

**THE EFFECTS OF EUROPEAN CORN BORER ON WHOLE-PLANT YIELD  
AND ROOT KNOT NEMATODE FITNESS IN CORN**

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## ABSTRACT

Field studies were conducted over two growing seasons to evaluate the effect of different levels of third instar European corn borer, *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae), on whole-plant dry matter in corn grown for silage. Mean ( $\pm$  SEM) whole-plant dry matter was significantly greater by 18.8% in uninfested control plants than in plants with an infestation level of 6 larvae/plant in 2004. Whole-plant dry matter in 2005 was significantly greater by 10.5% in control plants than in plants with an infestation level of 5 larvae/plant. Economic injury levels were calculated for each year using regression equations between whole-plant dry matter and European corn borer infestation level. Plant growth stage and infestation level had no effect on percent acid detergent fiber, neutral detergent fiber, and crude protein values for either year.

Greenhouse studies were conducted to examine the relationship between aboveground herbivory by European corn borer and belowground herbivory by root knot nematode, *Meloidogyne incognita* Chitwood (Tylenchida: Heteroderidae), in corn. Two experiments were conducted to measure belowground herbivory by *M. incognita* in juvenile penetrations and eggs/root system. In the first experiment, the main effects interaction was not significant for either *M. incognita* juvenile penetrations or eggs/root

system. Overall mean juvenile penetrations/root system across all three growth stages, at infestation levels of 1 and 3 larvae/plant were significantly less than in the non-infested control. In addition, overall mean eggs/root system at an infestation level of 3 larvae/plant were significantly less than in the control. In the second experiment, the main effects interaction was significant for both juvenile penetrations and eggs/root system. At the 8 and 10 leaf growth stages, juvenile penetrations/root system at infestation levels of 1 and 3 larvae/plant were significantly less than in the control. In addition, eggs/root system at an infestation level of 3 larvae/plant were significantly less than in the control, at all growth stages. In the reciprocal study, which examined the effect of different *M. incognita* inoculation levels on European corn borer stalk tunneling, no significant effect of inoculation level on European corn borer stalk tunneling was found.

## **DEDICATION**

This dissertation is dedicated to the loving memory of my father Shyam Sundar Tiwari, who passed away at 2:30 pm on October 30 2003 in Deoria, India. He was always a tremendous source of energy and guidance for me in every aspect of my life and will be missed always. Even during times of terminal illness, he never failed to inspire me with his courage and determination.

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## CHAPTER 1

### INTRODUCTION

Corn, *Zea mays* L., is one of the most important crops grown for grain and silage worldwide. The U.S. ranks as the number one corn-growing nation in the world and accounts for nearly one-fifth of annual worldwide corn production (Youngman and Tiwari 2004). In 2006, Virginia produced over 41.4 million bushels of corn grain (139,617 ha harvested) and 2.06 million metric tons of corn silage (52,609 ha harvested) (USDA-NASS 2007). Among the multitude of potential pests that attack field corn, European corn borer, *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae), is one of the most important (Mason et al. 1996, Youngman and Tiwari 2004, Dillehay et al. 2005). It has been estimated to cost U.S. corn growers approximately one billion dollars annually in management costs and crop losses (Mason et al. 1996).

European corn borer can be a serious pest of corn and other crops in Virginia (Despins and Roberts 1984). However, little research has focused on the relationship between European corn borer infestation level and yield loss in corn. The lack of research may be partly attributed to the infrequent and sporadic nature of the pest in the eastern part of the state. In a survey of cornfields in 14 eastern Virginia counties, Youngman et al. (1998, 1999, 2000) reported very low to moderate levels of European corn borer infestations (0.6 to 3.5%) in corn stalks. However, the situation in the western part of state is not the same where European corn borer causes greater economic

damage to corn (Youngman and Laub 2002). Youngman and Laub (2002) reported that out of 126 surveyed cornfields in western Virginia from 2000-2002, nearly 25% (31 fields) had 1 or more tunnels/stalk  $> 1.27$  cm (0.5”), and 19.8% (25 fields) had one or more tunnels/stalk  $> 2.54$  cm (1”). Considering an economic injury level of 1 or more tunnels/stalk, cornfields in 5 out of 10 counties surveyed in western Virginia averaged above threshold levels in 2002 (Youngman and Laub 2002). The findings indicate that European corn borer is a more serious pest of corn in western Virginia, and studies on the relationship between European corn borer and yield losses in corn can help in minimizing economic losses.

Most of the studies focusing on the relationship of European corn borer and yield losses in corn have focused on grain yield (Chiang et al. 1954, Jarvis et al. 1961, Calvin et al. 1988). The few studies that have focused on the relationship between European corn borer and yield losses in corn grown for silage have produced contradictory results (Thompson and White 1977, Raemisch and Walgenbach 1984, Myers and Wedberg 1999). It is clear that more research needs to be done to address the impact of European corn borer on corn grown for silage, especially in areas where corn is mostly grown for silage purposes. In Virginia, about 52,609 ha were harvested for silage purposes in 2006, which represents about 27.4% of the total hectares under corn production in the state (USDA-NASS 2007). At the national level, about 2,621,149 ha were harvested for silage in 2006, which is about 8.4% of total hectares under corn production (USDA-NASS 2007). The U.S. has the largest number of hectares under production for corn silage purposes of all the corn growing countries (Lauer et al. 2001).

Root feeding by plant-parasitic nematodes is another important factor that can interfere with successful corn production. The injury caused by plant-parasitic nematodes in corn is highly variable with location, and yield losses have been reported to range from 0-23% (McSorley et al. 1987) and 0-20% (Koenning et al. 1999). Potential losses to corn from nematodes in Virginia, with and without nematicide application are estimated at 1.3 and 6.5%, respectively (McSorley et al. 1987). One of the more important plant parasitic nematodes associated with corn is the root knot nematode, *Meloidogyne incognita* Chitwood (Tylenchida: Heteroderidae) (McSorley and Dickson 1989, Heffes et al. 1992, Tiwari et al. 2005). Under rare instances of heavy infestations, *M. incognita* can cause yield losses of  $\geq 30\%$  in corn (Windham and Williams 1994).

Several studies have investigated the association between spatially separated herbivores, such as that involving belowground herbivory by nematodes and aboveground herbivory by insects (Russin et al. 1989, Russin et al. 1993, Fu et al. 2001, Blossy and Hunt-Joshi 2003); however, detailed information on such interactions in corn is lacking. Given the potential economic losses from European corn borer and *M. incognita* to corn, research focusing on the interactions between two spatially separated pests may contribute to improving our understanding of basic ecological questions between these two species. Furthermore, the understanding of the interactions between these species will help in defining the impact of such interactions on corn pest management.

My research has four primary objectives:

- 1) Evaluate the effect of European corn borer on corn whole-plant dry matter.
- 2) Evaluate the effect of European corn borer on corn plant quality.
- 3) Develop European corn borer economic injury levels on corn grown for silage.
- 4) Evaluate the relationship between aboveground herbivory by European corn borer and belowground herbivory by root knot nematode in corn.

## LITERATURE REVIEW

### European Corn Borer

European corn borer was introduced into the eastern U.S. in the early 1900s in broomcorn from Hungary and Italy (Mason et al. 1996). Since then it has continued to spread across the U.S. moving westward to the Rocky Mountains, and by 1950 it was found in Mississippi, Alabama, and Georgia (Brindley and Dicke 1963). European corn borer can be a severe pest of field corn, with losses to U.S. corn growers estimated at one billion dollars annually (Mason et al. 1996).

Several authors have reviewed the life history and biology of European corn borer on corn (Brindley and Dicke 1963, Brindley et al. 1975, Mason et al. 1996, Calvin and Van Duyn 1999, Youngman and Tiwari 2004). The number of generations varies from a single generation in the Great Lakes region to as many as four generations in the southern corn producing states (Brindley and Dicke 1963). The fifth instar enters diapause in the fall in pieces of cornstalks, corn cobs, or suitable weed hosts where it remains until the following spring. In the spring, when temperature exceeds 10<sup>0</sup>C (50<sup>0</sup>F), the fifth instar resumes development and pupates. Adult emergence follows approximately 2-3 weeks after initiation of pupation (Mason et al. 1996). First generation eggs are usually deposited on whorl-stage corn plants, whereupon the newly eclosed first instars begin feeding on the leaves. Feeding initially occurs in the whorl, which is followed by the leaf axil, leaf sheath and stalk. Stalk tunneling typically begins

with the late third instar and continues through the fourth and fifth instars (Mason et al. 1996).

### **Yield Losses in Corn Grown for Grain**

The majority of research on the relationship of European corn borer injury and corn response has focused on corn grown for grain (Patch et al. 1942, Chiang et al. 1954, Kwolek and Brindley 1959, Jarvis et al. 1961, Raemisch and Walgenbach 1984, Umeozor et al. 1985, Bohn et al. 1999, Dillehay et al. 2005). The findings from these studies generally show a regional influence on the severity of European corn borer damage.

In an Ohio study, Patch et al. (1942) evaluated percent grain yield reduction/larva/plant in artificially infested non-hybrid (Clarage variety) and hybrid cornfields. In the non-hybrid fields that normally (i.e., non-infested) produced yields of 28 and 85 bushels/acre, the yield loss/larva/plant (4.9 and 3.7%, respectively) decreased as yield increased. In contrast, in the hybrid fields that normally (i.e., non-infested) produced yields of 85 and 105 bushels/acre, the yield loss/larva/plant (3.0 and 3.9%, respectively) increased as yield increased. In a Minnesota study involving natural infestation, Chiang et al. (1954) reported that second generation European corn borer feeding indirectly effects yield from stalk breakage and ear drop, but has little effect on ear growth. In another study conducted in Ohio, Minnesota, and Iowa involving both natural and artificial infestation, Kwolek and Brindley (1959) found conflicting results. In Ohio, the greatest yield losses resulted from second generation European corn borer

feeding. In contrast, first generation feeding caused the greatest yield losses in Minnesota. An intermediate situation involving an interaction of first and second generations on yield loss was found in Iowa. In another study conducted in Iowa by Jarvis et al. (1961), first generation feeding was found to cause greater yield reductions than second generation feeding. This study involved both naturally and artificially infested plants. Raemisch and Walgenbach (1984) reported that naturally infested second generation feeding resulted in 4.1% grain loss/tunnel, while the combination of first and second generation feeding resulted in 7.6% grain loss/tunnel. Umeozor et al. (1985) reported yield reductions ranging from 4.1 to 54.3%/tunnel in a North Carolina study on randomly selected plants under natural infestation. The authors concluded that yield reduction from European corn borer infestation is influenced by infestation date, infestation level, and rainfall. In a study conducted in Central Europe where there is only one generation/year, Bohn et al. (1999) using both naturally and artificially infested plants, found a 6% yield loss from one larva/plant. In a recent study conducted in Pennsylvania and Maryland, Dillehay et al. (2005) developed a model that predicted a yield loss/larva/plant of 2.69% on a regional level. Their model provided similar predictive outputs for other areas within climatic zones of 1100-1700 degree days (base threshold 12.5<sup>0</sup>C). Recognizing that mean yield losses can vary among corn growing areas as a result of differences in number of generations, insect-plant synchrony, and climatic conditions (Calvin et al. 1988), the yield loss estimation of 3% by one larva/plant by Patch et al. (1941, 1942) is considered to be the most widely accepted level of yield loss from European corn borer (Chiang et al. 1954, Lynch 1980).

### *Plant growth and yield loss*

Numerous research has been done on assessing the effects of tunneling on grain yield at different plant growth stages (Lynch 1980, Lynch et al. 1980, Calvin et al. 1988, Bode and Calvin 1990, Walker et al. 2000). Lynch (1980) in an Iowa study using artificial infestation found that yield reduction from European corn borer infestation was primarily due to physiological losses rather than from ear loss at the five growth stages: whorl, late whorl, pretassel, pollen shed, and blister. In another study using artificial infestation, Lynch et al. (1980) reported greater grain yield losses from stalk tunneling at pollen shed compared to the early whorl stage. In a Kansas study using artificially infested plants, Calvin et al. (1988) found that maximum yield reductions occurred from tunneling at the blister stage, with the impact of stalk tunneling decreasing with the growth of the corn plant. In contrast, a similar study conducted in Pennsylvania by Bode and Calvin (1990) using artificially infested plants found that stalk tunneling initiated at the 10-leaf or 16-leaf growth stages (vegetative growth stages) had a greater negative effect on grain yield than tunneling initiated at the blister or dough stages (reproductive stages). In a more recent study by Walker et al. (2000) on European corn borer injury among different transgenic Bt corn hybrids using artificial infestation, they found that not all Bt transgenic events provide the same level of protection against European corn borer injury. Event 176 was more effective in minimizing stalk tunneling by late instars during V12 and V16 than at R3 and R5 corn growth stages, whereas hybrids with events

Bt11, MON810, MON802, or CHB351 provided effective protection against tunneling at all growth stages (Walker et al. 2000).

