewer application costs (Metcalf 1982).

This study evaluated the efficacy of fall soil sampling, opposed to spring sampling, for predicting spring infestation of white grubs and wireworms in field corn. Pest densities from the standard soil sampling method were evaluated against a more efficient compact sampling method. In addition, replicated field trials were planted with low and high rates of a common seed-applied insecticide to determine the level of protection offered by seed treatments at varying pest densities. Development of sound methods for predictive scouting are immediately of great value to corn producers, while implementation in an integrated pest management program will help protect a valuable commodity and increase profit by limiting excessive pesticide use.

Methods & Materials

A two-year, replicated field study was started in fall 2005, and continued to spring 2007, to predict spring infestation levels of white grubs and wireworms in cornfields.

The following will address the development of a fall sampling method for white grubs and wireworms in corn.

2005-2006 Experiment. A total of 15 post-harvest soybean fields in Essex, Westmoreland, Caroline, and Accomack Counties, VA, were sampled in late October and early November and again in late March, two to three wks before corn planting. This study compared a 20.3 cm square by 15 cm deep soil sample (compact method) with a 30 cm square by 15 cm deep soil sample (standard method) to evaluate the potential of the compact method to correlate to the standard method after a 2.25 weighting factor (the standard method samples 2.25 x more volume of soil than the compact method).

In each field, 15 randomized pairs of the standard and compact method were sampled. The sample rows and samples within rows were ≈ 9.14 m apart. A square metal grid of the proper size was used to mark the perimeter of the sample area, and the soil was excavated to a depth of 15 cm. The soil was carefully sorted on a black plastic tarp to identify and collect soil insects. The soil fauna in each sample were recorded and collected in 70% ethyl alcohol. Collected specimens were returned to the laboratory and white grubs and wireworms were identified to genus and sometimes species using keys of common raster patterns of grubs and caudal features of wireworms (Vittum et al. 2001). Dominant soil textures in each sample location were determined from appropriate county soil maps (USDA 2007). The predominant soil texture in sampled fields was fine sandy loam.

Replicated field plots ($n = 14$) were planted no-till on 10 and 11 April with Trisler 5244-RR event MON863+GA21 corn seed (Augusta Seeds Corporation; Staunton, VA) following the cooperators' normal agronomic practices with the exception of soil applied

insecticides, such as granular or seed-applied insecticides. An Almaco 2-row Max-Emerge planter was used to plant all plots at a rate of 64,493 seeds per hectare. Randomized test strips with three seed treatments (untreated control; Poncho 250, 0.25 mg clothianidin / kernel; and Poncho 1250, 1.25 mg clothianidin / kernel) were planted into 14 fields. Insecticide-treated plots were two rows wide (76.2-cm row spacing) by 61 m long, while the untreated control was four rows wide by 61 m long.

On 10 May, approximately 30 d after planting, stand counts were taken by counting the number of plants in the center two rows per 30.5 row-m (no. plants per 61 row-m total of each plot). Plants that were unthrifty or dying were counted and subtracted from the treatment stand count total. Unthrifty plants were assessed on visual size and health evaluation.

Grain yields were taken on 23 and 24 August. Half-way in the plot, all main ears were removed from 15.2 row-m in the middle two rows (total of 30.5 row-m for each treatment). Ears were shelled using a tractor driven sheller, after which total grain weights and percent moisture were recorded for each treatment. Grain yield weights were converted to 15.5% moisture. Due to a lack of treated seed and other in-season complications, grain yield was determined in only 11 of the 14 fields.

2006-2007 Experiment. Since no significant difference was detected between the standard and compact sampling method in the previous year, only the compact method was used for soil samples following similar procedures from the previous year. In fall, 20 post-harvest soybean fields in Essex County, VA, and four varied practice fields in Accomack County, VA, were sampled from 3 to 10 November. On 27 and 28 March, only 21 fields were sampled after three fields were lost to wheat sown fields. Sampling

was performed 1 wk before spring planting. Unlike in 2006, soil temperature was recorded in all fields using a 10 cm analog temperature probe, and soil textures were determined from county soil maps (USDA 2007). The predominant soil textures of the fields were sandy loam and loamy sand.

