Effects of transgenic corn on the stalk borer, *Papaipema nebris*, and rearing techniques for the European corn borer parasitoid, *Macrocentrus cingulum*

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To Mom, who continues to feed my curiosity by over-explaining everything. Thank you for teaching me to approach a situation with both compassion and cynicism. To Dad, for taking me on those early morning bird walks and plant identification lessons. Thank you for teaching me the value of hard work and perseverance. I am still an expert parallel parker! Finally, to Darren, for your honest interpretations and your warm love. I look forward to the rest of our lives together.

TABLE OF CONTENTS

CHAPTER 1. INTRODUCTION 1
- Corn 1
- *Macrocentrus cingulum* 1
- *Papaipema nebris* 3
- Thesis organization 9
- References 9

CHAPTER 2. EFFECTS OF TRANSGENIC CORN ON THE STALK BORER, *PAPAIPEMA NEBRIS* 14
- Abstract 14
- Introduction 14
- Materials and Methods 18
  - Plant injury 18
  - Bioassay 19
- Results 20
  - Plant injury 20
  - Bioassay 21
- Discussion 23
- References 24
- Figure captions 27
- Figures 28
- Tables 36

CHAPTER 3. REARING TECHNIQUES OF A EUROPEAN CORN BORER PARASITOID, *MACROCENTRUS CINGULUM* 39
- Abstract 39
- Introduction 39
- Materials and Methods 40
- Discussion 42
  - Emergence 43
  - Adult care 43
  - Parasitization 43
- Conclusions 44
- References 45
- Table 47

Chapter 4. SUMMARY 48
- *Papaipema nebris* 48
- *Macrocentrus cingulum* 49
- References 50

CHAPTER 1. INTRODUCTION

Corn

Corn, *Zea mays*, is an economically important food crop, with approximately 77.4 million acres planted in the United States in 1999. (National Corn Growers Association 2000). Of the total acres planted in corn, 12.1 million acres were in Iowa. The European corn borer, *Ostrinia nubilalis* Hübner, is a major pest of corn in the United States, where more than 1 billion dollars is lost annually to yield loss and control expenses (Mason et al. 1996).

Macrocentrus cingulum

Macrocentrus cingulum Reinhard is an imported larval parasitoid of the European corn borer. Its natural range extends from Europe east to Japan (Parker 1931, Clausen et al. 1978). The adult female can lay from 200–300 eggs in her lifetime, and development in the host is polyembryonic (Parker 1931). After hatching, the parasitoid larvae feed internally on the fat bodies and some internal organs until it is time for them to molt to the fourth instar. Then they migrate to the exterior of the host larva, where they pierce a hole in its cuticle and begin to feed, consuming the remaining internal organs and muscles (Parker 1931). When feeding is complete, the parasitoid larvae spin individual cocoons attached to the remains of the host.

According to Clausen (1978), the only host *M. cingulum* has been reared from is the European corn borer. *Macrocentrus cingulum*'s life cycle is well synchronized with that of the European corn borer, and there may be one or two annual generations that coincide with single or multiple European corn borer generations

(Winnie and Chiang 1982). The peak of adult parasitoid emergence occurs around the same time as the peak of egg laying by the host (Parker 1931). The parasitoids overwinter within the host larvae as eggs. A survey of *M. cingulum* parasitism in Iowa from 1951 to 1980 concluded that rates were less than 5 percent through 1963, but have increased to up to 56 percent from 1964-1980 (Lewis 1982). In the early 1980s a survey of *M. cingulum* parasitism in Illinois revealed parasitism rates of 2-24 percent (Onstad et al. 1991). Onstad et al. (1991) also noted that parasitism rates were always higher in first generation larvae than in second-generation larvae.

Special conditions and needs of *M. cingulum* must be met in the laboratory to ensure successful pupation and parasitoid vitality. If the parasitoid larvae are placed on a flat surface when they are ready to pupate, they are unable to make a cocoon, and they die (Parker 1931). The ability of the host to produce a cocoon that surrounds the parasitic larvae seems to be an important factor in the ability of the larvae to successfully pupate (Orr et al. 1994b). *Nosema pyrausta* (Microspora: Nosematidae) is an entomopathogen that attacks European corn borer larvae (Orr et al. 1994a). It directly affects the European corn borer by infecting the Malpighian tubules and silk glands of the larvae (Cossentine and Lewis 1987). Indirectly it has been shown to infect parasitoids that attack *O. nubilalis* larvae, particularly *M. cingulum* (Cossentine and Lewis 1987, Orr et al. 1994b). By reducing the ability of *O. nubilalis* to produce a cocoon, the microsporidian indirectly reduces the ability of *M. cingulum* to pupate. Infection of corn borer larvae by *N. pyrausta* causes reduced fecundity, adult longevity, and eclosion of *M. cingulum*. The parasitoid larvae become infected with the microsporidian after they begin feeding externally on the host, or after feeding on host frass (Cossentine and Lewis 1987). As the spores of the microsporidian accumulate in the midgut of the host larva, the amount of digestible tissue available to the parasitoid larvae decreases (Brooks 1993). The subsequent malnutrition of the parasitoid larvae explains the reduced fecundity and longevity of the adult and reduced eclosion. Female adult parasitoids may not emerge from infected hosts (Cossentine and Lewis 1987). Health of the parasitoids will be increased by montioring and prevention of *N. pyrausta* infection in the parasitoid and European corn borer colonies.

Wishart (Wishart 1946) described rearing procedures for *M. cingulum*. No papers have been published detailing rearing of the wasp using multiple generation corn borers. There have also been advancements in the rearing of the European corn borer since the publication of Wishart's guide. Ding et al. (Ding et al. 1989) describes colony maintenance of *M. cingulum*, however, rearing was described only briefly in the context of their study concerning host selection behavior.

Van Achterberg and Haeselbarth (1983) synonymized *Macrocentrus grandii* Goidanich with *Macrocentrus cingulum* Reinhard, making *M. cingulum* the senior synonym.

Papaipema nebris

The stalk borer, *Papaipema nebris* (Guenée), is considered a pest of corn because of the damage it causes by injuring the corn plant. By definition, a pest is a species that interferes with human activities, property or health or is objectionable; pest status is the ranking of a pest based on the economics involved in managing the pest (Pedigo 1999).

Guenée first described the stalk borer in 1852, however the first two records of damage by a larva that was unmistakably *P. nebris* were in wheat in 1823 and 1840 (Decker 1931). Originally, Guenée described two color forms separately—light and dark—but made them one species after breeding both forms together. The stalk borer is a stem boring member of Noctuidae (Lepidoptera), native to the United States (Decker 1931). The stalk borer's range extends east of the Rocky Mountains to the Atlantic Coast. The larvae are reported to feed on 176 different species of plants (Decker 1931). Although corn is typically considered the primary economic host plant of the stalk borer, injury by the larva has been reported on other cultivated plants, including, but not restricted to, potato (*Solanum tuberosum* L.), tomato (*Lycopessicum esculentum* Mill), tobacco (*Nicotiana tabacum* L.), and soybeans (*Glycine max* L.) (Decker 1931, Rice and Pedigo 1996). Stalk borer larvae can also be pests of cultured hardwood trees, such as silver maple and sycamore, when they bore into the shoots of young plants (Solomon 1988). Decker (1931) reported significant populations in Iowa in the 1920s, citing farms where 80 to 100 percent of the corn plants were killed by stalk borer larvae. In his extensive study of the stalk borer, Decker found larvae in every county in Iowa. Although this pest appears to be common, there is a greater incidence of problems in corn fields in the Midwest associated with the practice of conservation tillage (Rubink and McCartney 1982, Stinner et al. 1984). As recently as 1996, farmers in western Iowa reported that 75 to 100 percent of their young corn plants were infested with stalk borer larvae (Rice and Pedigo 1996). The reason for the increasing economic significance of stalk borers begins with the ovipositional habits of the adults.

Adult stalk borers lay their eggs on the leaves of weeds, preferring members of the grass family to broadleaf species (Levine 1985). Although adult females have been observed to hold their eggs until death when only broadleaf plants are available for oviposition, a relationship has been suggested between the presence of broadleaf weeds and increased infestation of corn by stalk borer larvae (Highland and Roberts 1989, Pavuk and Stinner 1991). This relationship may occur because broadleaf plants usually have thicker stems than grasses, and thus may be a host to the larvae if the grass that was host to the egg is dead or the stem of the grass is too small (Pavuk and Stinner 1991). Also it takes less time for a stalk borer larva to bore into a corn plant than it does to enter the stem of a broadleaf plant (Alvarado et al. 1989). The females typically lay their eggs on the midrib of folding or curling leaves, which may provide protection from parasitization, predation, and desiccation (Levine 1985, Highland and Roberts 1989). Stalk borer larvae are cannibalistic and there is usually only one larva per plant (Decker 1931). Giant ragweed, *Ambrosia trifida* L., is a common, thick-stemmed host of larvae (Rubink and McCartney 1982). Waterways, terraces, fence rows, and weedy fields are attractive ovipositional sites to stalk borer females. The eggs are laid in late August and September and hatch in late April or early May (Decker 1931).

Initially, the larva tunnels into the stalk of the nearest acceptable plant, usually grass or corn, and feeds. When the initial host plant is a small-stemmed grass, soon the stalk borer is forced to leave the grass for one of two reasons: the grass stem becomes too small for the growing larva, or the grass dies because of herbicide application in cropping situations (Rubink and McCartney 1982). In both cases, the larva migrates out of the grass stem and into a different suitable plant, generally no more than a few meters away (Stinner et al. 1984). However, if the larvae are moving out of grassy areas—such as grassy waterways, field edges, or contour strips—that are adjacent to a cornfield, they usually infest the corn plants. Typically, only the first four to eight adjacent rows are damaged (Levine et al. 1984). In fields where conservation tillage is practiced, however, the stalk borers will move from herbicide-killed weeds in between the rows of corn to the seedling corn and damage can occur throughout the field.