### **Yield loss in Corn Grown for Silage**

Unlike the impact of European corn borer on grain yield in corn, the impact of this pest on whole-plant yield has not been well documented. Few studies have been conducted on the relationship between European corn borer and corn grown for silage (Thompson and White 1977, Raemisch and Walgenbach 1983, Raemisch and Walgenbach 1984, Myers and Wedberg 1999). In a study using natural infestations of European corn borer on corn grown in Prince Edward Island (Canada), Thompson and White (1977) found that although insecticide treatment significantly reduced the number of tunnels by European corn borer, whole-plant yields were unaffected. In a study conducted in South Dakota, Raemisch and Walgenbach (1983) reported that natural infestation by first generation European corn borer reduced whole-plant yields by more than 14% when compared to plots treated with cypermethrin at the rate of 0.11 kg ai/ha. In a subsequent study, Raemisch and Walgenbach (1984) reported that naturally occurring first generation European corn borer at an infestation level of 1 larva/plant caused 3.5, 4.9, and 8.2% loss of whole-plant yield in three locations, respectively. In a study using artificial infestation, Myers and Wedberg (1999) reported that whole-plant and stover (i.e., plants minus the ears) yields decreased as European corn borer infestation increased. Myers and Wedberg (1999) additionally reported that first

generation European corn borer populations caused greater yield reductions than second generation populations on whole-plant yields.

### **Economic Injury Levels**

Most crops harbor a wide variety of insects, but only a small portion of them actually cause economic damage on a recurring basis. The critical factor that determines the damaging capacity of an insect is its population level. The injury level concept was developed to identify the population level at which an insect causes economic damage in a specific crop. The most commonly used terms to indicate economically important population levels of insect pests are the economic injury level (EIL), and the economic threshold level (ETL) (Pedigo et al. 1986). Other terms used to indicate the same concept are action threshold, action level, threshold level, inaction threshold, and control threshold (Pedigo et al. 1986).

The original definition of the economic injury level as coined by Stern et al. (1959) is the “lowest population density that will cause economic damage”, where economic damage is referring to the “amount of injury which will justify the cost of artificial control measures”. The term “will justify” has been accepted to mean “the density of the pest at which the loss through damage just exceeds the cost of control” (Mumford and Norton 1984). Economic injury levels are most often used to support management decisions against occasional and perennial pests, where scouting and appropriate management programs are available (Pedigo et al. 1986). The mathematical

formula for calculating the EIL is expressed as follows (Pedigo et al. 1986, Pedigo 1989):

$$EIL = C/VID,$$

where EIL = economic injury level, density or intensity of insect population (e.g., insects/acre); C = cost of management/area (e.g., dollars/acre); V = market value/unit of produce (e.g., dollars/acre); I = injury units/insect/production unit (e.g., percent defoliation/insect/acre, expressed as a proportion); and D = damage/unit injury (e.g., bushels lost/acre/percent defoliation).

A similar formula developed by Bode and Calvin (1990) for calculating the EIL is as follows:

$$NL = TC/(CV \times 1 \times PL \times PC),$$

where NL is the number of larvae/plant; TC is the total control cost of planting Bt hybrid seeds (e.g., dollars/ha); CV = MV x EY, where CV is the crop value, MV is the expected market value (e.g., dollars/kg); 1 is the cost/benefit ratio at the break-even point; EY is the expected yield (e.g., kg/ha); PL is the expected average proportional yield reduction/larva/plant; and PC is the expected proportional control from insecticide application.

Although the mathematical relationship is straightforward, it is difficult to obtain values of the variables used in the formula to calculate the EIL (Poston et al. 1983, Mumford and Norton 1984, Pedigo et al. 1986). The four primary variables involved in calculating the EIL: market value, management cost, injury per insect density, and host damage/unit of injury, are affected by a number of complex secondary variables (Pedigo

et al. 1986). Determining the values for these four variables can be difficult because of their dependency on other complex biological processes, such as the impact of natural enemies on pest populations, impact of management decisions on natural enemy populations, presence of other pest species, changing weather conditions, etc. In addition, there is a growing appreciation to include an environmental cost (e.g., the cost of pollution or destruction of non-target organisms from broad spectrum insecticide applications) in the cost of the control variable (C) that is used in calculating the EIL (Pedigo and Higley 1992, Higley and Wintersteen 1992, Dent 2000).

Procedures for determining European corn borer EILs in corn have been developed for both grain and whole-plant yields (Bigler et al. 1990, Bode and Calvin 1990, Sayers et al. 1994, Mason et al. 1996, Myers and Wedberg 1999). These generally involve calculating unit yield reductions from a unit amount of stalk tunneling/plant or number of larvae/plant (Bode and Calvin 1990, Sayers et al. 1994, Mason et al. 1996, Myers and Wedberg 1999). European corn borer stalk tunneling has been considered as a more reliable index for measuring yield reductions in corn than other methods, such as number of larvae, and number of entrance holes (Kwolek and Brindley 1959, Jarvis et al. 1961). Bode and Calvin (1990) developed EILs for four different corn growth stages on the relationship of the number of larvae/plant on grain yield. They developed EILs using various control costs and crop values at each crop stage of infestation. Bode and Calvin (1990) assumed that the insecticide application provided 100% control of the pest population.

In a Wisconsin study comparing whole-plant yield reductions and associated monetary losses, Myers and Wedberg (1999) calculated EILs for European corn borer on corn grown for silage based on the percentage of artificially infested plants. Their study involved five different crop values and six different control costs for first and second generation populations separately. Calculating separate EILs for each generation was necessary because the type of damage and yield losses differ among the first and second generations.

Now with the advent of Bt corn hybrids, quantification of economic loss in corn by European corn borer has improved (Baute et al. 2002). Many studies have confirmed that Bt corn hybrids provide effective control of European corn borer, in terms of less leaf feeding, stalk tunneling, and shank tunneling compared to non-Bt corn hybrids (Lauer and Wedberg 1999, Clark et al. 2000). But very few studies have focused on the potential economic benefits that could be derived from this new technology (Baute et al. 2002). Studies evaluating the potential economic benefits of planting Bt corn hybrids have focused on corn grown for grain. Lauer and Wedberg (1999) reported that Bt corn hybrids provide economic benefits only during European corn borer outbreak years. During years when European corn borer is not endemic, non-Bt corn hybrids were able to perform as well as or better than the Bt corn hybrids (Lauer and Wedberg 1999). In another study comparing the economics of planting Bt corn hybrids with other management options, Ostlie et al. (1997) reported that planting Bt corn hybrids offers better economic benefits with returns of \$2.79/acre for all events against first generation borers. However, using no control measure against European corn borer resulted in a

loss of about \$6.57 and \$12.90/acre for first and second generation borers, respectively. The traditional IPM approach using scouting, economic thresholds, and insecticides resulted in a loss of about \$0.38 and \$4.07/acre for first and second generation borers, respectively. In a study in Eastern Canada, Baute et al. (2002) reported that the yield response from Bt corn hybrids was only realized when stalk tunneling exceeded 6 cm/plant. This was based on a premium price of \$34.58/ha Canadian (\$26.52/ha U.S.) and a market price of \$2.50/bushel Canadian (\$1.91/bushel U.S.) of corn. Hyde et al. (1999) reported that the economic benefits from planting Bt corn hybrids in Indiana are realized only during instances when there is a greater than average yield expectancy and 40% or greater probability of European corn borer infestation. In addition, Martin and Hyde (2001) stated that potential yield drag, impact of Bt technology on beneficial insects, and demand of Bt corn in the market must be considered before deciding to plant Bt corn hybrids for European corn borer.

### **Corn Pricing for Silage**

An important step in determining EILs is to have some idea of crop value, which in this case means calculating the value of corn grown for silage. The value of corn grown for silage is highly dynamic and depends on several factors, such as production costs, handling and storage costs, corn grain price, silage quality, moisture content at the time of harvest, supply and demand of corn silage, and availability of other feed alternatives (Lauer 1994, Hendrix 1996, Lauer and Undersander 2004). Of the aforementioned factors, the price of corn grain and moisture content of the standing crop

are the most frequently used factors in calculating the value of corn silage (Guyer and Duey 1986, Lauer 1994, Hendrix 1996, Lauer and Undersander 2004). The most common and simple method used in estimating the value of corn silage is to price a ton of corn grown for silage at 6 to 10 times the value of a bushel of corn grain (Guyer and Duey 1986, Lauer 1994, Hendrix 1996, Lauer and Undersander 2004). The value within the range of 6 to 10 depends on the percent dry matter and nature of the corn (i.e., harvested, standing, or ensiled). Standing corn with 30% dry matter can be priced at 6-times the value of a bushel of corn grain, whereas harvested corn with 40% dry matter can be priced at 10-times the value of a bushel of corn grain. Consequently, the price of corn grown for silage can be determined from the price of corn grain/bushel, percent dry matter, and nature of the corn. The price of corn silage/ton multiplied by the whole-plant yield in tons/ha, provides the value of corn grown for silage on a per hectare basis.

### **Nematodes in Corn**

Root feeding by plant-parasitic nematodes is another important factor that can interfere with successful corn production. More than 50 species of nematodes have been reported to cause injury in U.S. corn; however, only five species are reported to cause economic damage in corn (Pioneer Growing Point 2006). The damage caused by plant-parasitic nematodes in corn is highly variable with location, and yield losses have been reported to range from 0-23% (McSorley et al. 1987) and 0-20% (Koenning et al. 1999); however, yield losses of 5% are considered more common (Collins 2003). In general, the northern corn growing states experience the lowest level of damage from nematodes,

with the southern states experiencing the highest levels (Koenning et al. 1999). One of the more important plant parasitic nematodes associated with corn is the root knot nematode, *Meloidogyne incognita* Chitwood, (Tylenchida: Heteroderidae) (McSorley and Dickson 1989, Heffes et al. 1992, Tiwari et al. 2005). This nematode may cause yield losses of 30% or greater in corn under heavy infestations (Windham and Williams 1994).

### **Root Knot Nematode Biology and Damage**

The host range of *M. incognita* spans over 2,000 plant species, but studies on its relationship with the host plant is limited to a handful of species (Hussey 1985). The relationship of *M. incognita* to its host plant can be categorized as one of a sedentary endoparasitic type, involving a specialized and complex relationship with the host plant (Eisenback 1985).

The first stage *M. incognita* juvenile forms and molts into the second stage juvenile within the egg stage (Eisenback 1985, Hussey 1985, Gheysen and Fenoll 2002). Upon hatching, second stage juveniles are motile, but become sedentary as soon as they find a feeding site after penetrating into a root (Eisenback 1985). During the sedentary phase, the nematodes pass through three additional molts before reaching the adult stage (Eisenback 1985, Gheysen and Fenoll 2002).

Second-stage juveniles can enter plant roots through many points, such as directly behind the root cap, emergence point of lateral roots, galled tissue surrounding an adult female, or cut surfaces on the roots (Hussey 1985). The mechanism of

penetration involves mechanical thrusting of the oral stylet into the host plant along with the release of enzymatic substances that have cellulytic and pectolytic properties (Hussey 1985, Jasmer et al. 2003). Additional secretions injected into the roots by the juveniles cause the specific root cells to enlarge and form multinucleate giant cells (Huang 1985, Starr 1993, Trudgill and Blok 2001). These giant cells act as feeding cells transferring nutrients from the host plant to the developing juveniles (Trudgill and Blok 2001). Another important change in the plant that occurs soon after *M. incognita* starts feeding is the hypertrophic and hyperplastic changes in the root cells around the developing juveniles, which gives rise to the characteristic root galls usually associated with *M. incognita* infections (Hussey 1985, Trudgill and Blok 2001, Jasmer et al. 2003).

### **Root Knot Nematode on Host Plant Physiology**

The altered physiology of plants infected by *M. incognita* is exhibited in aboveground symptoms, such as stunted shoot growth, decreased shoot-root ratio, nutritional deficiencies in the leaves, temporary wilting, and reduced plant yields (Hunter 1958, O'Bannon and Reynolds 1965, McClure 1977, Hussey 1985, Heffes et al. 1992). These aboveground symptoms in *M. incognita* infected plants are the result of reduced water uptake, nutrient absorption, and translocation by the roots (Hussey 1985). Studies focusing on the altered physiology of *M. incognita* infected plants have reported changes in the respiration rate in the galls, imbalanced nutrient distribution, altered water uptake, and decreased photosynthesis rates (Hunter 1958, Owens and Rubinstein 1966, O'Bannon and Reynolds 1965, Loveys and Bird 1973, Meon et al. 1978).