Using the same seed and methods from the previous years, replicated field plots were planted no-till on 4 April ($n = 14$) in Essex County, VA, and on 11 April ($n = 4$) in Accomack County, VA. An Almaco 2-row Max-Emerge planter was used to plant plots at a rate of 64,493 seeds per hectare, with two border rows planted around the perimeter of the experiment area.

Stand counts were taken 35 d after planting (10 May). Grain was harvested on 3 September in Essex Co. and in late August and September in Accomack Co. following the same harvest procedures from 2006. Grain from all treatments was recovered in only eight of 18 fields due to extensive drought effects in the Mid-Atlantic region.

Statistics. The compact method was evaluated for its potential to correlate to the standard method after a 2.25 weighting factor. Pest density differences between the two soil sample methods were evaluated using paired t tests after adjusting the compact method weighting factor and then applying a square root $(x + 0.5)^{1/2}$ transformation to weighted compact and standard method data. Correlations were performed on transformed spring and fall field pest densities.

Harvest data were analyzed using analysis of variance (ANOVA) with linear contrasts in fields where all treatments were recovered, and similarly only in fields where white grub densities exceeded the action threshold of 0.9 white grubs per compact method (SAS Institute 2001). Additionally, fields with above-threshold wireworm

densities were removed from analysis to control for external treatment influences. Linear regression was used to describe the response of yield to pest density in the fall and spring. Proportion yield loss data were transformed ($\arcsin \sqrt{y}$) and pest count data were transformed (\sqrt{x} , $\log x$, $\ln x$, and $1/x$) to explore the relationship between pest densities and yield loss. Differences were considered significant at a $P \leq 0.05$ unless indicated otherwise. The optimal number of samples for sampling were determined from Taylor's power law (Taylor 1961):

$$N = (100 / c)^2 t^2 a m^{b-2}$$

where N is sample size, c is the accuracy level (25%), t is the value of the t distribution at $\alpha = 0.05$, m is the average number of white grubs per field across all sampling periods, and a and b are variables obtained from linear regression of log variance over log mean of mean white grubs within fields.

Results

Standard and compact sampling methods. In the first year of sampling, no significant differences in white grub densities were detected between the two soil sample methods in either the fall or spring results after correcting (2.25x) for differences in sampling volume (paired t test: fall, $t = -1.59$, $df = 224$, $P > 0.05$; spring, $t = -0.02$, $df = 224$, $P > 0.05$). When mean grub densities were averaged across all fields for the fall 2005 and spring 2006 sampling periods, the compact method had a slight but distinct numerical advantage over the standard method in detecting white grubs (Figure 3.1).

Because no difference was found between the two sample methods, only the compact method was used in the second year of sampling. Strong correlations were

observed between fall and spring densities in both sampling sequences (year 1: $F = 57.92$ $r = 0.91$, $df = 1, 13$, $P < 0.0001$; year 2: $F = 61.79$ $r = 0.88$, $df = 1, 19$, $P < 0.0001$).

Overall, a strong correlation was observed between fall and spring pest densities over both years ($F = 114.8$, $r = 0.88$, $df = 1, 32$, $P < 0.0001$). Transformation of pest density did not improve correlations over actual data. Therefore, actual data are presented in text and figures.

Soil Sampling. Soil temperatures at time of fall and spring sampling ranged from 6.7 – 19.4 °C in Essex Co. and from 13.9 – 16.7 °C in Accomack Co. With little fluctuation of mean daily temperature and some amount of lag time, soil temperatures down to 30 cm soil depth typically correspond to ambient air temperatures (Mail 1930, Villani and Wright 1990). Air temperature means fluctuated rapidly in the spring and fall with warm and cold spells contributing to a range of soil temperatures during sampling. All fields shared loam soil textures, though highest wireworm and white grub densities were found in fields with fine sandy loam in the upper soil profile ($\approx 0 - 30$ cm).

Assorted soil fauna were collected over several seasons of sampling, though white grubs followed by wireworms were the most abundant (Table 3.1). Of the grubs recovered in the first and second year of sampling, more than 97% and 99% were annual white grubs rather than true white grubs across all fields ($n = 39$). Therefore, provided the scarcity of true white grubs during both years, white grub densities were expressed as mean annual white grubs per compact method with reference to a spring nominal threshold of 0.9 annual white grubs per compact method, unless otherwise specified. In year one, 82% of the grubs were *P. japonica* and the rest predominantly of the genera *Maladera* and *Cyclocephala*. A different grub composition was observed in the second

year of sampling where more than 70% *M. castanea* and 25% *P. japonica* were collected. From spring to fall the proportion collected of *P. japonica* increased 6.5% and 11% in both years of study, respectively. In contrast, percent composition of *M. castanea* grubs decreased 4% and 6%.