The stalk borer injures corn by leaf feeding and by either tunneling into the whorl and down in young plants, or tunneling through the base of the stalk and up in older plants (Rubink and McCartney 1982). More damage will occur to corn that is attacked by the stalk borer before the growing point is above ground—approximately stage V6 (Ritchie et al. 1993). At this stage, the ear shoots and tillers are visible and the immature tassel is above ground level. Before V6, the plant is most likely to suffer dead heart due to stalk borer injury (Levine et al. 1984, Davis and Pedigo 1991a). Dead heart has been shown to significantly reduce yields, with barren rates ranging from 25.0 to 62.5 percent (Bailey and Pedigo 1986). Leaf feeding by stalk borer larvae, however, does not have a significant effect on yield. Damage can extend throughout the entire corn field if the field harbored stalk borer eggs before spring planting due to poor perennial-grass control (Stinner et al. 1984).

Management of the stalk borer has been problematic. Insecticides are effective only if the application is timed to coincide with larval migration (Davis and Pedigo 1990). If the chemical is not applied when the larvae are out of the weeds and searching for an acceptable host plant, the insecticide will have been wasted. Mowing or burning grass in the spring to reduce the stalk borer population works fairly well along field edges or grassy waterways and terraces. However, grassy weeds that are between corn rows may not burn well because they were flattened by snow or were not dense enough to burn properly (Rice and Pedigo 1996).

Biological control is an option that has not been explored thoroughly for stalk borers. Decker (1931) reported several insect predators and parasitoids attacking the stalk borer eggs and larvae including species from Coccinellidae and Carabidae, five species of Hemiptera, two species of Formicidae, several parasitic Hymenoptera and one species of Diptera. Host habitat specificity of certain stalk borer parasitoids was demonstrated in a study by Felland (1990) in northern Ohio. He examined parasitized stalk borer larvae from four major habitats: corn, potato, common ragweed, and giant ragweed. He observed different parasitoids as dominant in each of the four habitats. Decker (1931) cited "wilt disease" and a fungus as causing additional mortality of stalk borer larvae. In a field study, some inbred lines of resistant corn were shown to have significantly reduced damage when infested with a stalk borer larva when compared to non-resistant lines (Peterson et al. 1987).

Although a combination of these management techniques may begin to control the stalk borer population in a field, they are often not enough. Data on the economic losses due to injury by the stalk borer are unavailable; however, economic injury levels have been calculated for fields with grassy edges or terraces (Davis and Pedigo 1991b).

Transgenic corn, a new corn pest management technique, recently became available and may have some effect on the stalk borer. Transgenic corn is corn that has a foreign gene or genes incorporated into its DNA (Jansens et al. 1997). *Bacillus thuringiensis* (Bt) is a bacterium that produces insecticidal crystalline proteins during sporulation (Lambert et al. 1996). Bt was discovered in 1915 by Ernst Berliner (Beegle and Yamamoto 1992). When this bacterium was discovered to have a lethal effect on members of the order Lepidoptera, especially the European corn borer, it was used to prevent plant injury, and was usually applied as a liquid or dust (Beegle and Yamamoto 1992). When the technology became available, the insecticidal crystaline protein gene was incorporated into the corn plant. The result was transgenic Bt corn that acted as an insecticide to certain lepidopteran pests that fed on it.

One study has previously examined the efficacy of Bt corn against the stalk borer. Pilcher et al. (1997) evaluated event 176 (Cry1Ab) and found significantly less damage to the Bt corn than to the non-Bt corn, although there were no differences in the developmental response of the larvae to the Bt corn than to the non-Bt corn (Pilcher et al. 1997). Their data suggest that the *Bt* protein may have some effect on the feeding behavior of stalk borer larvae. Since their study, more Bt corn events have been introduced for the management of Lepidoptera pests, especially the European corn borer.

The objectives of this research were: 1) to assess the efficacy of transgenic Bt corn expressing different proteins on the stalk borer; 2) to evaluate the effects of transgenic Bt corn on stalk borer growth and development; and 3) to rear *M. cingulum* and develop an up-to-date rearing methodology.

Thesis Organization

This thesis is organized into an introduction, two papers that are to be submitted to journals for publication, and a general conclusion.

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CHAPTER 2. EFFECTS OF TRANSGENIC CORN ON THE STALK BORER, *PAPAIPEMA NEBRIS*

A paper to be submitted to the Journal of Economic Entomology

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Abstract

This study assessed the efficacy of two different genetic events, event Bt 11 (Cry1Ab) and event CBH351 (Cry 9C), in Bt corn against two instar classes of the stalk borer, *Papaipema nebris* (Lepidoptera: Noctuidae), across three different plant stages (V1, V3, and V5) of corn, *Zea mays*. Class A includes instars 1–2 and class B includes instars 3–4. Stalk borer response and development over time were measured and the data from 1999 and 2000 show that the Bt corn does have some effect on the feeding and development of *P. nebris*. Injury to the corn plant was reduced, although not eliminated. Stalk borer larvae caused significantly ($P=0.0001$) more injury to the non-Bt plants than to the Bt plants over time. Growth and development of the larvae were slowed and mortality was higher for Bt corn than for non-Bt corn. These data suggest that planting Bt corn may benefit growers by reducing, but not eliminating, stalk borer infestations and injury.

Introduction

The stalk borer, *Papaipema nebris* (Guenee) is a stem boring member of the Noctuidae (Lepidoptera) native to the United States (Decker 1931). The stalk borer's range extends east of the Rocky Mountains to the Atlantic Coast. The larvae are reported to feed on 176 different species of plants, including orchard grass, *Dactylis* *glomerata* L., pigweed, *Amaranthus retroflexus* L., giant ragweed, *Ambrosia trifida* L., and corn, *Zea mays* L. (Decker 1931). Since corn is an economically important food crop, the damage the stalk borer causes by injuring the corn plant gives it a pest status (Solomon 1988). In his extensive study of the stalk borer in 1931, Decker found larvae in every county in Iowa. Although this pest appears to be common, there is a greater incidence of problems in corn fields in the Midwest associated with the practice of conservation tillage (Rubink and McCartney 1982, Stinner et al. 1984). Initially, the larva tunnels into the stalk of the nearest acceptable plant, usually grass or corn, and feeds. When the initial host plant is a small-stemmed grass, soon the stalk borer is forced to leave the grass for one of two reasons: the grass stem becomes too small for the growing larva, or the grass dies because of herbicide application in cropping situations (Rubink and McCartney 1982). In both cases, the larva migrates out of the grass stem and into a different suitable plant, generally no more than a few meters away (Stinner et al. 1984). However, if the larvae are moving out of grassy areas that are adjacent to a cornfield—such as waterways, field edges, or contour strips—they have little choice, and usually infest the corn plants. Typically, only the first four to eight adjacent rows are damaged (Levine et al. 1984). In fields where conservation tillage is practiced, however, the stalk borers will move from herbicide-killed weeds in between the rows of corn to the seedling corn and damage can occur throughout the field.

The stalk borer injures corn by leaf feeding and by stalk tunneling. More damage will occur to corn that is attacked by the stalk borer before the growing point is above ground—approximately stage V6 (Ritchie et al. 1993). At this time, the plant is most likely to suffer dead heart due to stalk borer injury (Levine et al. 1984, Davis and Pedigo 1991a). Dead heart has been shown to significantly reduce yields (Bailey and Pedigo 1986). Leaf feeding by stalk borer larvae, however, does not have a significant effect on yield. Damage can occur throughout the entire cornfield if the field harbored stalk borer eggs before spring planting due to poor perennial-grass control the previous growing season (Stinner et al. 1984).

Management of the stalk borer has been problematic. Insecticides are effective only if the application is timed to coincide with larval migration and early-stage whorl feeding (Davis and Pedigo 1990a). Burning grassy areas in the spring to reduce the stalk borer population works fairly well along field edges or grassy waterways and terraces. However, a significant number of eggs may survive on grassy weeds that are between cornrows because the grass was not dense enough to burn properly (Rice and Pedigo 1996). Mowing grassy areas adjacent to the cornfield in the late summer makes these sites less attractive for egg laying.

Biological control is an option that has not been explored thoroughly for stalk borers. Decker (1931) reported several insect predators and parasitoids attacking the stalk borer larvae including species from Coccinellidae and Carabidae, four species of Hemiptera, several parasitic Hymenoptera and one species of Diptera. Decker (1931) also cited “wilt disease” and a fungus as causing additional mortality of stalk borer larvae. Felland (1990) examined parasitized stalk borer larvae from four major habitats and observed different parasitoids as dominant in each habitat. In a field study, some inbred lines of resistant corn were shown to have significantly reduced damage when infested with a stalk borer larva when compared to non-resistant lines (Peterson et al. 1987).

Although a combination of these management techniques may begin to reduce the stalk borer population in a field, they are often not enough to prevent economic damage. Data on the economic losses due to injury by the stalk borer are unavailable, however, economic injury levels have been calculated for fields with grassy edges or terraces (Davis and Pedigo 1991b).

Transgenic corn, a new corn pest management technique, recently became available and may have some effect on the stalk borer. Transgenic corn has a foreign gene or genes incorporated into its DNA (Jansens et al. 1997). *Bacillus thuringiensis* (Bt), a bacterium that produces insecticidal crystalline proteins during sporulation (Lambert et al. 1996), contains a gene that codes for different crystalline proteins. When the gene has been incorporated into corn the result is a transgenic Bt plant that is toxic to some species of lepidopteran pests that feed on it, especially the European corn borer *Ostrinia nubilalis* (Hübner) (Jansens et al. 1997).