Owens and Rubinstein (1966) reported that the respiration rates in galls of 40-day old tomato plants infected by *Meloidogyne* spp. were lower than in galls of non-infected plants, whereas the respiration rate in galls of 70-day old infected tomato plants were higher than in galls of non-infected plants. However, in both cases galls produced more carbon dioxide/mole of oxygen consumed compared to healthy tissues, indicating that a high rate of glycolysis is occurring in the galls. Hunter (1958) reported that the roots of *M. incognita acrita* infected tomato plants contained higher concentrations of N, P, K, and Mg than the roots of non-infected plants. O'Bannon and Reynolds (1965) reported that *M. incognita acrita* infected cotton plants consumed slightly more water than the non-infected plants, when water was supplied continuously, although the consumption rates were not statistically different. However, at 50% moisture content of soil capacity, the non-infected plants consumed significantly more water than the infected plants. The authors concluded that under limiting water conditions, decreased water uptake by the infected plants could be explained by the reduced growth and development of roots, and abnormality of the xylem elements. Meon et al. (1978) reported that the roots of tomato plants infected with *M. javanica* had greater suction pressure, which caused a greater flow of water into the roots than in the roots of non-infected plants. The authors attribute the greater suction pressure in the infected roots to the abnormality in the xylem elements caused by the giant cell formation. However, *M. javanica* infection caused no changes in the transpiration rate of the infected plants (Meon et al. 1978). Loveys and Bird (1973) reported that tomato plants infected with *M. javanica* resulted in a decreased rate of photosynthesis compared to non-infected plants.

The reduced photosynthesis rates in the infected plants was explained by the authors as a function of the altered physiology of the infected plants and plant growth stage. During the early plant growth stages, the reduced photosynthesis rates resulted from reduced translocation of root-derived factors, whereas during the later plant growth stages, reduced photosynthesis rates resulted from the smaller size of infected plants.

### **Interactions Between Belowground and Aboveground Herbivory**

Several studies have reported the association between diverse herbivores that are spatially separated on a common host plant. Examples include aboveground and belowground insects or belowground insects and nematodes (Russin et al. 1989, Moran and Whitham 1990, Russin et al. 1993, Fu et al. 2001, Bezemer et al. 2003, Blossy and Hunt-Joshi 2003, Carter-Wientjes et al. 2004, Wardle et al. 2004, Rasmann et al. 2005).

Studies conducted by Gange and Brown (1989), Masters and Brown (1992), Masters (1995), Masters et al. (1993, 2001), Fu et al. (2001), and Bezemer et al. (2003) have reported that belowground herbivory impacts positively on aboveground herbivory. While aboveground herbivory was found to have a negative impact on belowground herbivory (Masters and Brown 1992, Masters et al. 1993). However, Bezemer et al. (2003) reported that there was no effect of aboveground herbivory by *Spodoptera exigua* on the belowground herbivory by *Agriotes lineatus* on cotton plants. Russin et al. (1989, 1993) reported that aboveground herbivory increases the population densities of belowground herbivores, such as root knot nematode and cyst nematode.

In a greenhouse experiment on the effects of aboveground herbivory by soybean looper, *Pseudoplusia includens* (Walker), on the belowground herbivory by soybean cyst nematode, *Heterodera glycines*, Russin et al. (1989) reported that defoliation by soybean looper on soybean plants increased the population densities of juveniles and cysts of *H. glycines* in plant roots and soil. In another greenhouse study on the effect of aboveground herbivory by *P. includens* on *M. incognita* belowground herbivory, Russin et al. (1993) reported that the defoliation by soybean looper on soybean plants increased the population densities and percentage egg hatch of *M. incognita*. While studying the short-term impacts of aboveground herbivory on the activity of soil nematodes on corn, Fu et al. (2001) reported higher numbers of bacterivorous and fungivorous nematodes after 24 hours of a high level of herbivory from four pairs of grasshoppers. They also reported that  $^{14}\text{C}$  uptake in soil nematodes was increased at both low and high levels of aboveground herbivory when compared with plants without aboveground herbivory. In studying the effect of belowground herbivory by *M. incognita* on aboveground herbivory by *P. includens*, Carter-Wientjes et al. (2004) reported that *M. incognita* had no effect on the aboveground herbivory of *P. includens*.

Gange and Brown (1989) and Masters et al. (1993) explained that such interactions between spatially separated herbivores are a result of changes caused by the herbivores on the physiology of the plant. Belowground herbivores that feed on roots cause a stress response in the host plant that results in the accumulation of amino acids and carbohydrates in the aboveground parts. This in turn leads to increased aboveground herbivory. In contrast, aboveground feeding lowers the photosynthetic

ability of the host plant, resulting in a reduction of root biomass and less belowground herbivory. However, a study conducted by Holland et al. (1996) reported that aboveground herbivory by grasshoppers on corn plants resulted in increased carbon allocation to roots and reduced carbon allocation to shoots. Holland et al. (1996) concluded that aboveground herbivory positively impacts belowground organisms, especially microbial populations. Bezemer et al. (2003) found that the interaction between spatially separated herbivores is mediated by the production of secondary plant metabolites. They found that belowground feeding by *Agriotes lineatus* negatively impacts aboveground feeding by *Spodoptera exigua* on cotton plants by enhancing the terpenoids level in the leaves.

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## CHAPTER 2

### The Effect of European corn borer on corn whole-plant dry matter

#### ABSTRACT

Field experiments were conducted in 2004 and 2005 to determine the effect of different infestation levels of third instar European corn borer and growth stages of corn plant on whole-plant dry matter of corn grown for silage. In 2004, infestation level had a significant effect on whole-plant dry matter. Growth stage, and the interaction between infestation level and growth stage were not significant. Whole-plant dry matter was 18.8% greater ( $P \leq 0.05$ ) in non-infested control plants ( $318.3 \pm 11.4$  g/plant) than in plants with an infestation level of 6 larvae/plant ( $258.5 \pm 21.0$ ) in 2004. The results for 2005 were similar to the 2004 results, in which infestation level was found to have a significant effect on whole-plant dry matter. Likewise, growth stage, and the interaction between infestation level and growth stage were found to have no effect on whole-plant dry matter. Whole-plant dry matter in 2005 was 10.5% greater ( $P \leq 0.05$ ) in the non-infested control plants ( $315.3 \pm 7.5$ ) than in plants with an infestation level of 5 larvae/plant ( $282.3 \pm 10.8$ ). Economic injury levels calculated from the regression equations developed for each year between whole-plant dry matter and European corn borer infestation, are presented. Plant growth stage and infestation level had no effect on percent acid detergent fiber, neutral detergent fiber, and crude protein values for either year of this study.



## INTRODUCTION

Field corn is one of the most important crops grown for grain and silage worldwide (Youngman and Tiwari 2004). The U.S. accounts for nearly one-fifth of annual worldwide corn production and ranks as the number one corn-growing nation in the world (Youngman and Tiwari 2004). In Virginia, over 41.4 million bushels of corn grain (139,617 ha harvested) and 2.06 million metric tons of corn silage (52,609 ha harvested) were produced in 2006 (USDA-NASS 2007). In addition, corn grown for grain in Virginia was ranked second among all other field and fruit crops in terms of value of production (Virginia Agricultural Statistic Service 2006).

Among the multitude of potential pests that attack field corn, European corn borer, *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae), is one of the most important (Mason et al. 1996, Ostlie et al. 1997, Calvin and Van Duyn 1999, Youngman and Tiwari 2004, Dillehay et al. 2005). It has been estimated to cost U.S. corn growers approximately one billion dollars annually in management costs and crop losses (Mason et al. 1996). In addition, European corn borer has posed a potential risk to corn farmers in the Mid-Atlantic and southeastern U.S. for decades. However, most farmers have elected not to aggressively control this pest because of years of infrequent and sporadic infestations, and the cost and effort associated with traditionally managing this insect (Rice and Ostlie 1997). Biological control has been investigated for managing European corn borer on field corn, but has yet to achieve widespread success in North America (Youngman and Tiwari 2004).

The traditional way of managing second generation European corn borer infestations has consisted of aerially-applying granular insecticides on cornfields identified with above threshold eggmass counts. Although the traditional method has proven to be cost effective on corn grown for grain, it is not considered an economically viable option in many areas where corn is grown for silage (Thompson and White 1977, Myers and Wedberg 1999). Presently, the simple convenience of planting Bt corn hybrids that impede European corn borer feeding injury has dramatically changed the pest management scenario for this insect. Although planting Bt corn seed is straightforward, the main drawback is that it is not possible to predict at the time of planting whether a field will sustain enough European corn borer pressure to benefit from the additional cost of \$17-25/ha for the Bt corn seed (Hyde et al. 1999).

Surveys conducted by Youngman et al. (1998, 1999, 2000), and Youngman and Laub (2002) on second generation European corn borer damage in conventional (i.e., non-Bt) cornfields in eastern (1997-99) and western Virginia (2000-02) have provided partial insight on the role of Bt corn in Virginia. Economic loss was based on a threshold of 1 or more tunnels per plant > 1.3 cm in length for the fields in these surveys. It is important to realize, however, that this economic threshold was developed on corn grown for grain; not corn grown for silage. Less than 2% of the 172 fields surveyed in eastern Virginia from 1997-1999 experienced economic damage from European corn borer. The western Virginia survey findings, however, revealed a different picture (Youngman and Laub 2002). Nearly 25% of the 126 fields surveyed from 2000-2002 experienced economic damage. The significance of these survey findings is that the

aforementioned threshold can be applied with confidence to the eastern survey results because the vast majority (> 90%) of corn in eastern Virginia is grown for grain. In contrast, the majority of corn grown in the western half of the state is largely grown for silage and kept on-farm as feed for dairy animals. Therefore, it was important that research be done to determine the level at which European corn borer causes economic damage in corn grown for silage.

## **MATERIALS AND METHODS**

Field experiments were conducted during 2004 and 2005 at the Virginia Tech Kentland Research Farm in Montgomery Co. (80° 25' W, 37° 14' N; elevation ≈640 m), Virginia to quantify the effects of different levels of European corn borer infestation on the whole-plant dry matter of corn grown for silage. Pioneer 34B23 (non-Bt hybrid) and Pioneer 34B23 (Bt hybrid) (Pioneer Hi-Bred International, Johnston, Iowa) corn seed were planted no-till on April 4, 2004 and May 9, 2005 on 76.2-cm row spacing and plant population of 64,493 seeds/ha. All seed was treated with Kernel Guard<sup>®</sup> hopper box treatment at the rate of 56.7 g/bushel of corn seed. In addition, Force<sup>®</sup> 3G granular insecticide was applied at planting at the rate of 113.4 g/1000 row feet to eliminate any potential complications from soil pests such as western corn rootworm, white grubs, wireworms, and seedcorn maggot. The experimental design consisted of a split-plot randomized complete block design, with blocks replicated six times in 2004, and eight times in 2005. Each block consisted of three main plots, each main plot representing one of the following plant growth stages: 10-leaf, 16-leaf, and blister. Each main plot

consisted of subplots, with each subplot representing a different European corn borer infestation level. In 2004, infestation levels of 0, 1, 2, 4, and 6 were used; whereas in 2005, infestation levels were 0, 1, 2, 3, 4, and 5. Subplots were 4 rows wide (3.05 m) and 7.6 m long. Plants were selected for infestation from the middle two rows of each subplot (20 for 2004 and 16 for 2005), on the basis of uniformity in size and spacing. Any plant found to be naturally infested was not used for artificial infestation. In 2004, 6% (22 plants) of the 360 plants in the non-infested (control) subplots experiment-wide were found to be naturally infested. In 2005, 25% (105 plants) of the 384 plants in the non-infested (control) subplots experiment-wide were naturally infested. All infested plants in 2004 were marked with colored vinyl tape (non-adhesive) tied loosely around the base of the plant. In 2005, infested plants were marked with a fluorescent aerosol spray paint (Seymour, Sycamore Inc., Sycamore, Illinois) around the base of the plant.

The change in infestation levels from 2004-2005 was based on the 2004 results; mean whole-plant dry matter was found to be significantly different between infestation levels of 4 and 6 larvae/plant in 2004, and infestation level of 6 larvae/plant was significantly different from control unlike infestation level of 4 larvae/plant. Therefore to better approximate the highest infestation level at which yield loss starts occurring, I replaced infestation level of 6 larvae/plant with 5 larvae/plant. In addition, infestation level of 3 larvae/plant was added in 2005 studies to observe the effect of unit increase in infestation levels on whole-plant dry matter, from infestation levels of 0 to 5 larvae/plant. The number of blocks were increased from six (2004) to eight (2005) at the

expense of the number of plants/infestation level (subplot) to increase statistical robustness in the 2005 experiment.

### *Larval infestations*

Third instar European corn borer larvae used in the experiment were purchased from French Agricultural Research, Inc. (Lamberton, Minnesota). In 2004, corn plants at the 10-leaf stage were infested with European corn borer larvae using a gelatin capsule method (Bode and Calvin 1990). Individual third instars were loaded into gelatin capsules (size 0; 7.0 mm diameter) (Wonder Laboratories, White House, Tennessee) in the laboratory. The loaded gelatin capsules were then placed in plastic ice coolers on top of a cardboard barrier above a layer of cubed ice, and then transported to the field for infestation. A 0.1-0.2 mm deep circular hole was made in the plant using a 7.0 mm diameter disposable biopsy punch (Biopunch, Fray Products Corp. Buffalo, New York). One-half of a gelatin capsule containing the larva was inserted into the hole and lightly secured to the plant with scotch tape (Fig. 2.1). The intended number of larvae were introduced to the plant at the rate of one larva/internode. All gelatin capsules with dead or missing larvae were replaced with a fresh larva 24 hours after initial infestation.