In the first year of sampling, 85% and 57% of fields exceeded the nominal threshold in the fall (n = 12 of 14) and the spring (n = 8 of 14), respectively. In the second year of sampling, 82% and 65% of fields exceeded the action threshold in the fall (n = 11 of 17) and the spring (n = 9 of 17), respectively. Mean white grubs per compact method ranged from 0.4 – 4.5 and from 0.2 – 3.0 in fall 2005 and spring 2006, respectively. A greater range of white grub densities was found with means of 0.1 – 5.9 and 0.2 – 4.7 white grubs per compact method in fall 2006 and spring 2007, respectively.

White grub densities decreased an average of 34% across all sampling periods from fall to spring. This is consistent with the overwinter mortality rates of *Phyllophaga* spp. in the Midwest (NDSU 2007). Likewise, the number of fields above threshold in the fall decreased 33% and 21% in the spring in the first and second year of study, respectively.

More than 98% of wireworms collected were species of the genus *Conoderus*. In the first year of study, an average of 0.09 wireworms per compact method were found in twelve (of 15) fields. Mean wireworms per compact method ranged from 0 – 0.27. In the second year of study, two fields in the fall exceeded the nominal threshold of 0.44 wireworms per compact method with means of 1.13 and 0.53 wireworms. Densities in the following spring increased to 2.6 wireworms and dropped to 0 wireworms per compact method, respectively. One additional field with wireworms above threshold in

the spring (0.47) was below threshold in the fall (0.4 wireworms per compact method). Given the age variability of wireworms and the relatively low numbers collected, wireworm sampling data were not used in the data analysis.

Other soil fauna were collected including various arthropods found in or near the surface layer of soil. The most common soil fauna were ground beetle adults and larvae (Coleoptera: Carabidae), various pupae, *Tipula* larvae (Diptera: Tipulidae), millipedes (Diplopoda), and centipedes (Chilopoda).

The linear regression of log variance on log mean soil densities produced the model: $y = 0.127 + 1.09x$ ($r^2 = 0.87$; $F = 420.29$; $df = 1, 63$; $P < 0.0001$). According to this model and Taylor's power law (Taylor 1961), at least four samples per field were needed for 95% certainty that the mean sample densities of white grubs are within 25% of the true value (Table 3.2). Furthermore, with a level of 15% accuracy of the true value, at least ten samples per field were needed.

Insecticide seed treatments. Corn plant stand was evaluated ≈ 30 d after planting for each year of study. Treatment was a significant source of variation in stand in the first and second year of study ($F = 4.34$, $df = 12, 20$; $P < 0.01$; ANOVA; $F = 2.69$; $df = 15, 26$; $P < 0.05$; ANOVA). Stand was significantly higher in Poncho 1250 plots versus Poncho 250 and the check over both years (year 1: $n = 11$; $F = 12.52$; $df = 1, 10$; $P < 0.01$, ANOVA and linear contrast; year 2: $n = 14$, $F = 26.17$; $df = 1, 13$; $P < 0.0001$; ANOVA and linear contrast). Plant stands in Poncho 250 treated plots were consistently lower than the untreated plots; however, this difference was not significant ($P > 0.05$).

A similar relationship between insecticide treatment and plant stand was observed in yield analysis. Corn grain yield data were collected and evaluated for fields in the first

($n = 11$) and second ($n = 7$) years of this study. Fewer fields were recovered in the second year due to an extensive drought in the Mid-Atlantic region. In the first and second years of study, 73% ($n = 8$) and 43% ($n = 3$) of fields exceeded the nominal threshold with white grubs densities that ranged from 1.0 to 3.0 grubs per compact method. Fields below threshold in the first ($n = 3$) and second ($n = 4$) years of study had white grub densities that ranged from 0.1 to 0.8 grubs per compact method, respectively.