One study has previously examined the efficacy of Bt corn against the stalk borer. Pilcher et al. (1997) evaluated event 176 (Cry1Ab) and found significantly less damage to the Bt corn than to the non-Bt corn. However, there were no differences in the developmental response of the larvae to the Bt corn than to the non-Bt corn (Pilcher et al. 1997). Their data seem to suggest that the Bt protein may have some effect on the feeding behavior of stalk borer larvae.

The objectives of this study were to assess the efficacy of transgenic Bt corn expressing Cry1Ab (event Bt11) and Cry9c (event CBH351) proteins on the stalk borer and to evaluate the effects on stalk borer growth and development.

Materials and Methods

Plant Injury

Corn plants were grown in 3.8 liter (1 gallon) pots, one plant per pot, in a greenhouse at Iowa State University, Ames, IA. The following corn hybrids were evaluated: 1) Novartis N4640Bt, YieldGard®, Cry1Ab, event Bt11; 2) Novartis N4494, non-Bt near isogenic; 3) Garst 8600BLT, StarLink®, Cry9C, event CBH351; and 4) Garst 8600IT, non-Bt near isogenic. Instar classes were separated in the greenhouse. Within each instar class, pots were arranged in a randomized complete block design with eight blocks per instar class. Each block contained 12 plants representing the combination of one of 3 plant stages with one of 4 corn hybrids. There were 96 plants per instar class. Pots were placed in plastic trays and watered from the bottom.

Stalk borers adults were collected in the fall and allowed to oviposit on bromegrass (*Bromus inermis* Leyss), which was kept in a jar filled with wet paper towels in an outdoor cage. After the moths died, the grass was allowed to dry, removed from the cage, and placed in a chamber that was maintained at 5°C for approximately 6 months. One week before initiation of the experiment, eggs were removed from the chamber and allowed to develop at room temperature (approximately 22°C). The method given by Davis and Pedigo (Davis and Pedigo 1990b) was used to rear the larvae. Larvae that were not immediately placed on a corn plant after hatching were fed a modified black cutworm diet (Reese et al. 1972) and kept in an environmental growth chamber at 26ºC and a photoperiod of 16:8 (L:D). Each hybrid was infested with one of two instar classes of *P. nebris*. Class A includes instars 1–2 and class B includes instars 3–4. Each instar class was placed on each of three plant stages (V1, V3, and V5) for each hybrid. When the eggs hatched, the larvae to be used for instar class A infestations were immediately placed on the corn plants. The larvae to be used for instar class B infestations were individually placed in plastic cups and fed the cutworm diet until they reached instars 3 or 4, approximately 8-12 days later (Decker 1931).

A single larvae from instar class A or B was placed in the whorl of a corn plant with a camel hair brush. The cotton end of half a cotton swab was placed in the whorl of the plant for the first 48 hours of infestation to act as a barrier to deter insect dispersal and encourage insect establishment.

Plants were rated twice weekly for injury using the six-point scale of Davis and Pedigo (Davis and Pedigo 1990b): 1) plant uninfested or minor leaf feeding present; 2) plant tunneled, very little leaf feeding, and growing point is not injured; 3) heavy leaf feeding, growing point not injured; 4) dead heart, growing point not injured; 5) dead heart and plant tillers; and 6) plant killed. Stalk borer survival was also recorded. The experiment was terminated at the end of three weeks, and was performed twice, once in 1999 and again in 2000.

Bioassay

A corn plant at stage V2 from each of the four hybrids was removed from its pot in a greenhouse, rinsed with distilled water, and placed in a plastic box (0.79 x

10.95 x 1.13 centimeters; Pioneer Plastics, Inc., Dixon, KY). Each plant was infested with a single *P. nebris* larva from one of two instar classes. Larvae were placed into the whorls of the plants. Each larva was weighed before placement in the plant using an electronic scale (Mettler AE 100). Plastic boxes containing larvae and plants were randomly placed within an environmental growth chamber at 26ºC and a photoperiod of 16:8 (L:D). Larvae were reweighed every 7 days and mortality was assessed. Because some of the stalk borer larvae consumed all of the corn tissue before 7 days had passed, all larvae were given a fresh plant in the middle of the week. Larvae that were still alive after 7 days were returned to the box, supplied with a fresh plant from the same treatment, and placed back into the environmental chamber. The experiment terminated at 28 days.

Each of the four hybrids was a treatment for each instar class and each treatment was replicated 15 times. Data were analyzed using analysis of variance with significance at $P < 0.05$ (SAS 1999). Differences between hybrids were determined using t-tests or least squared means and contrasts were used to determine the differences between and among the classes for the class by hybrid interaction.

Results

Plant Injury

In 1999, stalk borer larvae caused significantly (P=0.0001, 15 d.f., F=7.57) more injury to the non-Bt plants (N4494 and Garst 8600IT) than to the Bt plants (N4640Bt and Garst 8600BLT) over time (Fig. 1, Table 1). By the second observation date, the non-Bt plants were showing significantly higher injury ratings than the Bt plants. There were no significant differences between the two Bt hybrids or between the two non-Bt hybrids ($P < 0.05$). On the last day of observations, the highest average injury rating over hybrids was 3.1 for Garst 8600IT and the lowest was 1.3 for N4640BT, event Bt11. Over all four hybrids, the injury rating was significantly higher for corn plant stage V1 than for stage V3 or V5 ($P = 0.0001$, 10 d.f., $F = 6.33$) (Fig. 2). By the final day of observations, the average injury rating for plants infested at stage V1 was 3.4 and the average injury ratings for plants infested at stages V3 and V5 were 1.5 and 2.0 respectively.

In 2000, stalk borer larvae caused significantly more damage to the non-Bt Garst 8600IT than to all the other hybrids tested over time ($P = 0.0002$, 15 d.f., $F = 2.93$) (Fig. 3, Table 1). The highest average injury rating over hybrids was 2.4 for Garst 8600IT and the lowest was 1.2 for N4640BT, event Bt11. Hybrid by instar class ($P = 0.38$, 3 d.f., $F = 1.04$) and hybrid by plant stage interactions were non-significant; ($P = 0.83$, 3 d.f., $F = 0.29$). The plant stage study was not conducted this year because there were not enough stalk borer larvae to complete the experiment.

Bioassay

In 1999, Bt hybrids caused significantly higher mortality to stalk borer larvae than non-Bt hybrids ($P=0.0001$, 3 d.f., $F=94.34$) (Fig. 4, Table 2). At the first observation, the two Bt events were significantly different, with 90% mortality observed for event Bt11 and 83% for event CBH351. Only 27% and 30% mortality was observed for non-Bt hybrids N4494 and 8600IT respectively, both significantly different from the Bt hybrids. By the second week 93% mortality was observed for event Bt11 and 93% by the third week for event CBH351. By the end of the experiment, the surviving larvae that fed on event Bt11 weighed significantly less than all other surviving larvae except for those that fed on event CBH351 (P=0.0001, 3 d.f., F=23.28) (Fig. 5).

The results in 2000 were similar to the results from 1999. Although the percent mortality overall was lower than in 1999, the Bt hybrids again caused significantly higher mortality to stalk borer larvae than non-Bt hybrids (P=0.0001, 3 d.f., F=82.96) (Fig. 6, Table 3). At the first week, 50% mortality was observed for both Bt hybrids. The non-Bt hybrids N4494 and 8600IT were significantly different, with 17% and 7% mortality respectively. The second week, 60% mortality was observed for event Bt11 and 57% for event CBH351, with no significant differences between the two Bt hybrids. The two non-Bt hybrids were significantly different from each other, with 27% mortality observed for N4494 and 20% mortality for 8600IT. We observed 70% by the 3rd week for event CBH351, but only 63% mortality for event Bt11, and the two Bt hybrids were significantly different from each other. We observed 83% by the 4th week for event CBH351, while mortality for event Bt11 was the same as the 3rd week, with a significant difference. Larvae in instar class B that had survived on event Bt11 by the third and fourth weeks of observations had significantly lower weights than all other surviving larvae (P=0.0022, 2 d.f., F=7.16) (Fig. 7). High mortality in all hybrids for instar class A prevented analysis of the data for that instar class. Time was a significant factor in the mortality of larvae, and mortality can be described as a linear increase over time. The instar class by hybrid interaction was also a significant factor in the mortality of stalk borer larvae (P<0.0001). Mortality has been averaged over weeks to emphasize the interaction of class and hybrid (Fig. 8). The Bt hybrids caused significantly more mortality to instars 1-2 (class A) than the non-Bt hybrids. However, there was no difference between the mortality of instars 3-4 (class B) caused by the Bt hybrids compared to the non-Bt hybrids. When comparing the instar classes, class A had significantly higher mortality than class B for the Bt hybrids, but there was no difference in mortality between the classes for the non-Bt hybrids. Although the two Bt events are genetically different, there was not a significant difference between them in either 1999 (P=1.0) or 2000 (P=0.34).

Discussion

This study indicates that Bt corn expressing genetic events Bt11 or CBH351 has a negative effect on stalk borer larvae. Although the effect is not 100% mortality, as studies have shown it to be with the European corn borer (Armstrong et al. 1995, Ostlie et al. 1997), suppression does occur. When larvae are exposed to the toxin, significant mortality occurs relative to non-Bt hybrids, and it takes longer for the larvae to develop. Injury to corn plants is reduced, although not eliminated, and older corn plants had lower injury ratings over all hybrids than younger corn plants.

Larval development is slowed, as is indicated by the reduced weight of surviving larvae on Bt corn plants. Longer development times mean longer periods of exposure to adverse weather conditions, pathogens, and natural enemies, which translates into a potential for higher larval mortality.

Younger larvae exhibited significantly higher mortality than the older larvae in all hybrids. In field conditions where weeds in the field are killed by herbicide application, the stalk borer is forced to move out of the weed stem and into corn plants sooner than usual. The presence of Bt corn in the field would result in fewer surviving larvae on young corn plants, which are otherwise less resistant to stalk borer injury than older corn plants.