Owing to the high number of replacements (approximately 80%) required on plants infested with the gelatin capsule method, all further artificial infestations for 2004 and 2005 were done using a wire-nut technique (Tiwari unpublished data). The wire-nut technique involved the WingGard™ (Gardner Bender, Milwaukee, Wisconsin) plastic wire connector (size 10-086) hereinafter referred to as ‘wire-nut’. The wire-nut is

shaped like a capsule, with an open end (1.1 cm inside diameter) that is slightly wider than the closed end (0.8 cm diameter). The wire-nut measures 3.0 cm in length and has two side flanges on the open end. In order to fasten the wire-nut to a cornstalk, an approximate 0.25-cm notch was cut into each flange using a Dremel® rotary drilling tool (Dremel, Robert Bosch Tool Corporation, Racine, Wisconsin). In addition, the flat circular open end of the wire-nut was slightly curved with a 1.90 cm diameter drum sander tool (Ali GatorGrit Inc., Fairborn, Ohio) to better conform to the natural curvature of the cornstalk. This was necessary to minimize escape of larvae at the point of attachment to the stalk. Individual larvae were placed into the wire-nuts and capped with corks prior to infestation and transportation to the trial site. The capped wire-nuts were then placed in plastic ice coolers on top of a cardboard barrier above a layer of cubed ice. The wire-nut was secured onto a cornstalk with size 10 or 12 rubber bands (Alliance Advantage, Hotsprings, Arizona) (Fig. 2.2). In general, size 10 rubber bands were used for 10-leaf stage plants, and size 12 rubber bands were used for 16-leaf and blister stage plants.

For 10-leaf stage plants, all larvae were introduced into the internodes below the whorl at the rate of 1 larva/internode. For the 16-leaf and blister stages, half the number of total larvae were placed on the internodes above the primary ear with the remaining half placed on the internodes below the primary ear, at a rate of 1 larva/internode. At the infestation level of 1 larva/plant, the larva was placed on the internode just below the whorl for 10-leaf stage plants, but on the internode just above the primary ear for 16-leaf

and blister stages plants. All wire-nuts with dead or missing larvae were replaced with a fresh larva 24 hours after initial infestation.

### *Harvest*

Artificially infested plants were cut manually approximately 5 cm above the soil surface at the half kernel milkline stage. Each plant was split longitudinally from the tassel to the base to record the number of tunnels per plant and the length of each tunnel. Given that some plants may be naturally infested by European corn borer, plants found with tunnels in internodes other than the area of infestation were excluded from statistical analysis.

Percent dry matter and forage quality evaluations were performed on four complete corn plants arbitrarily selected from within each infestation level subplot. Plants were then chopped into approximately 2.5 cm pieces using a Tomahawk® Chipper/Shredder (Troy-Bilt Manufacturing Co., Troy, NY). A 1 kg subsample of the chopped material was placed in a cotton harvest bag (50 x 100 cm) and then dried in a forced air dryer at 70<sup>0</sup>C (158<sup>0</sup>F) at a Virginia Tech forage drying facility, Blacksburg, VA. After the plant material was completely dry, it was removed from the dryer and weighed. Dry matter values were then used to determine the percent moisture content of the subsamples at harvest. All yields were adjusted to 100% dry matter. In addition, a 20 g dried portion of each subsample was taken to the Virginia Tech Forage Analysis Laboratory, Blackstone, VA, for forage quality analysis. Near infrared reflectance

spectroscopy analysis procedures were performed to determine the percent acid detergent fiber, neutral detergent fiber, and crude protein in each sample.

### *Economic injury level*

The economic injury level (EIL) for each plant growth stage was determined based on regression analysis using different crop values (\$/ha) and control costs (\$/ha). Economic injury levels were calculated using the equation developed by Bode and Calvin (1990) with the following modifications: the wording, 'planting Bt corn seed' replaced 'insecticide applications' for TC (total control cost) and PC (proportional control). Thus, the formula used for calculating EIL is as follows:

$$NL = TC / (CV \times PL \times PC),$$

where NL is the number of larvae per plant; TC is the total control cost of planting Bt corn seed (\$/ha);  $CV = MV \times EY$ , where CV is the crop value, MV is the expected market value (\$/kg); EY is the expected yield (kg/ha); PL is the expected average proportional yield reduction/larva/plant (PL values were calculated from regression equations developed for each growth stage); PC is the expected proportional control from planting Bt corn. A PC of 1 signifies 100% control. When PC is less than 1, the EIL can be obtained by dividing the EIL for 100% control by the percent control achieved from planting Bt corn. It should be noted that not all Bt hybrids provide 100% control against European corn borer, Bt hybrids with event MON810 provides 96% control of European corn borer (Ostlie et al. 1997).

## Statistics

Two-way analysis of variance (ANOVA) was used to analyze for differences in whole-plant dry matter with growth stage and infestation level as main effects, for each year separately (SAS Institute 2001). When significant main effect interactions were found, separate ANOVAs, and Fisher's protected LSD mean separation tests were performed to inspect for whole-plant dry matter differences among infestation levels within a growth stage (SAS Institute 2001). Unless specified otherwise, a  $P \leq 0.05$  was used for assigning significance. The general linear model procedure (SAS Institute 2001) was used to perform ANOVA and Fisher's protected LSD mean separation tests. Linear regression models for each year were generated by regressing mean whole-plant dry matter of each subplot against infestation level. The general linear model procedure (SAS Institute 2001) was used for all regression analyses.

Two-way analysis of variance (ANOVA) was used to analyze for differences in percent acid detergent fiber, neutral detergent fiber, and crude protein values with growth stage and infestation level as main effects, for each year separately (SAS Institute 2001). When significant main effect interactions were found, separate ANOVAs, and Fisher's protected LSD mean separation tests were performed to inspect for percent acid detergent fiber, neutral detergent fiber, and crude protein value differences among infestation levels within a growth stage (SAS Institute 2001).

## RESULTS

Although two controls (Pioneer 34B23 [non-Bt hybrid] and Pioneer 34B24 [Bt hybrid]) were used in this study, for purposes of analysis only the non-Bt control was used in both the years.

**2004:** The experimental unit used in 2004 was mean whole-plant dry matter of the number of plants per subplot with the requisite number of tunnels. Therefore, at the most, eighteen whole-plant dry matter means per infestation level were available for the overall analysis, and six whole-plant dry matter means per infestation level were available for the growth stage analyses. However, because eight of eighteen subplots lacked any plants with 6 tunnels, mean whole-plant dry matter for this infestation level (i.e., 6 larvae/plant) was based on the 32 plants from the remaining 10 subplots. The large standard error for mean whole-plant dry matter for the 6 larvae/plant infestation level is attributed to the fewer number of plants with the requisite number of tunnels.

Infestation level reduced whole-plant dry matter (g/plant) ( $F = 3.90$ ;  $df = 4, 60$ ;  $P = 0.0070$ ), whereas the main effect of plant growth stage ( $F = 0.63$ ;  $df = 2, 60$ ;  $P = 0.5360$ ) and the interaction between plant growth stage and infestation level ( $F = 0.95$ ;  $df = 8, 60$ ;  $P = 0.4811$ ) had no significant effect on whole-plant dry matter. Mean ( $\pm$  SEM) whole-plant dry matter was significantly greater in the non-infested control plants ( $318.3 \pm 11.4$ ) than in plants infested with 6 larvae/plant ( $258.5 \pm 21.0$ ), indicating an 18.8% yield loss (Table 2.1).

Regression analysis showed a significant relationship between mean whole-plant dry matter and infestation level ( $P = 0.0025$ ) (Table 2.2). Further regression analyses

revealed no significant quadratic or cubic component in either relationship. Separate regression analysis for each growth stage was not developed, as growth stage main effect was not found to have significant effect on the whole-plant dry matter. Regression equation was used to calculate the mean percent yield reduction/larva/plant for each growth stage. The mean percent whole-plant dry matter reduction/larva/plant calculated from the regression equation was used to calculate EILs at varying control costs and crop value levels (Table 2.3). The EILs were calculated assuming 100% control achieved by planting Bt hybrids; however, the EIL for 100% control can be adjusted for lower levels of protection. The EIL decreases as crop value increases, but increases as control costs increase.

Forage analysis indicated no significant effect of growth stage, infestation level, or the interaction between growth stage and infestation level on percent acid detergent fiber, neutral detergent fiber, or crude protein. This suggests that the quality of the forage remains unchanged even at an infestation level of 6 larvae/plant (Tables 2.4).

**2005:** The experimental unit used in 2005 was mean whole-plant dry matter of the number of plants per subplot with the requisite number of tunnels. Therefore, at the most, twenty-four yield means per infestation level were available for the overall analysis, and eight whole-plant dry matter means per infestation level were available for the growth stage analyses.

Infestation level had a significant negative effect on whole-plant dry matter ( $F = 3.26$ ;  $df = 5, 119$ ;  $P = 0.0086$ ), whereas the main effect of plant growth stage ( $F = 1.56$ ;

df = 2, 119;  $P = 0.2148$ ) as well as the interaction between plant growth stage and infestation level ( $F = 1.39$ ; df = 10, 119;  $P = 0.1932$ ) had no significant effect on whole-plant dry matter. The infestation levels for 0, 1, and 3 larvae/plant had significantly greater whole-plant dry matter than the infestation level of 5 larvae/plant (Table 2.1). Mean whole-plant dry matter was 10.5% significantly greater in the non-infested control plants ( $315.3 \pm 7.5$ ) than in plants infested with 5 larvae/plant ( $282.3 \pm 10.8$ ), (Table 2.1).

Regression analysis revealed significant relationships between whole-plant dry matter and infestation level ( $P = 0.0015$ ) (Table 2.2). Further regression analyses revealed no significant quadratic or cubic component involved in either relationship. The mean percent whole-plant dry matter reduction/larva/plant calculated from the regression equation was used to calculate EILs at varying control costs and crop value levels (Table 2.5). The EILs were calculated assuming 100% control achieved by planting Bt hybrids; however, the EIL for 100% control can be adjusted for lower levels of protection. The EIL decreases as crop value increases, but increases as control costs increase.

Forage analysis indicated no significant effect of growth stage, infestation level, or the interaction between growth stage and infestation level on percent acid detergent fiber, neutral detergent fiber, or crude protein. This suggests that the quality of the forage remains unchanged even at the infestation level of 5 larvae/plant (Table 2.4).

## DISCUSSION

In both years of study, whole-plant dry matter was significantly lowest in the plants with the highest infestation levels (i.e. 6 in 2004 and 5 in 2005) when compared with the non-infested control plants. Based on the mean whole-plant dry matter at different infestation levels for each year, it could be concluded that corn whole-plant dry matter starts declining at an infestation level of above 4 larvae/plant. However, this level of infestation rarely occurs in Virginia, where corn is largely grown for silage (Youngman et al. 1998, 1999, 2000). In a study conducted by Raemisch and Walgenbach (1983) involving natural infestation of European corn borer, whole-plant dry matter was significantly reduced in the untreated control plots compared to the cypermethrin (0.11 kg ai/ha) treated plots by 14.1 and 14.7% at two test sites, respectively. In another study conducted by Myers and Wedberg (1999) using artificial infestations of European corn borer, they found a highly negative correlation between the percent of European corn borer infested plants and whole-plant dry matter. In their studies, whole-plant dry matter was significantly reduced, when the percent of infested plants increased from 0 to 84. In contrast, a two-year study conducted by Thompson and White (1977) found no significant differences in whole-plant dry matter when mean European corn borer levels ranged from 2 to 37% in 1974, and 5 to 32% in 1975. However, grain yield was significantly higher in the treatment that averaged 2% infested plants when compared to the treatment that averaged 37% infested plants in 1974.

In both years of my study, mean whole-plant dry matter in plants infested with 1 larvae/plant was slightly higher than the non-infested control plants, although the

difference was not significant. This increase in whole-plant dry matter could be attributed to an over-compensatory response exhibited by the corn plants. Poston et al. (1983) suggested that an over-compensatory response occurs when a plant that experiences low level injury, such as that caused by an insect feeding, is stimulated to produce more plant matter.

My results for both years showed that the growth stage during which larvae initiated tunneling had no significant effect on whole-plant dry matter. In contrast, Bode and Calvin (1990) and Myers and Wedberg (1999) reported a significant negative effect on yield as a function of the growth stage during which tunneling was initiated. Bode and Calvin (1990) found greater reductions in grain-yield from infestations made at the blister stage than at the dough stage. Myers and Wedberg (1999) reported that infestation during the early growth stages (i.e., V6 to V11 stages) caused greater reductions in whole-plant dry matter compared to infestations during later growth stages (i.e., R1 to R<sup>2</sup> stages).

Economic injury levels (number of larvae/plant) were calculated using varying control costs (cost of planting Bt hybrids/ha) and crop values (\$/ha). The control costs were calculated in terms of the cost involved in planting a field with a Bt hybrid (\$/ha). Bt hybrids that target European corn borer have been widely adopted by the U.S. corn growers (Pilcher et al. 2002). A three year survey conducted across the six major corn growing states by Pilcher et al. (2002) revealed that the percentage of corn growers using Bt hybrids has increased from 10.5 (1996) to 40.7 (1998). According to a 2001 survey

by Wilson et al. (2005), it was reported that growers plant an average of 46.4% of their corn acreage in Bt corn hybrids for managing European corn borer.