In the first year, there was no significant treatment effect for grain yield when all fields ($n = 11$) were included in analysis ($F = 0.966$; $df = 2, 20$; $P = 0.4$; ANOVA). When the three, below-threshold fields were removed from the analysis, the treatment effect for grain yield was significant ($F = 3.11$; $df = 2, 14$; $P = 0.076$, ANOVA). Poncho 1250 yielded significantly higher than the check and Poncho 250 treatments ($F = 6.18$; $df = 1, 14$; $P = 0.026$; ANOVA and linear contrast) (Figure 3.2). Likewise, in the second year of study, no significant treatment effect was observed when all fields ($n = 7$) were included in analysis ($F = 1.60$; $df = 2, 12$; $P = 0.24$; ANOVA). Though no significant treatment effect was observed when the below-threshold fields were removed from analysis ($F = 1.16$; $df = 2, 4$; $P = 0.40$; ANOVA), the same numerical trend of yield performance for Poncho 1250 treated plots was observed when compared to the Poncho 250 treated plots and the untreated check (Figure 3.2).

Of seven fields below threshold across both years of study, not including two that had only two treatments, mean grain yields were not significantly different between the control or insecticide-treated plots ($F = 0.42$; $df = 2, 4$; $P = 0.7$; ANOVA; $F = 1.08$; $df = 2, 6$; $P = 0.4$; ANOVA) (Figure 3.3). The untreated control numerically yielded more

than both treatments in the first year of study; however, the treated plots yielded more than the untreated control in the second year.

After observing a significant treatment effect on yield for the first year of study, the regression of spring pest density on yield benefit in Poncho 1250 and Poncho 250 treated plots relative to the check resulted in significant and non-significant linear relationships. No clear relationship was observed between fall pest density and yield benefit from any treatment. The best linear relationship was observed between spring pest density $(x + 0.5)^{1/2}$ and yield benefit $(\arcsin \sqrt{y})$, where $y = 0.5 + \text{proportion yield benefit} / 2$ of Poncho 1250 treated plots relative to the check, which produced the following regression model: $y = -0.076 + 0.043x$ ($r^2 = 0.42$; $F = 6.48$; $df = 1, 9$; $P < 0.05$). Thus, it was determined that the economic injury level (EIL) for yield loss would occur in untreated plots if spring pest density was ≥ 1.04 (± 0.06) white grubs per compact method. This corresponds to an economic treatment threshold (ET) of 0.76 white grubs per compact method (75% of the EIL). A spring pest density of 1.04 white grubs corresponds to a fall pest density of 1.6 (± 0.17) white grubs per compact method. Therefore, based on an EIL of 1.6 grubs per compact method, the ET for fall sampling is 1.2 white grubs per compact method.

Discussion

Management techniques for soil pests generally rely on producer agronomic practices and pest incidence. For example, planting corn year after year in the same field will attract corn rootworms, corn after soybean will attract white grubs, and corn following pasture and hay crops typically will attract more than 10 insect pests such as

white grubs, wireworms, and seedcorn maggots (Luckmann and Metcalf 1982). In addition, a knowledge of historical pest activity combined with some form of soil insect sampling technique is the basis for a comprehensive treatment plan (McLeod et al. 1999). Although moderate to high early season soil pest densities will not always result in yield loss, a pest density above some specified threshold is usually the best indicator for treatment. The results of my study suggest that it is possible to relate fall white grub density to subsequent spring white grub density. Furthermore, the results show a relationship between spring grub density and potential stand and yield damage following in fields planted with corn.

My results indicate no difference between the two sample methods, which strongly suggests that sampling for white grubs is more efficient and less time consuming with smaller soil samples, i.e., the compact method than with the standard method. The numerical advantage of detecting white grubs with the compact method, especially in the fall, may be the result of slightly aggregated groups of grubs that have not migrated down in the soil profile. Clumped distributions were consistent across most fields. There were correlations between fall and spring pest density for both years of the study. In addition, regressing spring pest density against fall pest density produced a model that accounted for 75% of the model variability. This relationship was expected because white grub overwinter mortality should be consistent across similar environments.

White grubs densities can be sampled within 25% of the actual mean with four compact samples per field. However, an inverse relationship exists for wireworm sampling where successively more samples were required for sample sizes smaller than 30 cm square (Jones 1937, Fenton 1947). The number of samples required for white grub

sampling were not adequate for accurate wireworm sampling. Therefore, it is reasonable to exclude the wireworm data from analysis. Although incidence of wireworms should not be ignored, damaging populations most commonly occur in areas of historical activity.