Although Bt corn does not eliminate stalk borers, farmers planting Bt corn to control the European corn borer may benefit from reduced stalk borer infestations, especially if the larvae are first or second instars and the corn plants are at stage V3 or older. The reduction in numbers of stalk borer would be more significant when combining Bt corn with other management techniques, such as mowing or burning grassy areas in the spring and encouraging the presence of natural enemies.

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Figure Captions

Figure 1. Injury rating (on a 6 point scale) of corn plants averaged over all plant stages for four different corn hybrids (N4640Bt, event Bt11; N4494, non-Bt, 8600BLT, event CBH351; and 8600IT, non-Bt), 1999. Observations began when injury was first observed.

Figure 2. Injury rating of corn plants when infested at three specific plant stages, for all four corn hybrids (N4640Bt, event Bt11; N4494, non-Bt, 8600BLT, event CBH351; and 8600IT, non-Bt), 1999. Observations began when injury was first observed.

Figure 3. Injury rating (1-3 of a 6 point scale) of corn plants averaged over all plant stages for four different corn hybrids (N4640Bt, event Bt11; N4494, non-Bt, 8600BLT, event CBH351; and 8600IT, non-Bt), 2000. Observations began when injury was first observed.

Figure 4. Cumulative percent mortality of stalk borer larvae (n = 15) on four different corn hybrids (N4640Bt, event Bt11; N4494, non-Bt, 8600BLT, event CBH351; and 8600IT, non-Bt), 1999.

Figure 5. Average weekly weight of stalk borer larvae (n = 15) when fed four different corn hybrids (N4640Bt, event Bt11; N4494, non-Bt, 8600BLT, event CBH351; and 8600IT, non-Bt), 1999.

Figure 6. Cumulative percent mortality of stalk borer larvae (n = 15) on four different corn hybrids (N4640Bt, event Bt11; N4494, non-Bt, 8600BLT, event CBH351; and 8600IT, non-Bt), 2000.

Figure 7. Average weekly weight of instar class B stalk borer larvae (n = 15) when fed four different corn hybrids (N4640Bt, event Bt11; N4494, non-Bt, 8600BLT, event CBH351; and 8600IT, non-Bt), 2000.

Figure 8. The interaction of instar class and corn hybrid when mortality is averaged for each hybrid (N4640Bt, event Bt11; N4494, non-Bt, 8600BLT, event CBH351; and 8600IT, non-Bt), 2000.