Benefits derived from Bt hybrids in corn grown for grain are similar in corn grown for silage. Specifically there are no qualitative differences in silage quality between Bt and non-Bt hybrids, in terms of digestibility (Barrière et al. 2001, Donkin et al. 2003), dry matter intake (Folmer et al. 2002, Donkin et al. 2003), fat-corrected milk production (Barrière et al. 2001, Folmer et al. 2002, Donkin et al. 2003), milk composition (Barrière et al. 2001, Folmer et al. 2002, Donkin et al. 2003), and nutrient composition (Donkin et al. 2003).

The value of corn grown for silage is highly dynamic and depends on several factors, such as production costs, handling and storage costs, grain corn value, silage quality, moisture content at the time of harvest, the supply and demand of corn silage and other feed alternatives (Lauer 1994, Hendrix 1996, Lauer and Undersander 2004). Several researchers have reported that the value of corn grown for silage is based on the price of corn grain and moisture content of the standing crop (Guyer and Duey 1986, Lauer 1994, Hendrix 1996, Lauer and Undersander 2004). The most common and simple method involves estimating the price of a ton of corn grown for silage at 6 to 10 times the value of a bushel of grain corn (Guyer and Duey 1986, Lauer 1994, Hendrix 1996, Lauer and Undersander 2004). The value within the range of 6-10 depends on the percent dry matter and nature of the corn (either harvested or standing or ensiled). Standing corn at 30% dry matter can be priced at 6 times the value of a bushel of grain corn, whereas corn harvested at 40% dry matter can be priced at 10-times the value of a

bushel of grain corn. Therefore, the price of corn grown for silage can be determined using price of grain corn /bushel, percent dry matter and nature of the corn (either harvested or standing or ensiled) (Table 2.9). Furthermore, the price of corn silage (per ton) multiplied against the whole-plant dry matter (tones/hectare) provides the crop value on a per hectare basis.

The EILs calculated in my study for corn whole-plant dry matter were higher than those calculated by Bode and Calvin (1990) for grain yield. At a control cost of \$20/ha and crop value of \$250/ha at 10-leaf stage, Bode and Calvin (1990) calculated an EIL of 1.35 larvae/plant. Assuming the same control cost and crop value for corn silage the EILs for my study are 2.76 and 3.79 larvae/plant for 2004 and 2005, respectively, suggesting that corn grown for silage can withstand higher numbers of larvae per plant than corn grown for grain.

Forage analysis indicated no significant effect of growth stage, infestation level, or the interaction between growth stage and infestation level on percent acid detergent fiber, neutral detergent fiber, and crude protein values. This suggests that the quality of the forage remains largely unchanged even at infestation levels as high as 6 larvae/plant. Similar results were found in a study conducted by Myers and Wedberg (1999), where infestation had no effect on silage quality when measured in terms of milk per ton. A study on the effect of western corn rootworm (*Diabrotica virgifera virgifera*) on corn silage quality also revealed no significant losses in silage quality in terms of digestibility factors (Davis 1994).

The overall findings of my study indicates that a significant reduction in whole-plant dry matter occurs when plants average greater than 4 European corn borer larvae/plant. The importance of this finding for Virginia corn growers is that this level of infestation rarely occurs in Virginia, where corn is largely grown for silage (Youngman et al. 1998, 1999, 2000). Another important outcome of my research concerns the non-significant effect that European corn borer has on forage quality. This suggests that Virginia growers who grow corn for silage should strongly consider selecting non-Bt hybrids that exhibit the best agronomic traits suited to their particular farming operation before deciding on the Bt hybrid. Planting non-Bt hybrids can cut down production costs by \$17-25/ha.

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Table 2.1. Mean ( $\pm$ SEM) whole-plant dry matter at different infestation levels of European corn borer.

No. of larvae/plant	Dry matter (g/plant)	Plants with requisite number of tunnels
2004		
0	318.3 $\pm$ 11.4ab	338
1	324.8 $\pm$ 11.6a	202
2	290.5 $\pm$ 12.6bc	140
4	298.3 $\pm$ 14.1ab	82
6	258.5 $\pm$ 21.0c	32
2005		
0	315.3 $\pm$ 7.5ab	279
1	327.0 $\pm$ 7.8a	229
2	299.6 $\pm$ 6.6bc	188
3	307.4 $\pm$ 7.0ab	156
4	300.4 $\pm$ 11.6bc	138
5	282.3 $\pm$ 10.8c	125

Means within each year followed by different letters are significantly different (Fisher's protected LSD,  $P \leq 0.05$ ).

Table 2.2. Linear regression equations relating whole-plant dry matter (dry matter in g/plant) to different infestation levels of European corn borer in 2004 and 2005.

Regression equation	SEM		$r^2$	$P > F$
	Slope	Intercept		
2004				
$Y = 323.1 - 9.4X$	3.00	9.02	0.1116	0.0025
2005				
$Y = 322.3 - 6.8X$	6.35	2.10	0.0686	0.0015

Table 2.3. Economic injury level values for European corn borer larval populations at various control costs and crop values on whole-plant dry matter in 2004 (PC = 1, assuming 100% control).

Crop Value (\$/ha)	Control cost (\$/ha)						
	20	25	30	35	40	45	50
100	6.90	8.62	10.34	12.07	13.79	15.52	17.24
150	4.60	5.75	6.90	8.05	9.20	10.34	11.49
200	3.45	4.31	5.17	6.03	6.90	7.76	8.62
250	2.76	3.45	4.14	4.83	5.52	6.21	6.90
300	2.30	2.87	3.45	4.02	4.60	5.17	5.75

Table 2.4. ANOVA for percent acid detergent fiber, neutral detergent fiber, and crude protein at different levels of European corn borer (ECB) infestation and growth stages.

2004							
Source	<i>df</i>	Acid detergent fiber		Neutral detergent fiber		Crude protein	
		<i>F</i> Value	<i>P</i> > <i>F</i>	<i>F</i> Value	<i>P</i> > <i>F</i>	<i>F</i> Value	<i>P</i> > <i>F</i>
Growth stage	2	2.64	0.0780	2.45	0.0933	0.22	0.8038
ECB level	4	0.44	0.8219	0.45	0.8104	0.23	0.9495
Growth stage x ECB level	8	1.12	0.3560	1.00	0.4508	0.77	0.6526
2005							
Growth stage	2	1.28	0.2829	0.91	0.4055	2.05	0.1324
ECB level	5	1.50	0.1940	1.38	0.2355	1.28	0.2783
Growth stage x ECB level	10	1.82	0.0633	1.85	0.0584	1.61	0.1108

Table 2.5. Economic injury level values for European corn borer larval populations at various control costs and crop values on whole-plant dry matter in 2005 (PC = 1, assuming 100% control).

Crop Value (\$/ha)	Control cost (\$/ha)						
	20	25	30	35	40	45	50
100	9.48	11.85	14.22	16.59	18.96	21.33	23.70
150	6.32	7.90	9.48	11.06	12.64	14.22	15.80
200	4.74	5.92	7.11	8.29	9.48	10.66	11.85
250	3.79	4.74	5.69	6.64	7.58	8.53	9.48
300	3.16	3.95	4.74	5.53	6.32	7.11	7.90

Table 2.6. Corn silage values (per ton) based on moisture content and price of corn grain.

Price of corn grain (\$/bushel)	Value of corn silage at 30% dry matter (in-field)	Value of corn silage at 40% dry matter (in-field)	Value of corn silage at 40% dry matter (harvested and ensiled)
1.75	10.5	14.0	17.5
2.00	12.0	16.0	20.0
2.25	13.5	18.0	22.5
2.50	15.0	20.0	25.0
2.75	16.5	22.0	27.5



Fig. 2.1. Infesting third instar European corn borer using gelatin capsule method.



Fig. 2.2. Infesting third instar European corn borer using wire-nut method.

## CHAPTER 3

### **The effect of European corn borer, *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae), stalk tunneling on root knot nematode, *Meloidogyne incognita* Chitwood (Tylenchida: Heteroderidae), fitness on corn**

#### **ABSTRACT**

Greenhouse studies were conducted in 2004, 2005, and 2006 to examine the reciprocal effects of aboveground herbivory by European corn borer, *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae), and belowground herbivory by root knot nematode, *Meloidogyne incognita* Chitwood (Tylenchida: Heteroderidae), on one another at three growth stages of corn, *Zea mays* L. Two separate experiments were conducted to study the effect of aboveground herbivory on the number of *M. incognita* juvenile penetrations and eggs/root system. Conversely, the effect of belowground herbivory on European corn borer was determined by the length of stalk tunneling, in two separate experiments. In the first experiment, the main effects interaction was not significant for either *M. incognita* juvenile penetrations/root system ( $P = 0.4055$ ) or *M. incognita* eggs/root system ( $P = 0.8115$ ). Overall mean *M. incognita* juvenile penetrations/root system across all three growth stages, at infestation levels of 1 and 3 larvae/plant were significantly less than in the non-infested control. In addition, overall mean *M. incognita* eggs/root system at an infestation level of 3 larvae/plant were significantly less

than in the control. In the second experiment, the main effects interaction was significant for both *M. incognita* juvenile penetrations/root system ( $P = 0.0422$ ) and *M. incognita* eggs/root system ( $P = 0.0134$ ). At the 8 and 10 leaf growth stages, *M. incognita* juvenile penetrations/root system at infestation levels of 1 and 3 larvae/plant were significantly less than in the control. In addition, *M. incognita* eggs/root system at an infestation level of 3 larvae/plant were significantly less than in the control, at all growth stages. In the reciprocal study, which examined the effect of different *M. incognita* inoculation levels on European corn borer stalk tunneling, no significant effect of inoculation level on European corn borer stalk tunneling was found.

## INTRODUCTION

Several studies have reported an association between diverse herbivores, such as aboveground and belowground insects or belowground insects and nematodes that are spatially separated on a common host plant (Bezemer et al. 2003, Blossy and Hunt-Joshi 2003, Carter-Wientjes et al. 2004, Wardle et al. 2004, Rasmann et al. 2005). These studies on the interactions between spatially separated herbivores have yielded different results that are likely due to differences in host plant species and the type/class of herbivores used in the studies. Studies conducted by Gange and Brown (1989), Moran and Whitman (1990), Masters and Brown (1992), Masters et al. (1993), Masters (1995), and Masters et al. (2001) have reported that aboveground herbivory had a negative impact on belowground herbivores. In contrast, Russin et al. (1989) and Russin et al. (1993) reported that aboveground herbivory by soybean looper, *Pseudoplusia includens* (Walker) (Lepidoptera: Noctuidae), positively impacts soybean cyst nematode, *Heterodera glycines* Ichinohe (Tylenchida: Heteroderidae), and root knot nematode, *Meloidogyne incognita* Chitwood (Tylenchida: Heteroderidae). Similarly, Fu et al. (2001) found that aboveground herbivory by grasshoppers increased the numbers and <sup>14</sup>C activity of belowground nematodes on corn plants. With regard to the effects of belowground herbivores on aboveground herbivores, studies conducted by Gange and Brown (1989), Masters and Brown (1992), Masters et al. (1993), Masters (1995) and Masters et al. (2001) have reported that belowground herbivory impacts aboveground herbivores positively. However, Carter-Wientjes et al. (2004) reported that belowground herbivory by *M. incognita* had no effect on aboveground herbivory by *P. includens*. The

aforementioned studies on the interactions between spatially separated herbivores have yielded different results that are likely due to differences in host plant species and the type/class of herbivores used in the studies.

European corn borer, *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae), is a key pest of corn that annually costs U.S. growers approximately one billion dollars in management costs and yield losses (Mason et al. 1996). Early in the growing season, first and second instar larvae feed in the whorl, which causes a shothole appearance after the leaves expand (Youngman and Tiwari 2004). Late third and subsequent instars tunnel into the stalks, ears, or ear shanks (Youngman and Tiwari 2004).

Root feeding plant-parasitic nematodes are also important pests that can interfere with successful corn production. More than 50 species of nematodes have been reported to injure corn in the U.S.; however, only five species are reported to cause economic damage (Pioneer Growing Point 2006). The damage caused by plant-parasitic nematodes in corn is highly variable with geographic location, and yield losses have been reported to range from 0-23% (McSorley et al. 1987) and 0-20% (Koenning et al. 1999); however, yield losses of 5% are considered more common (Collins 2003). In general, the northern corn growing states experience the lowest level of damage from nematodes, with the southern states experiencing the highest levels (Koenning et al. 1999). One of the more important plant parasitic nematodes found to be associated with corn is the root knot nematode, *Meloidogyne incognita* Chitwood (Tylenchida: Heteroderidae), (McSorley and Dickson 1989, Heffes et al. 1992, Tiwari et al. 2005).

This nematode may cause yield losses of 30% or greater in corn under heavy infestations (Windham and Williams 1994).