The variable white grub composition of soil samples may have been a result of actively recurring populations, crop rotation practices, and environmental conditions including soil moisture, temperature, and texture. Peak *P. japonica* flight and egg laying typically occurs in mid July and may extend into September in Virginia. High rainfall in July 2005 may have contributed to optimum soil conditions for *P. japonica* oviposition. In contrast, the below average rainfall in the egg-laying months of July through September over the second and third years of my study may have contributed to lower *P. japonica* soil populations. Low wireworm diversity may be the result of sampling method, low local diversity and or consistently low wireworm abundance at sample locations. The high number of soil samples required to reduce sampling error, in addition to labor intensive wireworm soil extraction techniques that were not used in this study may have limited wireworm recovery and diversity. However, the lack of other non-white grub pests, such as wireworms and seed-corn maggots, reveals no unexplainable findings in my stand and yield results.

Overwintering behavior of white grubs may explain composition changes from the fall to the spring. While most *P. japonica* overwinter as third instar grubs, *M. castanea* overwinter as second and third instar grubs. Various factors in late summer and fall may prevent development beyond the second instar and result in a higher proportion of second instars that overwinter. It is plausible that the second instar *M. castanea*

require more feeding and development time than third instars in the spring, though overwinter survival decreases when the third instar is not reached. Years with a high density of overwintering second instar *M. castanea* may increase the potential for reduction in stand and yield than will a high density of third instars feeding in the spring. The decrease of *M. castanea* proportionally in the spring, relative to the fall, may be the result of overwinter mortality of second instar grubs. Though both species exhibit the following behavior, the proportional increase of *P. japonica* in the spring may occur after the white grubs move upward in the soil profile to within a few centimeters of the surface layer and commence feeding in the spring.

It is clear from the stand loss in untreated corn that both *P. japonica* and *M. castanea* are equally strong indicators of stand loss. Despite no clear benefit of seed treatment in below-threshold treated plots, extraneous factors like water stress in the second year of study may reveal treatment benefits that would not otherwise be realized in years of adequate moisture. However, *P. japonica* infestations above threshold resulted in yield loss in untreated corn. The compact method was accurate at predicting yield loss in fields above the EIL and ET in the fall and spring. For fields above the estimated EIL in the fall and spring, 57% and 75% of field yields benefited from the Poncho 1250 treatment. In addition, in fields above the ET in both fall and spring, 75% of fields benefited from the Poncho 1250 treatment. A 6% yield benefit was observed with Poncho 1250 treatments. The significant yield advantage of Poncho 1250 treated seed in comparison to Poncho 250 is especially troublesome, given that white grub control is stated on the Poncho 250 label and because of the much higher cost of Poncho 1250 treated seed relative to Poncho 250 treated seed.

Current management of white grubs includes some form of baiting or soil sampling method (Youngman and Tiwari 2004). The baited wire trap (Youngman et al. 1993) and bait stations (Ward and Keaster 1977) may predict potential white grub damage to corn. However, my results showed a direct relationship between white grub soil density and subsequent stand and yield loss. Although spring white grub densities accounted for < 39% of the variability in subsequent yield loss, the predictive information obtained in this study will be an immediate benefit to corn producers in Virginia, and possibly other corn growing regions.

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Table 3.1. White grubs (Scarabaeidae) and wireworms (Elateridae) collected in soil samples in the fall (F) and spring (S) for both years of study in post-harvest soybean (n = 32) and mixed practice fields (n = 4) being planted into corn.

Order	Family	Scientific name	Common name	Year 1		Year 2	
				F	S	F	S
Coleoptera:	Scarabaeidae	<i>Popillia japonica</i> Newman	Japanese beetle	920	518	77	70
		<i>Maladera castanea</i> (Arrow)	Asiatic garden beetle	105	30	254	165
		<i>Cyclocephala borealis</i> Arrow or	northern masked chafer or				
		<i>C. immaculata</i> Arrow	southern masked chafer	82	39	12	6
		<i>Phyllophaga</i> spp.	May / June beetle	10	3	0	0
		<i>Cotinus nitida</i> (Linnaeus)	green June beetle	8	0	1	2
		<i>Ataenius spretulus</i> (Haldeman)	black turfgrass ataenius	0	1	0	0
	Elateridae	<i>Conoderus</i> spp.		57	53	46	22
		<i>Melanotus</i> spp.		1	1	0	0
		<i>Limonium</i> spp.		1	0	0	0

Table 3.2. Minimum number of samples in the fall and spring needed to be 95% confident within a specified percentage of the actual mean, according to Taylor's power law.