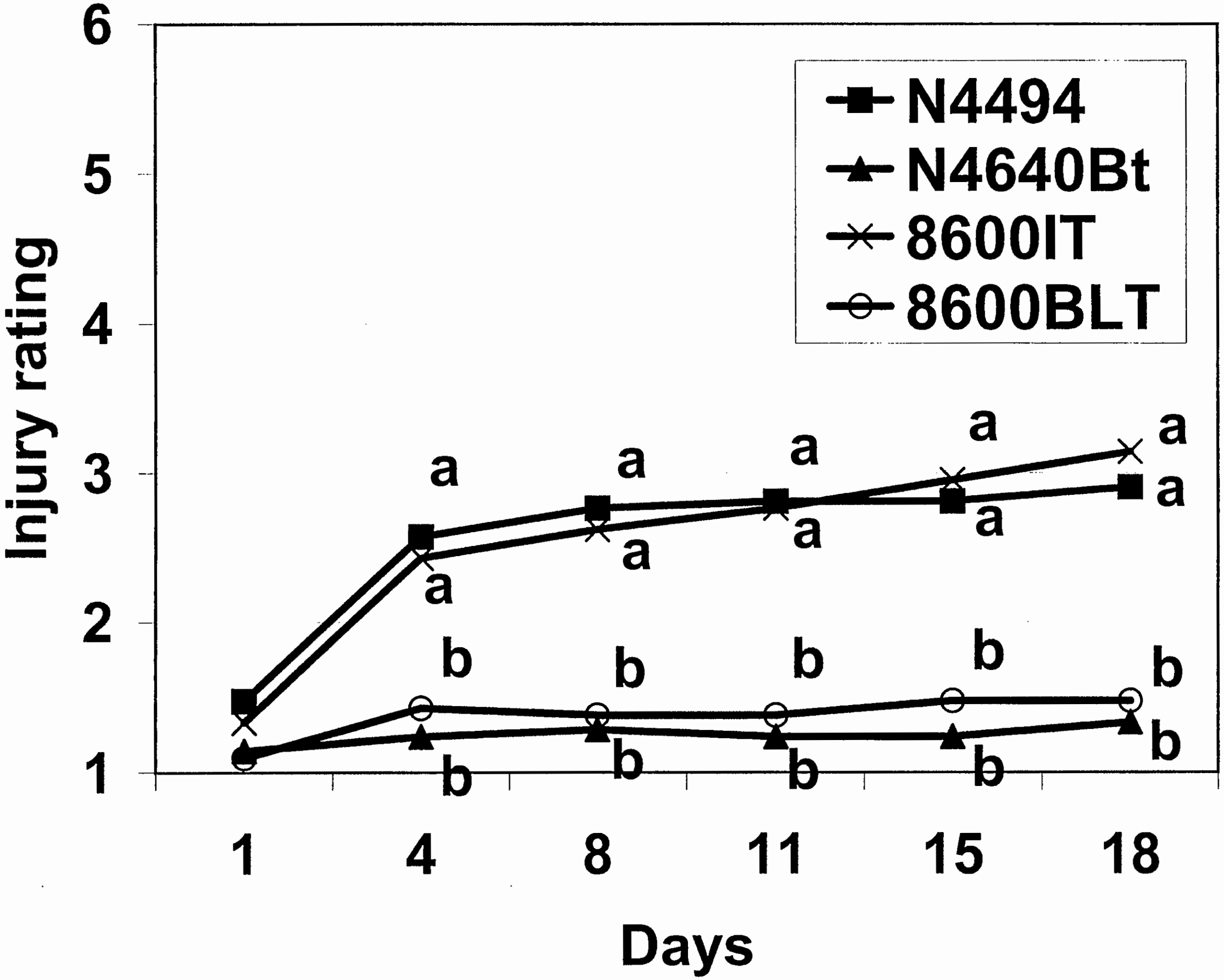

6
5
4
3
2
1
Injury rating
1
4
8
11
15
18
Days
N4494
N4640Bt
8600IT
8600BLT
a
b

Figure 1

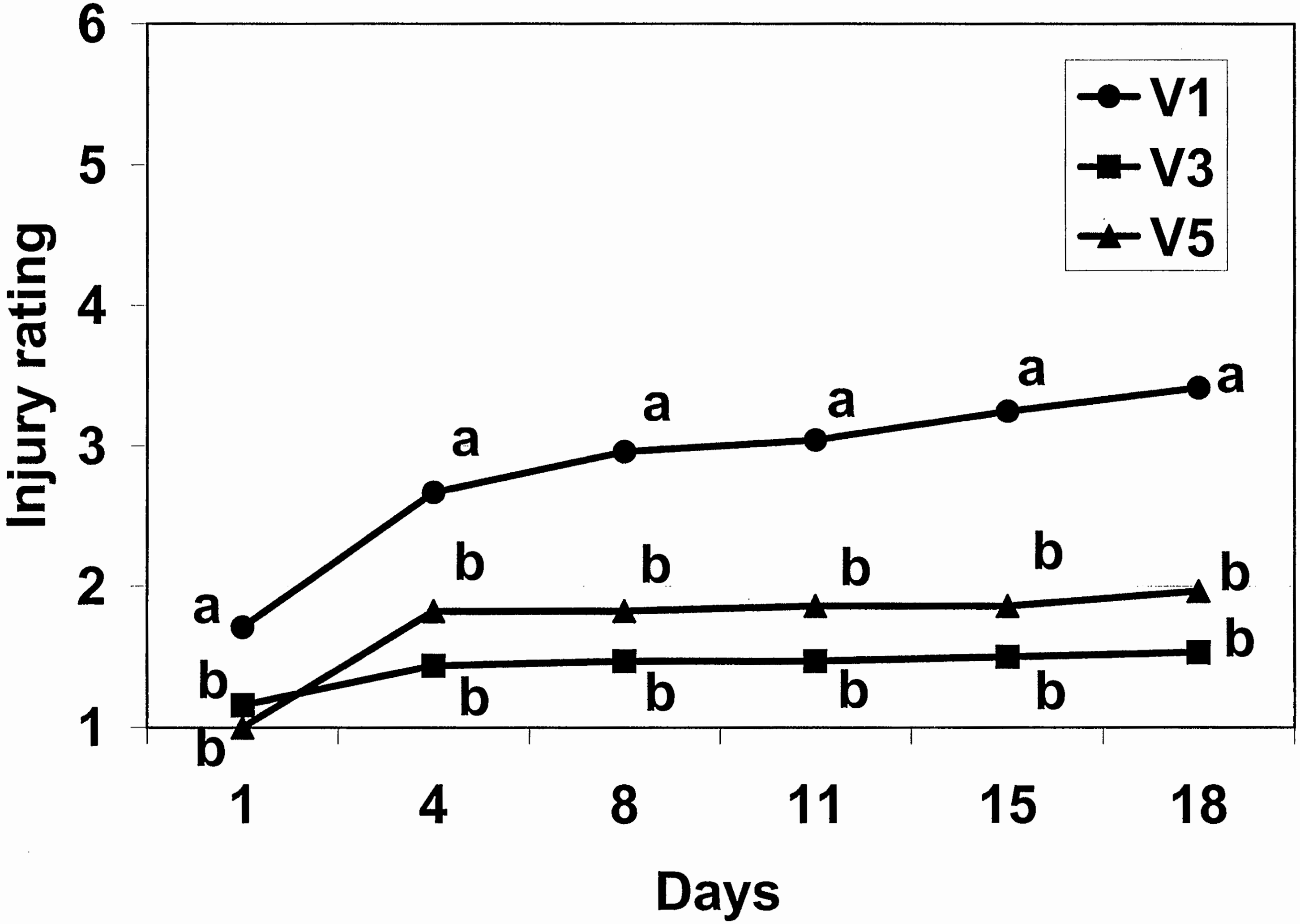
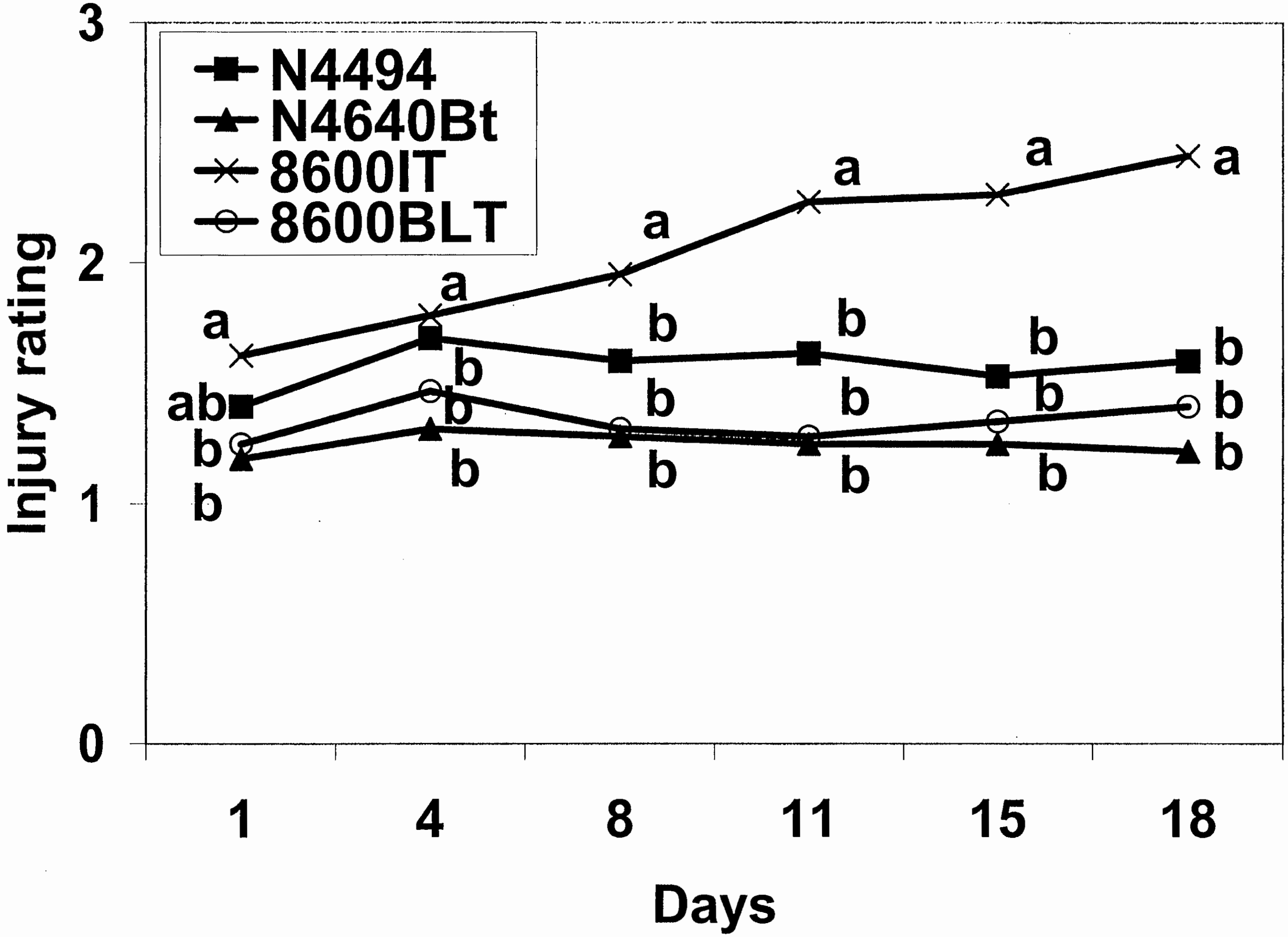

Injury rating
6
5
4
3
2
1
Days
1
4
8
11
15
18
V1
V3
V5
a
a
a
a
a
a
b
b
b
b
b
b
b
b
b
b
b
b

Figure 2


3
2
1
0
Injury rating
N4494
N4640Bt
8600IT
8600BLT
a
a
a
a
a
a
ab
b
b
b
b
b
b
b
b
b
b
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b
b
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b
b
1
4
8
11
15
18
Days

Figure 3

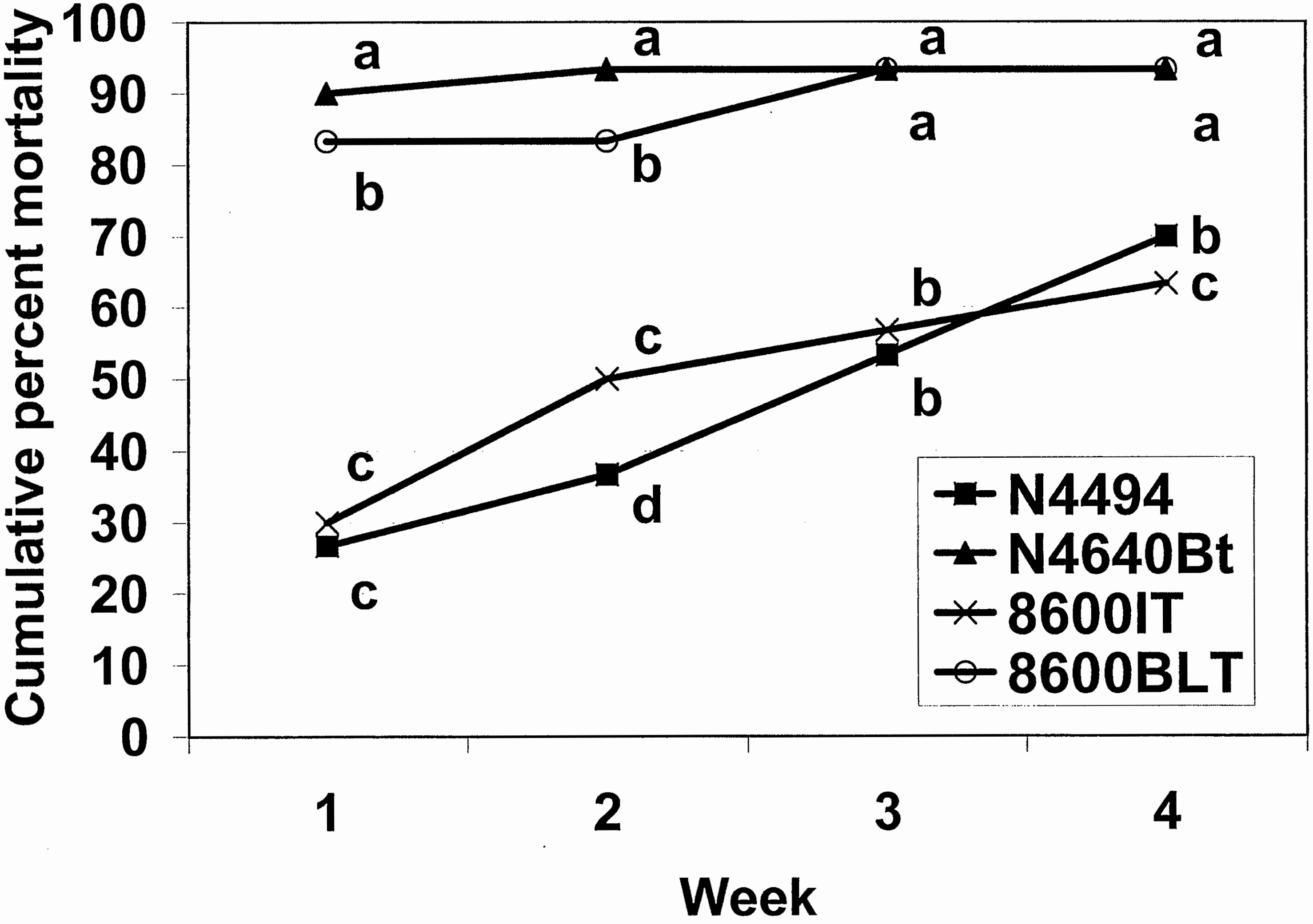

Cumulative percent mortality
100
90
80
70
60
50
40
30
20
10
0
Week
1
2
3
4
a
a
a
a
b
b
a
a
b
c
b
c
b
c
c
d
c
N4494
N4640Bt
8600IT
8600BLT