*M. incognita* injury starts soon after the second stage juveniles enter the roots and start initiating the formation of multinucleate giant cells (Eisenback 1985, Hussey 1985, Trudgill and Blok 2001). These giant cells act as feeding cells transferring nutrients from the host plant to the developing juveniles (Trudgill and Blok 2001). Another important change in the plant that takes place soon after *M. incognita* starts feeding is the hypertrophic and hyperplastic changes in the root cells around the developing juveniles that give rise to the characteristic root galls usually associated with *Meloidogyne* spp. infections (Jasmer et al. 2003). The consequences of these physiological changes are reduced shoot growth, decreased shoot-root ratio, nutritional deficiencies in the leaves, temporary wilting, and reduced plant yields in the infected host plants (Hunter 1958, O'Bannon and Reynolds 1965, McClure 1977, Hussey 1985, Heffes et al. 1992).

Numerous studies have been conducted on corn to better understand the direct effects of European corn borer (Calvin et al. 1988, Bode and Calvin 1990, Dillehay et al. 2005) and root knot nematode (McSorley and Dickson 1989, McSorley and Gallaher 1991, Johnson et al. 1999) feeding and their potential damage to the crop in isolation of each other. However, no literature exists on the relationship between these two spatially separated herbivores on one another in corn. Given the potential economic loss from nematodes, as well as from aboveground insect pests of corn, research focusing on the interaction between two spatially separated pests may provide valuable insight into

furthering our understanding of the complexity of such processes through a common host plant. In addition, findings from this study may provide insight on the interactions between spatially separated herbivores on other host plants.

## **MATERIALS AND METHODS**

Experiments were conducted during 2004-2006 at a greenhouse facility located at Virginia Tech, Blacksburg, VA. All experiments were conducted using Pioneer 34B23 (non-Bt hybrid) (Pioneer Hi-Bred International, Johnston, Iowa) corn plants. Two seeds per pot were planted 1.5 cm deep in 3.79 liters (1 gallon) plastic pots containing sand and potting mixture in the ratio of 1:1. One seedling was removed from each pot after plant emergence. Ambient temperature (22-30<sup>0</sup>C) and relative humidity (65-75%) in greenhouse was monitored throughout the studies.

### ***M. incognita* Egg Extraction**

Eggs and juveniles of *M. incognita* race 4 used in the experiments were collected from a culture maintained on tomato 'Rutgers' plants in the greenhouse. Nematode infected roots were washed, cut into 1-2 cm pieces, and placed in a 150 ml beaker containing water and chlorine bleach (5.25% NaOCl) in a ratio of 1:10. The beaker along with the contents was covered with several layers of parafilm and vigorously agitated for approximately 2 minutes. The contents of the beaker were passed through a 200-mesh (75  $\mu$ m) sieve, nested over a 500-mesh (25  $\mu$ m) sieve to collect the eggs. While flowing through the sieves, the contents from the beaker were passed through

fresh water to rinse off residual chlorine bleach. All eggs and juveniles were used within 24 h of extraction.

### **European corn borer Infestation**

Third instar European corn borer, purchased from French Agricultural Research, Inc. (Lamberton, Minnesota), were used in all the experiments. Plants were infested with European corn borer larvae using a gelatin capsule method described by Bode and Calvin (1990). Third instars were loaded individually into gelatin capsules (size 0; 7.0 mm diameter) (Wonder Laboratories, White House, Tennessee) in the laboratory. The loaded gelatin capsules were then placed in plastic ice coolers on top of a cardboard barrier above a layer of cubed ice, and transported to the greenhouse for infestation. A 0.1-0.2 mm deep circular hole was made on the side of each plant using a 7.0 mm diameter disposable biopsy punch (Biopunch, Fray Products Corp., Buffalo, New York). One-half of a gelatin capsule containing the larva was inserted 0.1-0.2 mm deep into each 7.0 mm diameter hole on the plant and lightly secured to the plant with adhesive tape. The intended numbers of larvae were introduced at the rate of 1 larva/internode. After 24 hours, all gelatin capsules with dead or missing larvae were replaced with a fresh one.

### **European Corn Borer Herbivory on *M. incognita* Juvenile Penetrations and Eggs/Root System**

Two experiments were conducted as a 3 x 3 factorial design in a randomized complete block design in 2004. In each experiment, treatments consisted of 3 levels of European corn borer infestation (0, 1, and 3 larvae/plant), and 3 levels of plant growth stages (6-, 8-, and 10-leaf stages). Each treatment combination was replicated ten times. At each plant growth stage, 10 plants were infested with 1 larva, 10 plants with 3 larvae, and 10 plants were left uninfested (control). Seventy-two hours after larval infestation, a total of six thousand *M. incognita* eggs and juveniles suspended in water were inoculated at the base of each plant. Of the ten replicates for each treatment combination, five were harvested after two weeks to record the number of *M. incognita* juvenile penetrations/root system, with the remaining five harvested after five weeks to record the number of eggs/root system.

To count the number of *M. incognita* juvenile penetrations/root system, roots were removed from the pots and flushed with water for approximately 2-3 minutes to remove soil particles. The roots were then stained using an acid-fuchsin technique (Byrd et al. 1983), which involved further washing and then cutting the roots into 1-2 cm pieces. The roots were then placed in a 150 ml beaker containing chlorine bleach (5.25% NaOCl) and water in a 1:10 final volume. The solution was occasionally agitated over a 4-minute period, followed by rinsing the root pieces under fresh water for approximately 2-3 minutes to rinse off residual chlorine bleach. The root pieces were additionally soaked in fresh water for 15 minutes to remove any remaining chlorine bleach. The root pieces were transferred to another beaker containing 30 ml water and 1 ml of acid fuchsin stain (3.5 g acid fuchsin, 250 ml acetic acid, and 750 ml distilled

water), and heated to boiling. After boiling for about 30 seconds, the contents were allowed to cool to room temperature, and then removed from the stain and rinsed again under fresh water for approximately 2-3 minutes to remove excess stain from the root pieces. The root pieces were then placed into another beaker containing 20-30 ml of acidified glycerine, and heated to boiling. After boiling, the contents were allowed to cool to room temperature. The root pieces were then pressed between glass plates for observation under a dissecting microscope (Fig. 3.1). Observations were made on the number of juvenile penetrations/root system. To count the number of *M. incognita* eggs/root system, eggs were extracted using the aforementioned procedure.

### ***M. incognita* on European Corn Borer Herbivory**

Two experiments were conducted as a 4 x 3 factorial experiment in a randomized complete block design in 2005 and 2006. The treatments consisted of 4 levels of nematode inoculation (0, 2000, 4000, and 6000 *M. incognita* eggs and juveniles/plant), and 3 levels of plant growth stage (6-, 8-, and 10-leaf stages). All treatment combinations were replicated ten times.

At each plant growth stage, 10 plants were inoculated with 0, 2000, 4000, or 6000 *M. incognita* eggs and juveniles. The desired number of *M. incognita* eggs and juveniles suspended in 10 ml water were inoculated at the base of each plant. All *M. incognita* eggs and juveniles were used within 24 h of extraction from the roots.

After 2 weeks of *M. incognita* inoculation, plants were infested with a single third instar European corn borer, using the method described earlier. Seventy-two hours

after European corn borer placement on the plant, each plant was cut just above the soil surface and dissected vertically to measure tunnel length. The roots of each plant were observed for any unusual differences, in root development or health. Plants with any abnormal root growth, such as reduced size or biomass were discarded.

## Statistics

A separate two-way factorial analysis of variance (ANOVA) was used to analyze for differences among each response variable: *M. incognita* juvenile penetrations, *M. incognita* eggs/root system, and European corn borer tunnel lengths/plant. All the response variables for each experiment were analyzed separately. For analyses involving *M. incognita* juvenile penetrations and eggs/root system as response variables, European corn borer infestation level and plant growth stages were treated as main effects. For analyses involving European corn borer tunnel lengths/plant as a response variable, *M. incognita* inoculation level and plant growth stages were treated as main effects. *M. incognita* juvenile penetrations and eggs/root system were subject to  $(X + 0.5)^{1/2}$  transformation before analysis. Untransformed means are presented in tables and text. When significant main effect interactions were found, separate ANOVAs, and Fisher's protected LSD mean separation tests were performed to inspect for *M. incognita* juvenile penetrations and eggs/root system differences among infestation levels within a growth stage. Statistical significance was preset at  $P \leq 0.05$ , although all  $P$  values obtained are presented in the result section. The general linear model procedure (SAS Institute 2001) was used for performing ANOVA and Fisher's protected LSD mean

separation tests. Separate correlation analyses were performed on European corn borer tunnel lengths/plant, and *M. incognita* juvenile penetrations/root system and *M. incognita* eggs/root system. Correlation analyses were performed on untransformed data.

## RESULTS

### **European Corn Borer Herbivory on *M. incognita* Juvenile Penetrations and Eggs/Root system**

*Experiment I.* The main effect of European corn borer infestation level on *M. incognita* juvenile penetrations/root system was significant ( $P = 0.0005$ ); however, the main effect of plant growth stage and the interaction of European corn borer infestation level and plant growth stage were not significant ( $P > 0.05$ ) (Table 3.1). Overall mean *M. incognita* juvenile penetrations/root system across all three growth stage, at infestation levels of 1 and 3 larvae/plant were significantly less than in non-infested control plant. In the 1 and 3 larvae/plant treatments, mean ( $\pm$  SEM) *M. incognita* juvenile penetrations/root system were  $4.0 \pm 2.26$  and  $10.6 \pm 0.68$ , respectively at the 10-leaf growth stage (Table 3.2). These treatments were significantly less than the control ( $30.0 \pm 2.39$ ) at the same growth stage (Table 3.2). European corn borer stalk tunnel lengths and *M. incognita* juvenile penetrations/root system were significantly negatively correlated ( $n = 28$ ,  $P \leq 0.05$ , Pearson Correlation Coefficient 'r' = -0.4438) (Fig. 3.2).

The main effects of European corn borer infestation level and the plant growth stage on *M. incognita* eggs/root system were significant ( $P = 0.0562$  and  $P < 0.0001$ ,

respectively), however, the interaction of European corn borer infestation level and plant growth stage was not significant ( $P > 0.05$ ) (Table 3.1). Overall mean *M. incognita* eggs/root system at an infestation level of 3 larvae/plant were significantly less than in non-infested control plant. The mean eggs/root system ( $10,320 \pm 1,970$ ) in the 3 larvae/plant treatment was significantly less than the control ( $18,880 \pm 1,398$ ) at the 10-leaf growth stage (Table 3.2). European corn borer stalk tunnel lengths and *M. incognita* eggs/root system were significantly negatively correlated ( $n = 30$ ,  $P \leq 0.05$ , Pearson Correlation Coefficient 'r' = -0.4161) (Fig. 3.3).

*Experiment II.* The main effects of European corn borer infestation level and plant growth stage, and the interaction of European corn borer infestation level and plant growth stage on *M. incognita* juvenile penetrations/root system were significant ( $P = 0.0424$ ,  $P = 0.0041$  and  $P = 0.0422$ , respectively) (Table 3.3). ANOVAs and Fisher's protected LSD mean separation tests were performed to inspect for differences in *M. incognita* juvenile penetrations/root system within each plant growth stage. In the 1 and 3 larvae/plant treatments, juvenile penetrations/root system were  $13.4 \pm 1.36$  and  $12.0 \pm 0.84$ , respectively for the 8-leaf growth stage, and  $8.8 \pm 0.8$  and  $8.0 \pm 0.84$ , respectively for the 10-leaf growth stage. These treatments were significantly less than the control for the 8-leaf ( $21.6 \pm 1.17$ ) and 10-leaf ( $15.2 \pm 1.24$ ) growth stages (Table 3.4). European corn borer stalk tunnel lengths and *M. incognita* juvenile penetrations/root system were significantly negatively correlated ( $n = 3$ ,  $P \leq 0.05$ , Pearson Correlation Coefficient 'r' = -0.5168) (Fig. 3.4).

The main effects of European corn borer infestation level and plant growth stage, and the interaction of European corn borer infestation level and plant growth stage on *M. incognita* eggs/root system were all highly significant ( $P < 0.0001$ ,  $P < 0.0001$  and  $P = 0.0134$ , respectively) (Table 3.3). ANOVAs and Fisher's protected LSD mean separation tests were performed to inspect for differences in *M. incognita* eggs/root system within each plant growth stage. The control treatment averaged significantly more eggs/root system than the 1 or 3 larvae/plant treatments at all three plant growth stages (Table 3.4). European corn borer stalk tunnel lengths and *M. incognita* eggs/root system were also significantly negatively correlated ( $n = 30$ ,  $P \leq 0.05$ , Pearson Correlation Coefficient 'r' = -0.5053) (Fig. 3.5).