Sampling Period	% within actual mean			
	10%	15%	20%	25%
Fall	21.5	9.5	5.4	3.4
Spring	33.1	14.7	8.3	5.3

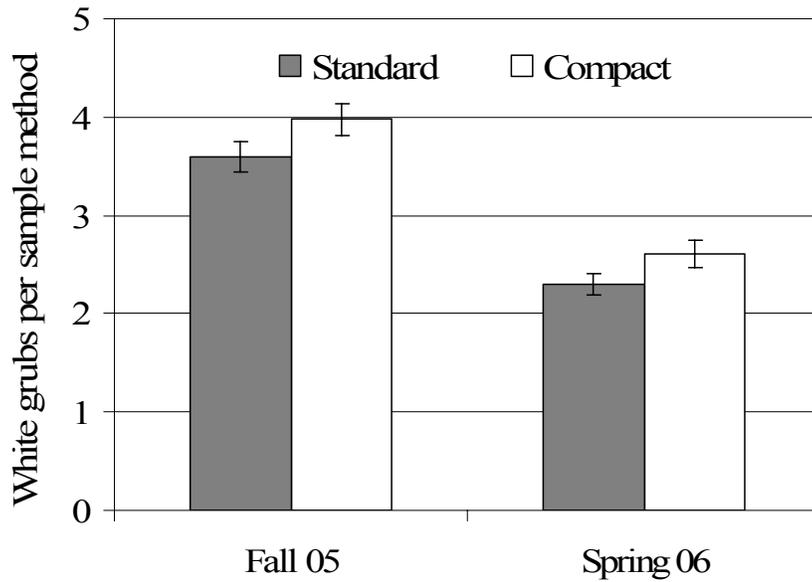


Figure 3.1. Overall mean white grubs across fields ($n = 14$) per standard (30 cm square by 15 cm deep) and compact (20.3 cm square by 15 cm deep) (after 2.25x weighting factor) sample methods between fall 2005 and spring 2006. Differences not significant, paired t test ($P > 0.05$).