Figure 4

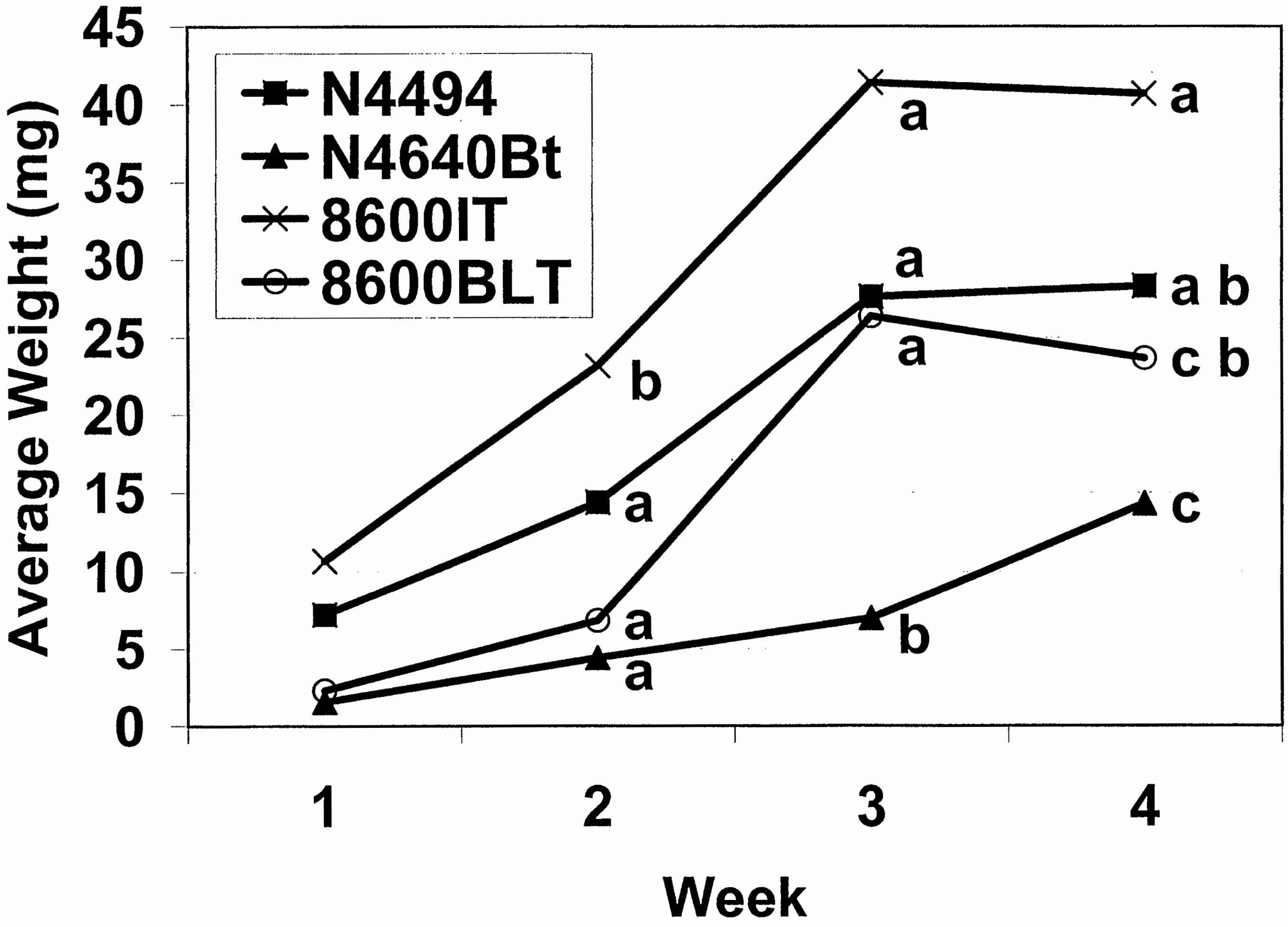
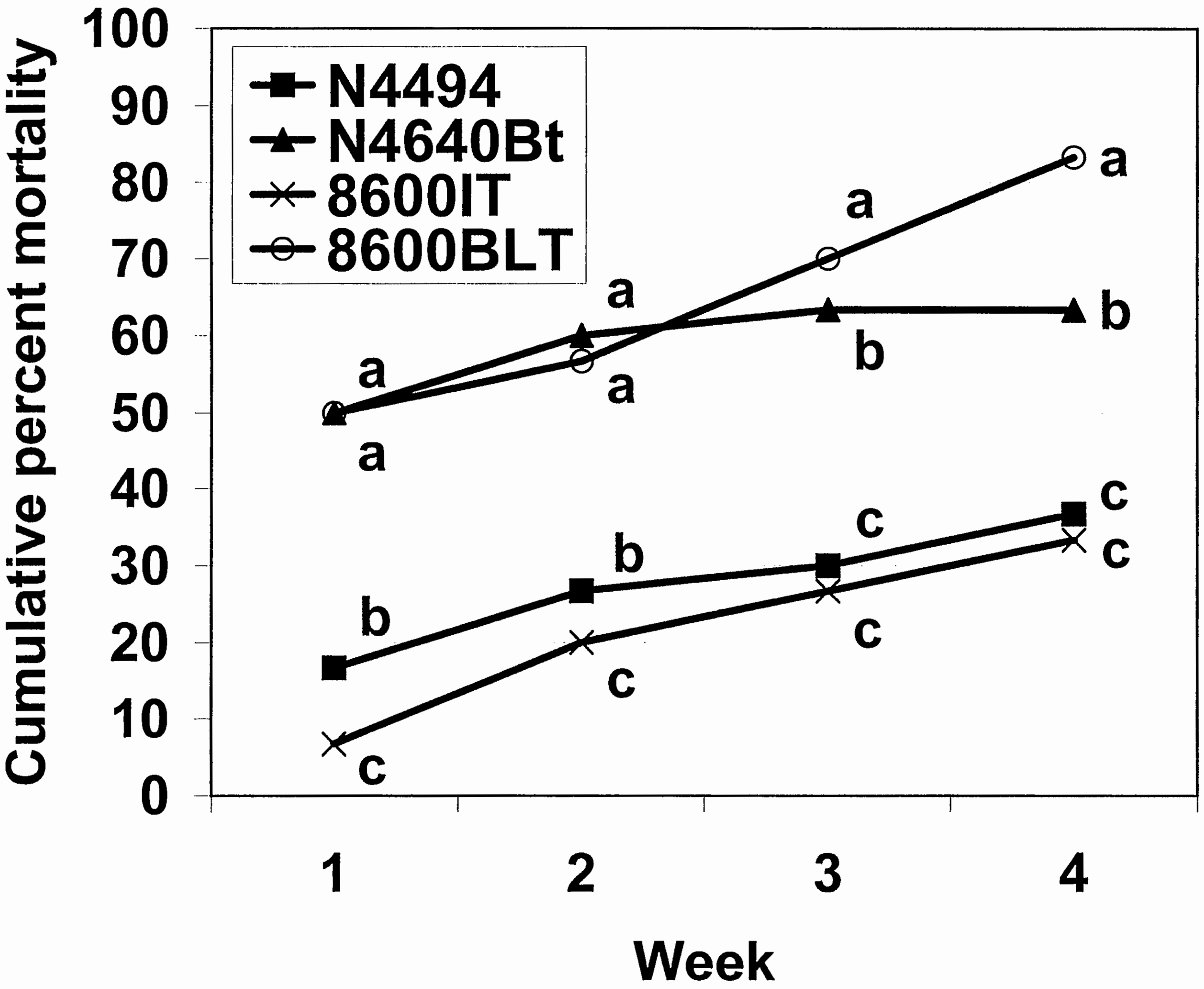
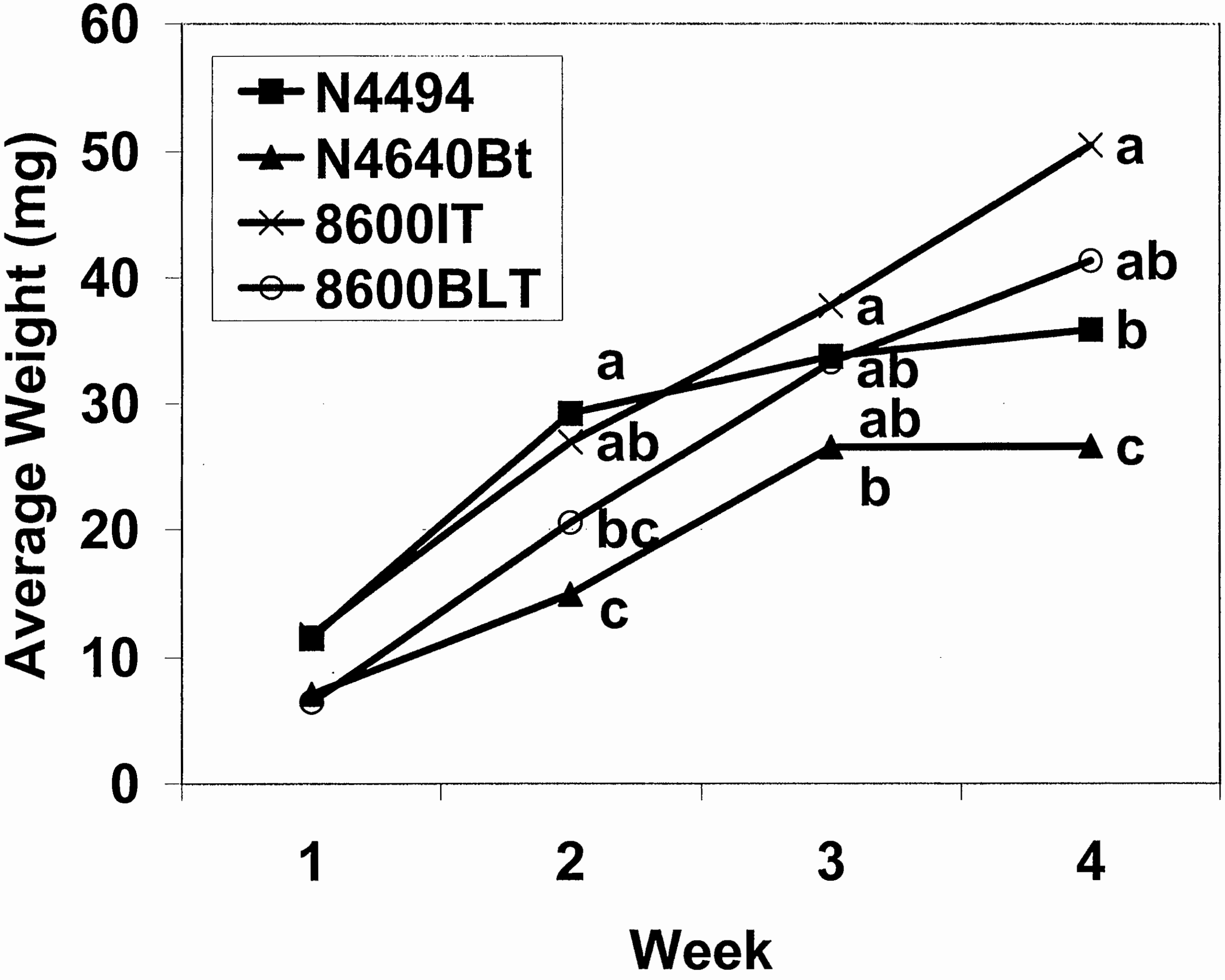