### **Effect of *M. incognita* on European Corn Borer Herbivory**

*Experiments III and IV.* Four different inoculation levels of *M. incognita* eggs and juveniles/plant (i.e., 0, 2,000, 4,000, and 6,000) were used to determine the effect of belowground herbivory on European corn borer tunneling at three plant growth stages. In both experiments, no significant ( $P > 0.05$ ) differences in stalk tunneling were found for the main effects of *M. incognita* inoculation level, plant growth stage, and the interaction of *M. incognita* inoculation level and plant growth stage (Table 3.5). Mean ( $\pm$  SEM) European corn borer tunnel lengths/plant were  $2.65 \pm 0.32$ ,  $2.63 \pm 0.35$ ,  $2.48 \pm 0.34$ , and  $2.63 \pm 0.33$  at the *M. incognita* inoculation levels of 0, 2,000, 4,000, and 6,000 eggs and juveniles/plant, respectively for the third experiment. Likewise, mean European corn borer tunnel lengths/plant were  $1.10 \pm 0.17$ ,  $0.70 \pm 0.15$ ,  $0.95 \pm 0.17$ , and

0.95 ± 0.14 at the *M. incognita* inoculation levels of 0, 2,000, 4,000, and 6,000 eggs and juveniles/plant, respectively for the fourth experiment.

## DISCUSSION

In my study, *M. incognita* juvenile penetrations and eggs/root system were negatively correlated with European corn borer tunnel lengths. In addition, in general number of *M. incognita* juvenile penetrations and eggs/root system decreased with increasing infestation levels. My findings support those of Moran and Whitman (1990) and Masters and Brown (1992). Masters et al. (1993) developed a model that states that aboveground insect herbivory results in the reduction of root biomass causing in turn less belowground insect herbivory.

In contrast, I found no significant effect of *M. incognita* belowground herbivory on European corn borer herbivory. However, studies conducted by Gange and Brown (1989), Masters and Brown (1992), Masters et al. (1993), Masters (1995) and Masters et al. (2001) found that belowground herbivory by insects positively impacts aboveground herbivory by insects. The model developed by Masters et al. (1993) states that belowground herbivory positively impacts aboveground herbivory through a stress response in the plant, which results in the accumulation of amino acids and carbohydrates in the foliage of the plant. The variation in my findings could be the result of a nematode, rather than an insect as was done in the aforementioned studies, for the belowground herbivory. Therefore, considering the differences in the types of injury these two classes of herbivores cause, and the differences in host plant response, it is

plausible that aboveground herbivores respond differently depending on which type of belowground herbivore is used.

The lack of a significant effect of *M. incognita* belowground herbivory on European corn borer aboveground herbivory in my study could be that the *M. incognita* inoculation levels were not high enough to bring about a measurable change in tunnel length. In addition, the model developed by Masters et al. (1993) involved herbivory by foliage feeding insects, rather than from a stalk tunneling insect as was the case in my study. In my study, European corn borer larvae were placed on the internodes to initiate tunneling. Consequently, the Masters et al. (1993) model stating that increased accumulation of amino acids and carbohydrates in plant foliage that occurs as a function of belowground herbivory had little relevance for my study, which focused on a stalk tunneling insect. Although herbivory in my study was measured as stalk tunnel length rather than the amount of foliage consumed, my findings agree with those of Carter-Wientjes et al. (2004), who report that *M. incognita* herbivory on soybean had no effect on the amount of foliage consumed by *P. includens*.

To my knowledge, my study is the first to investigate the reciprocal effects of aboveground herbivory by European corn borer and belowground herbivory by *M. incognita* on one another in corn. Additional studies under field conditions may provide further insight into these interactions.

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Table 3.1. Effect of European corn borer (ECB) infestation level and plant growth stage on *M. incognita* juvenile penetrations/root system and *M. incognita* eggs/root system; Experiment I.

Juvenile penetrations/root system*			
Source	df	F value	P value
ECB Level	2	9.62	0.0005
Plant growth stage	2	0.54	0.5851
ECB level x Plant growth stage	4	1.03	0.4055
Eggs/root system*			
ECB level	2	3.12	0.0562
Plant growth stage	2	15.38	<0.0001
ECB level x Plant growth stage	4	0.39	0.8115

\* Data were subject to subject to  $(X + 0.5)^{1/2}$  transformation before analysis.

Table 3.2. Effect of European corn borer infestation level and plant growth stage on mean ( $\pm$ SEM) \* number of *M. incognita* juvenile penetrations/root system and *M. incognita* eggs/root system; Experiment I.

# of larvae/ plant	6-leaf	8-leaf	10-leaf	Mean Across All Growth Stage
Juvenile penetrations/root system				
0	40.20 $\pm$ 16.85a	25.20 $\pm$ 0.86ab	30.00 $\pm$ 2.39a	31.80 $\pm$ 5.52a
1	18.40 $\pm$ 6.07a	27.60 $\pm$ 3.09a	14.00 $\pm$ 2.26b	20.00 $\pm$ 2.68b
3	11.60 $\pm$ 3.52a	15.20 $\pm$ 6.21b	10.60 $\pm$ 0.68b	12.47 $\pm$ 2.27b
Eggs/root system				
0	49,400.00 $\pm$ 13,655.48a	13,560.00 $\pm$ 2,271.92a	18,880.00 $\pm$ 1,397.99a	27,280.00 $\pm$ 6,020.69a
1	31,980.00 $\pm$ 7,427.41a	13,320.00 $\pm$ 3,200.37a	15,920.00 $\pm$ 3,020.99ab	20,406.67 $\pm$ 3,458.72ab
3	28,800.00 $\pm$ 9,973.57a	10,000.00 $\pm$ 2,097.62a	10,320.00 $\pm$ 1,970.38b	16,373.33 $\pm$ 3,972.23b

Means followed by different letters within a column for each response variable are significantly different (Fisher's protected LSD,  $P \leq 0.05$ ). Data were subject to subject to  $(X + 0.5)^{1/2}$  transformation before analysis.

\* Untransformed means and SEMs are presented in the table.

Table 3.3. Effect of European corn borer (ECB) infestation level and plant growth stage on *M. incognita* juvenile penetrations/root system and *M. incognita* eggs/root system; experiment II.

Source	df	F value	P value
Juvenile penetrations/root system*			
ECB Level	2	3.46	0.0424
Plant growth stage	2	6.43	0.0041
ECB level x Plant growth stage	4	2.76	0.0422
Eggs/root system*			
ECB Level	2	39.72	<0.0001
Plant growth stage	2	15.25	<0.0001
ECB level x Plant growth stage	4	3.66	0.0134

\* Data were subject to subject to  $(X + 0.5)^{1/2}$  transformation before analysis.

Table 3.4. Effect of European corn borer infestation level and plant growth stage on mean ( $\pm$ SEM)\* number of *M. incognita* juvenile penetrations/root system and *M. incognita* eggs/root system; Experiment II.

# of larvae/ plant	6-leaf	8-leaf	10-leaf
Juvenile penetrations/root system			
0	14.40 $\pm$ 2.23a	21.60 $\pm$ 1.17a	15.20 $\pm$ 1.24a
1	17.00 $\pm$ 1.27a	13.40 $\pm$ 1.36b	8.80 $\pm$ 0.80b
3	20.00 $\pm$ 6.91a	12.00 $\pm$ 0.84b	8.00 $\pm$ 0.84b
Eggs/root system			
0	19,750.00 $\pm$ 617.45a	20,570.00 $\pm$ 2,101.64a	11,155.00 $\pm$ 1,070.76a
1	11,900.00 $\pm$ 857.47b	9,378.00 $\pm$ 482.06b	10,570.00 $\pm$ 2,399.77a
3	9,300.00 $\pm$ 532.68c	9,842.00 $\pm$ 627.19b	5,000.00 $\pm$ 779.74b

Means followed by different letters within a column for each response variable are significantly different (Fisher's protected LSD,  $P \leq 0.05$ ). Data were subject to subject to  $(X + 0.5)^{1/2}$  transformation before analysis.

\* Untransformed means and SEMs are presented in the table.

Table 3.5. Effect of *M. incognita* inoculation level and plant growth stage on European corn borer tunnel lengths/plant.

Experiment III			
Source	df	<i>F</i> value	<i>P</i> value
<i>M. incognita</i> level	2	0.06	0.9801
Plant growth stage	3	0.08	0.9217
<i>M. incognita</i> level x Plant growth stage	6	1.30	0.2652
Experiment IV			
<i>M. incognita</i> level	2	1.09	0.3565
Plant growth stage	3	1.49	0.2302
<i>M. incognita</i> level x Plant growth stage	6	0.34	0.9130

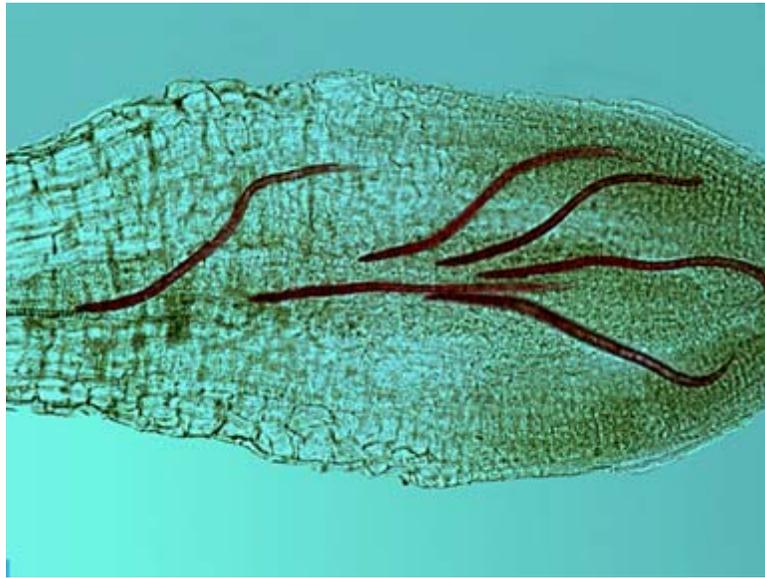


Fig. 3.1. Stained roots for counting *M. incognita* juvenile penetrations/root system  
(Source: Mactode Productions, J. D. Eisenback).

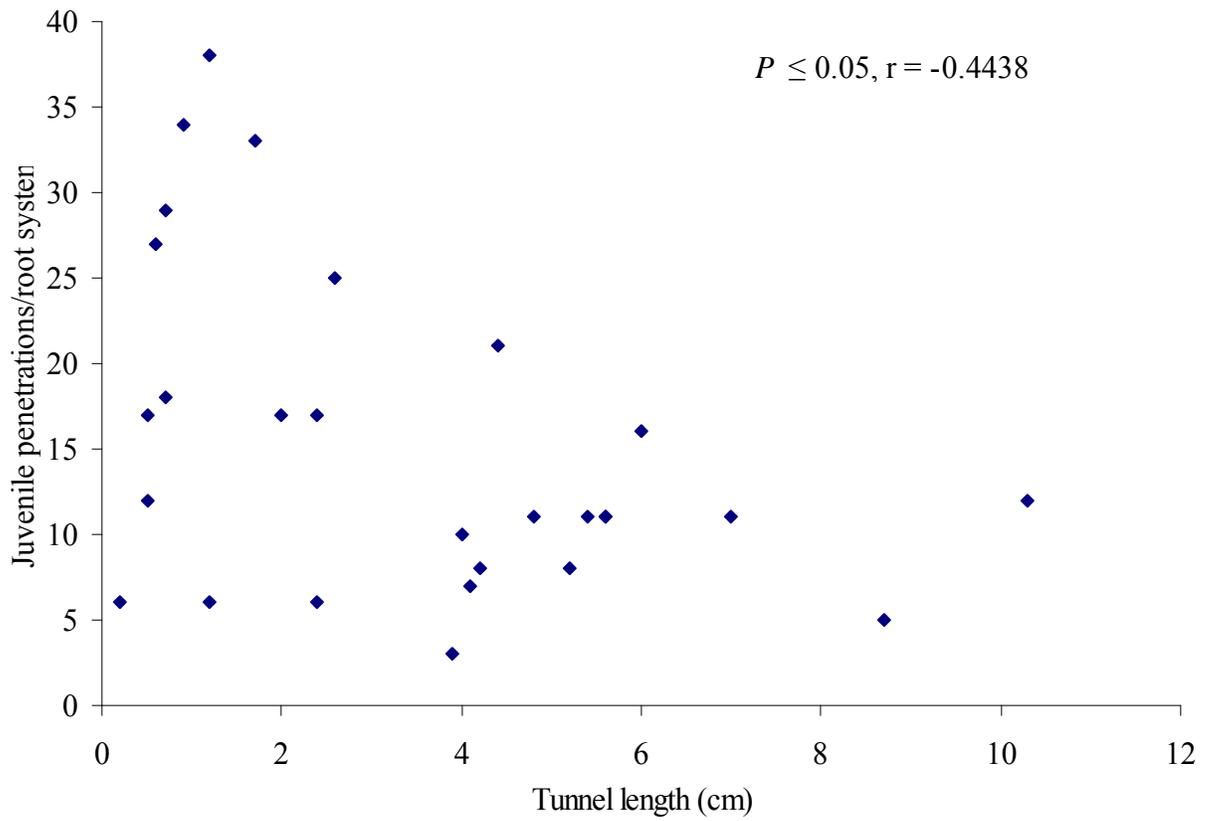


Fig. 3.2. Relationship between European corn borer tunnel length and *M. incognita* juvenile penetrations/root system; experiment I.