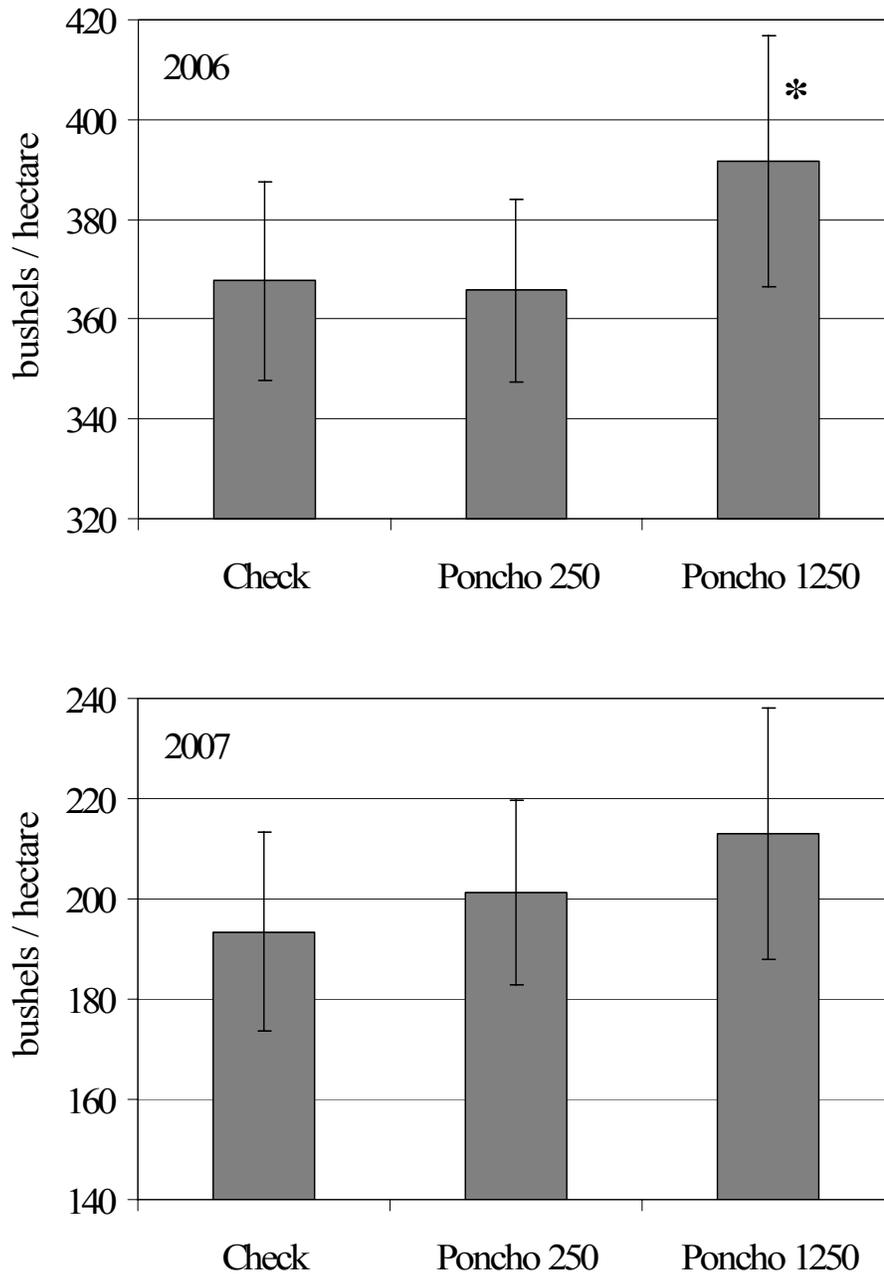


Figure 3.2. Mean (\pm SE) corn grain yield (bushels/hectare at 15% moisture) in fields with spring pest densities above action threshold of 0.9 white grubs per compact method (n = 8 in 2006; n = 4 in 2007). *Column mean with asterisk is significantly different from columns without asterisk (ANOVA, linear contrast; $P < 0.05$).

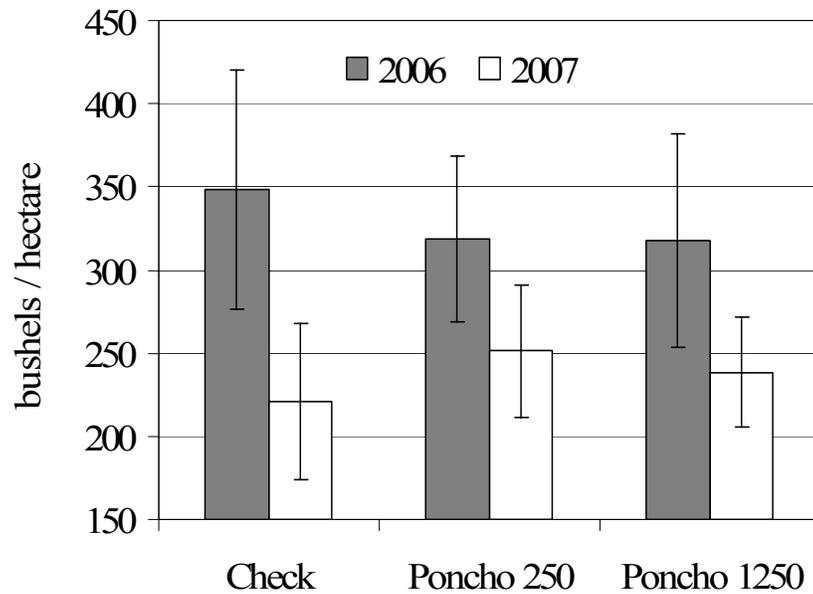


Figure 3.3. Mean (\pm SE) corn grain yield (bushels/hectare at 15% moisture) across a non-treated check and two insecticide treated plots in fields with spring pest densities below action threshold of 0.9 white grubs per compact method (n = 3 in 2006; n = 4 in 2007).