Average Weight (mg)
45
40
35
30
25
20
15
10
5
0
N4494
N4640Bt
8600IT
8600BLT
b
a
a
a
a
a
a
a
b
a
a b
c b
c
1
2
3
4
Week

Figure 5



Cumulative percent mortality
100
90
80
70
60
50
40
30
20
10
0
N4494
N4640Bt
8600IT
8600BLT
a
a
a
a
a
a
a
b
b
b
a
b
b
c
c
c
c
c
c
1
2
3
4
Week

Figure 6



60
50
40
30
20
10
0
Average Weight (mg)
N4494
N4640Bt
8600IT
8600BLT
a
ab
bc
c
a
ab
ab
b
a
ab
b
c
1
2
3
4
Week

Figure 7

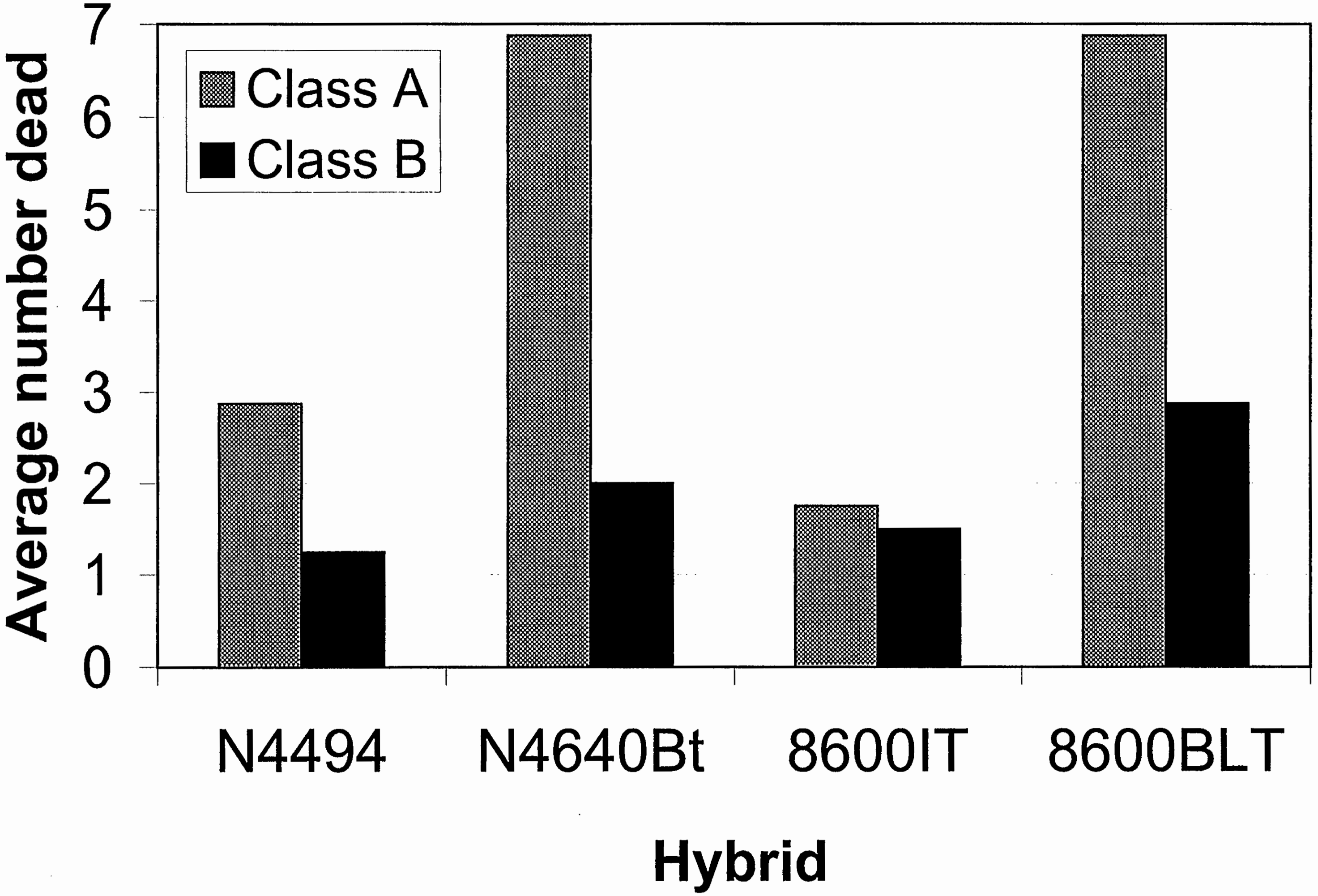

Average number dead
7
6
5
4
3
2
1
0
Class A
Class B
N4494
N4640Bt
8600IT
8600BLT
Hybrid

Figure 8

Table 1. Injury ratings (on a 6 point scale) on the final week of observation, averaged over two instar classes (A and B).

	1999		2000	
Hybrid	Mean	Range	Mean	Range
N4494	2.9	1-6	1.6	1-5
8600IT	3.1	1-6	2.4	1-6
N4640Bt	1.3	1-5	1.2	1-5
8600BLT	1.5	1-5	1.4	1-6

Table 2. Number of surviving stalk borer larvae per week on four corn hybrids, averaged over two instar classes (A and B), 1999.

	Week			
Hybrid	1	2	3	4
N4494	11.0	9.5	7.0	4.5
8600IT	1.5	1.0	1.0	1.0
N4640Bt	10.5	7.5	6.5	5.5
8600BLT	2.5	2.5	1.0	1.0

Table 3. Number of surviving stalk borer larvae per week on four corn hybrids, averaged over two instar classes (A and B), 2000.

	Week			
Hybrid	1	2	3	4
N4494	12.5	11.0	10.5	9.5
8600IT	14.0	12.0	11.0	10.0
N4640Bt	7.5	6.0	5.5	5.5
8600BLT	7.5	6.5	4.5	2.5

CHAPTER 3. REARING TECHNIQUES OF A EUROPEAN CORN BORER PARASITOID, *MACROCENTRUS CINGULUM*

A paper to be submitted to the Environmental Entomology

Rachel Binning, Marlin Rice

Abstract

This study developed an up-to-date rearing guide for the European corn borer parasitoid, *Macrocentrus cingulum* (Hymenoptera: Braconidae), using literature and observations. Techniques were modified from a previous publication and advancements in the rearing of the European corn borer were included.

Introduction

Macrocentrus cingulum Reinhard is a larval parasitoid imported to the United States for control of the European corn borer, *Ostrinia nubilalis* Hübner (Clausen et al. 1978). Its natural range extends from Europe east to Japan (Parker 1931, Clausen et al. 1978). The adult female can lay from 200–300 eggs in her lifetime, and development in the host is polyembryonic (Parker 1931). After hatching, the parasitoid larvae feed internally until it is time for them to molt to the fourth instar. As they molt, they migrate to the exterior of the host larva, where they pierce a hole in the cuticle and begin to feed. When feeding is complete, the parasitoid larvae spin individual cocoons attached to the remains of the host. If the parasitoid larvae are placed on a flat surface when they are ready to pupate, they are unable to make a cocoon, and they die (Parker 1931). The ability of the host to produce a cocoon that surrounds the parasitic larvae seems to be an important factor in the ability of the parasitoid larvae to successfully pupate (Orr et al. 1994).

According to Clausen et. al. (1978), the only host *M. cingulum* has been reared from is the European corn borer. *Macrocentrus cingulum*'s life cycle is well synchronized with that of the European corn borer, and there may be one or two annual generations that coincide with single or multiple European corn borer generations (Winnie and Chiang 1982). The peak of adult parasitoid emergence occurs around the same time as the peak of egg laying by the host (Parker 1931). The parasitoids overwinter within the host larvae as eggs.

Wishart (1946) described rearing procedures for *Macrocentrus gifuensis* (*M. cingulum*) using single generation European corn borer larvae. Although Wishart had some success in blocking the tendency of the European corn borer larvae to enter diapause, no papers have been published detailing rearing of the parasitic wasp in the lab using multiple generation corn borers, the most common corn borer in the Midwest. There also have been advancements in the rearing of the European corn borer since Wishart (Wishart 1946). Ding et al. (1989b) describe colony maintenance of *M. cingulum*, however, rearing was described only briefly in the context of their study concerning host selection behavior.

The objective of this study was to rear *M. cingulum* and develop an up-to-date rearing guide using literature and observations of the parasitoid.

Materials and Methods

The colony of *M. cingulum* was started by collecting diapausing European corn borer larvae from corn stalks in several fields in Iowa, including fields in

Allerton, Ames, Anita, Decorah, Eddyville, Houghton, Melbourne, Mount Vernon, Oelwein, Plainfield, and Rembrandt between the dates of November 9, 1999 and January 14, 2000. Larvae were collected by the USDA/ARS Corn Insect Research Unit. The larvae were held in a cold chamber and allowed to continue diapause for 1-2 more months. After diapause was broken, each larva was placed individually into a glass vial (5 cm, 1.5 cm dia.) with a 1x4 cm piece of filter paper (FISHER*brand*®, P5). A brass screen (1 mm^2 openings) was used as a plug to prevent larval dispersal, and vials were placed at an angle onto a pallet with a screened bottom to allow even light distribution throughout the growth chamber. Larvae were placed in a growth chamber at 24°C and 16:8 (L:D) hours, misted daily with distilled water, and allowed to pupate. Larvae were checked daily for signs of parasitization. Larvae that successfully pupated, showed signs of disease, or died, were removed from the growth chamber and discarded. After parasitoid pupation, the pupae were left in the growth chamber and were not misted, but observed daily for parasitoid emergence. Vials were laid horizontally in rows onto the pallet.