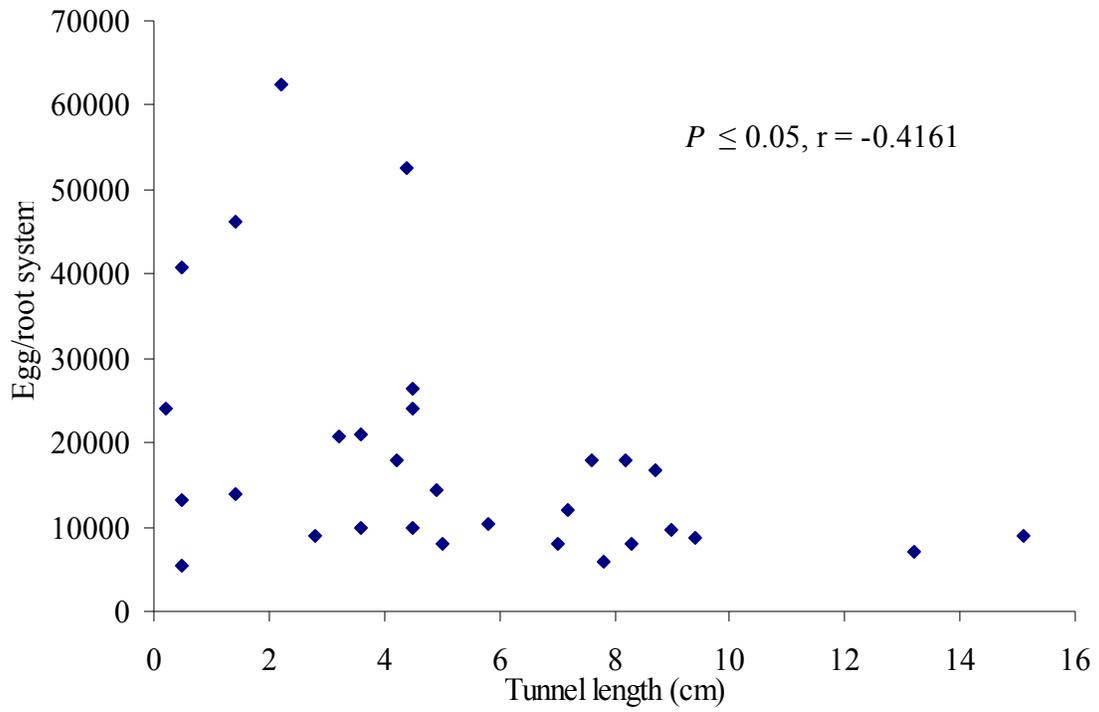


Fig. 3.3. Relationship between European corn borer tunnel length and *M. incognita* eggs/root system; experiment I.

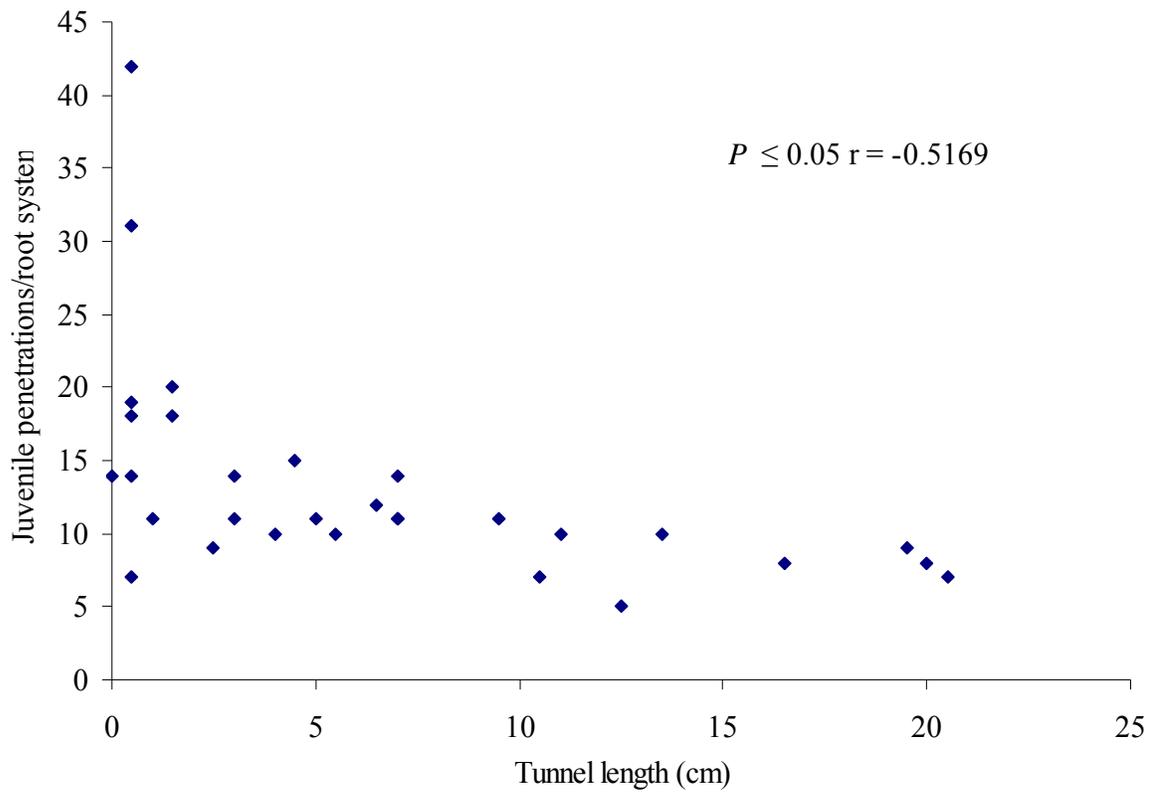


Fig. 3.4. Relationship between European corn borer tunnel length and *M. incognita* juvenile penetrations/root system; experiment II.

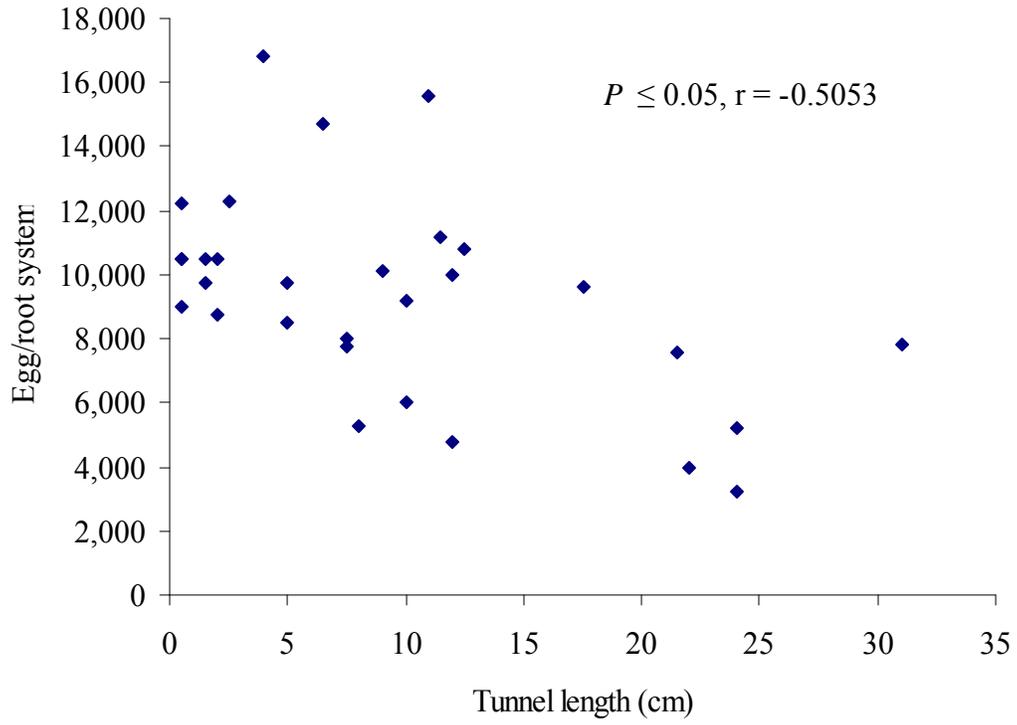


Fig. 3.5. Relationship between European corn borer tunnel length and *M. incognita* eggs/root system; experiment II.

## CHAPTER 4

### Conclusion

European corn borer, *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae), is one of the most economically important pests of corn. It is estimated to cause more than a billion dollars loss to U.S. corn growers in yield losses and management expenditures (Youngman and Tiwari 2004). The economic importance of this pest has led to several studies focusing on the relationship between European corn borer and yield losses in corn. However, most of the studies have focused on corn grown for grain (Chiang et al. 1954, Jarvis et al. 1961, Bode and Calvin 1990). The few studies on the effect of European corn borer on corn grown for silage were conducted in various geographical locations, which have produced contradictory results (Thompson and White 1977, Raemisch and Walgenbach 1984, Myers and Wedberg 1999). Considering the importance of geographical location on the life history and feeding injury of European corn borer (Mason et al. 1996), results found in one location might not agree with other locations. Therefore, the first objective of my study was to evaluate the effect of different levels of European corn borer on corn grown for silage under Virginia conditions.

Field studies were conducted over two growing seasons to evaluate the effect of different levels of third instar European corn borer on whole-plant dry matter in corn grown for silage in 2004 and 2005. Mean whole-plant dry matter was significantly

greater by 18.8% in uninfested control plants than in plants with an infestation level of 6 larvae/plant in 2004. Whole-plant dry matter in 2005 was significantly greater by 10.5% in control plants than in plants with an infestation level of 5 larvae/plant. The findings of my research indicate that whole-plant dry matter loss in corn grown for silage begins at or above an infestation level of 5 larvae/plant. Although the quantity of whole-plant dry matter was found to be significantly less at or above an infestation level of 5 larvae/plant, the quality of whole-plant dry matter as measured in percent acid detergent fiber, neutral detergent fiber, and crude protein was not affected. The importance of these findings for Virginia corn growers is that this level of infestation rarely occurs, where corn is largely grown for silage in Virginia (Youngman et al. 1998, 1999, 2000). This suggests that Virginia growers who grow corn for silage should strongly consider selecting non-Bt hybrids that exhibit the best agronomic traits suited to their particular farming operation before deciding on using a Bt hybrid. Planting non-Bt corn hybrids can lower production costs as much as \$17-25/ha.

Another important pest of corn is the root knot nematode, *Meloidogyne incognita* Chitwood (Tylenchida: Heteroderidae). In Virginia, the root knot nematode has been reported to cause up to 7% losses (McSorley et al. 1987). However, under rare instances of heavy infestations, *M. incognita* can cause yield losses of  $\geq 30\%$  in corn (Windham and Williams 1994). In light of several studies on the effect of feeding by aboveground pests and belowground pests through a common host plant (Fu et al. 2001, Bezemer et al. 2003, Blossy and Hunt-Joshi 2003, Carter-Wientjes et al. 2004, Wardle et al. 2004, Rasmann et al. 2005), the second part of my research focused on evaluating the

relationship between aboveground herbivory by European corn borer and belowground herbivory by root knot nematode in corn.

Belowground herbivory by root knot nematode was measured in juvenile penetrations and eggs/root system. The findings of my study show that aboveground herbivory by European corn borer results in fewer root knot nematode juvenile penetrations and eggs/root system. In the reciprocal study, which examined the effect of root knot nematode inoculation levels on European corn borer stalk tunneling, no significant effect of different root knot nematode inoculation level was found on European corn borer stalk tunneling. My studies were conducted under greenhouse conditions. Further studies under field conditions may shed additional insight into these findings.

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## VITA

Siddharth Tiwari was born on November 30 1976 in Uttar Pradesh, India. He received his Bachelors in Agriculture and Animal Husbandry degree with honors from G. B. Pant University of Agriculture and Technology, Pantnagar, India in 1998. In fall 1998, he began his studies on the rearing techniques of *Corcyra cephalonica* and *Trichogramma* spp. in Department of Entomology at G. B. Pant University of Agriculture and Technology, Pantnagar, India for his Master's research under the supervision and direction of Dr. M. A. Khan. His Master's work was a part of the research project entitled "Mass production of *Trichogramma* spp. and *Sturmiopsis inferens*" sponsored by the Department of Biotechnology, Ministry of Science and Technology, Govt. of India. After completing his Master's in fall of 2000, he continued to work as Senior Research Fellow under Dr. Khan till he joined Virginia Tech for his Ph.D. He began his doctoral research on "the effects of European corn borer on whole-plant yield and root knot nematode fitness in corn" in fall 2002, under Dr. Youngman. During his doctoral research, he received Virginia Crop Production Scholarship in 2004, a research grant from Southern Region USDA Program on Sustainable Agriculture Research and Education (SARE) in 2005, and research grants from Graduate Research Development Program (Virginia Tech) in 2004-2006. He successfully defended his dissertation on April 26 2007. Siddharth married Sonal Mishra on December 10, 2005 at Delhi, India.