As the adult *M. cingulum* emerged, they were placed into 30.5 cm by 17.8 cm plastic boxes with most of the lid cut out and replaced with "No-See-Um" mesh (Balson-Hercules Group, Pawtucket, RI). The mesh was misted daily with distilled water. A cotton sugar water wick was placed inside the box with yellow tape marking it to make it more attractive to the wasps (Udayagiri et al. 1997). About 40-50 wasps were kept in a container, and each container was marked with the date of emergence. Three to four days after emergence, the females were ready to oviposit (Parker 1931).

The European corn borer larvae to be parasitized were obtained from a lab colony provided by the USDA/ARS Corn Insect Research Unit. They were fed a meridic diet (Guthrie et al. 1985) until they reached the third or fourth instar. They were then transferred to petri dishes (9 cm dia.) containing corn slices (about 1 cm thick) cut from hybrid Novartis N4494, with approximately 15-20 larvae per dish. Lids were placed on the dishes and sealed with parafilm. The parasitoids are attracted to volatiles that are given off by the corn plant or by frass when the European corn borer larvae feed on a corn plant (Ding et al. 1989a). The larvae were allowed to feed on the corn disks for at least 3 hours in an environmental chamber at 24°C and 16:8 (L:D) hours. Petri dishes were then placed without a lid into a box containing the *M. cingulum* adults for approximately 3 hours. Larvae were introduced to the parasitoids three times a day until oviposition was no longer observed or the parasitoids died.

After the European corn borers were parasitized, they were placed into a fresh dish of meridic diet with a corrugated cardboard ring to facilitate pupation (Guthrie et al. 1985). Dishes containing parasitized larvae were kept in an environmental chamber at 24°C and 16:8 (L:D) hours and observed for pupation twice a week. Once the larvae started to pupate, all were removed from the cardboard ring and the diet and placed into individual glass vials. Procedures followed to allow parasitoid pupation were the same as with field collected larvae.

Discussion

Advancements on and similarities to Wishart's (1946) original rearing paper are summarized in Table 1.

Emergence

Adult *M. cingulum* seemed most likely to emerge when the light first turns on in the environmental chamber after the eight hours of dark. Wishart (1946) observed the most adult emergence in the "forenoon." Movement of the vial containing parasitoid pupae seemed to stimulate emergence, an observation that was also made by Wishart (1946).

Adult care

If the mesh of the cage lid is always misted in the morning, the parasitoids will drink. The addition of daily misting and a sugar water wick increased adult longevity from approximately two days to nearly two weeks. Multiple generations of the parasitoid can be achieved if a continuous supply of European corn borer larvae is available. However, maintaining the colony past five or six months is difficult, possibly because of a combination of disease or lack of genetic diversity.

Parasitization

It is important to remove the sugar water wick from the cage before placing the petri dishes in, because the corn borers will leave the corn slices for the sugar water and it will be harder for the wasps to find the corn borer larvae. Place as many petri dishes into the cage as will fit without overlapping. Before opening the lid, face one side of the box toward an uncovered, closed window or a cool light source. The parasitoids are highly attracted to natural light, less to artificial light, and will gather at the end of the box by the light source (Parker 1931, Wishart 1946). It is then easy to open the opposite side of the lid and slide the petri dishes into the box. Parasitoids that escape during transfer can be collected with an aspirator, or between two fingers, and placed back into the box. After parasitization, European corn borer larvae should be immediately removed from the corn slices and placed into a fresh dish of meridic diet because they will soon exhaust their food source in the petri dish.

Following the technique outlined here will produce approximately 2.6-8.5 parasitized European corn borer larvae per hour. Each parasitized European corn borer larva will produce about 10-20 adult parasitoids (Parker 1931). Thus, approximately 26-170 adult parasitoids can be produced per hour.

Conclusions

Although the basic steps provided in this paper are similar to Wishart (1946) (Table 1), recent publications concerning the behavior and biology of *M. cingulum* as well as advancements in the rearing of the host, *O. nubilalis*, have helped to develop a more complete and up-to-date rearing guide for this important parasitoid. Significant additions include the knowledge of volatiles in European corn borer frass and the importance of these volatiles for the parasitoid to locate the host, the importance of a daily misting and the presence of a sugar water wick, how to start a colony of parasitoids, and the availability of artificial diet for European corn borer larvae.

Further research into maintaining the health of the colony and reasons for the difficulty in maintaining a colony past five or six months is needed. Making a second field collection of first generation European corn borer larvae in the summer and incorporating the resulting parasitoids into the lab colony should be investigated as a means of increasing genetic diversity and health of the colony.

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Table 1. Comparison and contrast of two methods for rearing *M. cingulum* in the lab

Wishart, 1946	Binning, 2001
	1 a. Start the *M. cingulum* colony with field collected European corn borers
	b. If necessary, break diapause, then place larvae in a glass vial with a piece of filter paper
	c. Keep *M. cingulum* colony in an environmental growth chamber at 24 degrees C, 16:8 (L:D)
	d. Mist larvae daily with distilled water until parasitoid larvae emerge and pupate
1. Put newly emerged wasps in cage and allow to mate	e. Put newly emerged wasps in a cage with a partial mesh lid and allow to mate
	2 a. Provide wasps with a sugar water wick and mist mesh daily with distilled water
2. Wait 3-4 days for females to be ready to oviposit	b. Wait 3-4 days for females to be ready to oviposit
	3 a. Feed European corn borers slices of corn in a petri dish for at least 3 hours
3. Place European corn borers into the cage with adult *M. cingulum*	b. Put dish of European corn borers into the cage with adult *M. cingulum*
	4 a. Allow 2-5 hours for parasitization
4. Remove European corn borers after parasitization is observed	b. Remove dishes containing European corn borers from the cage
	5 a. Remove European corn borers from the corn slices and place them in a fresh dish of meridic diet
	b. Continue parasitization procedure until all female wasps are dead
	c. Observe European corn borer larvae twice a week for pupation
5. Place European corn borer larvae into glass vials and feed green string beans until they stop eating	d. Once pupation is observed, remove European corn borer larvae from the diet and place in glass vials
6. Put piece of filter paper into vial to facilitate parasitoid pupation	6 Put a piece of filter paper into vial to facilitate parasitoid pupation
7. Repeat starting at step 1	7 Repeat starting at step 1c

CHAPTER 4. SUMMARY

Papaipema nebris

Corn is an economically important crop in the United States that also has many insect pests, the stalk borer and the European corn borer among them. Control of and resistance to these pests is important to corn growers to increase their annual yield.

This study indicates that Bt corn expressing genetic events Bt11 or CBH351 has a negative effect on stalk borer larvae. Although the effect is not 100% mortality, as studies have shown it to be with the European corn borer (Armstrong et al. 1995, Ostlie et al. 1997), suppression does occur. When larvae are exposed to the toxin, significant mortality occurs relative to non-Bt hybrids, and larval development is slowed, as is indicated by the reduced weight of surviving larvae on Bt corn plants. Longer development times mean longer periods of exposure to adverse weather conditions, pathogens, and natural enemies, which translates into a potential for higher larval mortality. Injury to corn plants is reduced, although not eliminated, and older corn plants had lower injury ratings over all hybrids than younger corn plants.

Younger larvae exhibited significantly higher mortality than the older larvae in all hybrids. In field conditions where weeds in the field are killed by herbicide application, the stalk borer is forced to move out of the weed stem and into corn plants sooner than usual. The presence of Bt corn in the field would result in fewer surviving larvae on young corn plants, which are otherwise less resistant to stalk borer injury than older corn plants.

Although Bt corn does not eliminate stalk borers, farmers planting Bt corn to control the European corn borer may benefit from reduced stalk borer infestations, especially if the larvae are first or second instars and the corn plants are at stage V3 or older. The reduction in numbers of stalk borer would be more significant when combining Bt corn with other management techniques, such as mowing or burning grassy areas in the spring and encouraging the presence of natural enemies.

This study shows that Bt corn can provide partial resistance to the stalk borer without requiring extra time on the grower's part to scout or spray for larvae.

Further study should be done to determine timing of corn planting and weed spraying so that the young larvae are moving into older corn. Proper timing would insure reduced injury to the corn plant and increased mortality of the stalk borer.

Macrocentrus cingulum

The European corn borer is an economically important pest of corn and other crops in the United States. There are several larval parasitoids other than *M. cingulum* that have been imported to the United States for control of the European corn borer. It is important to have a current, easy to follow guide to rearing these parasitoids so that research into the biological control of such an important pest of corn can be completed quickly and efficiently.

Although the basic steps provided in this paper are similar to Wishart (1946), recent publications concerning the behavior and biology of *M. cingulum* as well as advancements in the rearing of the host, *O. nubilalis*, have helped to develop a more complete and up-to-date rearing guide for this important parasitoid. Significant additions include the knowledge of volatiles in European corn borer frass and the importance of these volatiles for the parasitoid to locate the host, the importance of a daily misting and the presence of a sugar water wick, how to start a colony of parasitoids, and the availability of artificial diet for European corn borer larvae.

Further research into maintaining the health of the colony and reasons for the difficulty in maintaining a colony past five or six months is needed. Making a second field collection of first generation European corn borer larvae in the summer and incorporating the resulting parasitoids into the lab colony should be investigated as a means of increasing genetic diversity and health of the colony.

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