Larval feeding behaviors of the black cutworm Agrotis ipsilon on Herculex I

Nina May Richtman

Iowa State University

Follow this and additional works at: https://lib.dr.iastate.edu/rtd

Recommended Citation
https://lib.dr.iastate.edu/rtd/19032

This Thesis is brought to you for free and open access by the Iowa State University Capstones, Theses and Dissertations at Iowa State University Digital Repository. It has been accepted for inclusion in Retrospective Theses and Dissertations by an authorized administrator of Iowa State University Digital Repository. For more information, please contact digirep@iastate.edu.
Larval feeding behaviors of the black cutworm *Agrotis ipsilon* on Herculex™ I

by

**Nina May Richtman**

A thesis submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Entomology

Program of Study Committee:
Jon Tollefson, Major Professor
Bryony Bonning
Robert Hartzler
Stephen Lefko

Iowa State University
Ames, Iowa
2006

Copyright © Nina May Richtman, 2006. All rights reserved.
Graduate College
Iowa State University

This is to certify that the master's thesis of

Nina May Richtman

has met the thesis requirements of Iowa State University

Signatures have been redacted for privacy
"Follow your bliss."
-Joesph Campbell, The Power of Myth

I dedicate this to all those who have enabled me to follow my bliss.
# TABLE OF CONTENTS

## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>vi</td>
</tr>
</tbody>
</table>

## LIST OF TABLES

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>viii</td>
</tr>
</tbody>
</table>

## CHAPTER 1. General Introduction

- Thesis Organization
- Literature Review
  - *Agrotis ipsilon* in corn
  - Oviposition
  - Feeding preferences
  - Damage
  - Herculex™ I for control of *Agrotis ipsilon*
  - Pathogens for control of *Agrotis ipsilon*
- Rationale
- References

## CHAPTER 2. Feeding of the black cutworm *Agrotis ipsilon* (Lepidoptera: Noctuidae) larvae on Herculex™ I with and without an alternative host

- Abstract
- Introduction
- Materials and Methods
  - Field studies
  - Greenhouse studies
  - Analysis
- Results
- Discussion
- Acknowledgements
- References Cited
CHAPTER 3. Susceptibility of the black cutworm (Lepidoptera: Noctuidae) to Herculex™ I and Agrotis ipsilon nucleopolyhedrovirus

Abstract 32
Introduction 32
Materials and Methods
   Insects, plants, virus 34
   Effects of Cry1F on larval weight 35
   Effects of Cry1F with virus exposure 35
Results
   Effects of Cry1F on larval weight 36
   Effects of Cry1F with virus exposure 36
Discussion 37
References Cited 39

CHAPTER 4. General Conclusions 45
   Future research 45

ACKNOWLEDGEMENTS 47

APPENDIX. Ethovision black cutworm trials 48
LIST OF FIGURES

CHAPTER 2.

Figure 1. Field design, showing individual barrier size. Bold dashed lines in the center of the plots represent treatment rows of Herculex™ I or isoline. Outer dashed lines represent the rows of alternative host plants.

Figure 2. Mean percent of cut corn plants in the field with treatments of Herculex™ I (HX), Herculex™ I with weeds removed (HXWR), isoline (IS) and isoline with weeds removed (ISWR) at the time of weed removal. Bars indicate percentage of cut plants. Lines with each bar indicate standard errors.

Figure 3. Mean percent of cut corn plants in the field with treatments of Herculex™ I (HX), Herculex™ I with weeds removed (HXWR), isoline (IS) and isoline with weeds removed (ISWR) at the time of weed removal and at the end of the experiment. Bars indicate percentage of cut plants. Lines with each bar indicate standard error at the end of the experiment.

Figure 4. Difference in percentage of cut corn plants in the field between the time of weed removal and the end of the experiment with treatments of Herculex™ I (HX), Herculex™ I with weeds removed (HXWR), isoline (IS) and isoline with weeds removed (ISWR). Bars indicate percentage of cut plants.

Figure 5. Mean percent of cut corn plants in the greenhouse with treatments of Herculex™ I (HX), Herculex™ I with weeds removed (HXWR), isoline (IS) and isoline with weeds removed (ISWR) at the time of weed removal. Bars indicate percentage of cut plants. Lines with each bar indicate standard error.

Figure 6. Mean percent of cut corn plants in the greenhouse with treatments of Herculex™ I (HX), Herculex™ I with weeds removed (HXWR), isoline (IS) and isoline with weeds removed (ISWR) at the time of weed removal and at the end of the experiment. Bars indicate percentage of cut plants. Lines with each bar indicate standard error at the end of the experiment.

Figure 7. Difference in percentage of cut corn plants in the greenhouse between the time of weed removal and the end of the experiment with treatments of Herculex™ I (HX), Herculex™ I with weeds removed (HXWR), isoline (IS) and isoline with weeds removed (ISWR). Bars indicate percentage of cut plants.
CHAPTER 3.

Figure 1. Difference in black cutworm larval weight gain between those fed Herculex™ I and isoline for 7 days. Bars with different letters represent values that are significantly different from one another (P < 0.01).

Figure 2. Distribution of weight differences in black cutworm larvae fed for 7 days on Herculex™ I or isoline.

Figure 3. Percent mortality by AgipMNPV for black cutworm larvae on treatments of Herculex™ I or isoline diets. Lines with each bar indicate standard errors. Bars with different letters represent values that are significantly different from one another.

APPENDIX.

Figure 1. Ethovision tracks of larval movement on 3 treatments: No food, Herculex™ I, isoline.
LIST OF TABLES

CHAPTER 2.

Table 1. Mean proportion of cut corn plants in the field at the time of weed removal (A) and at the end of the experiment (B) and standard error (lower, upper).

Table 2. Mean proportion of cut corn plants in the greenhouse at the time of weed removal (A) and at the end of the experiment (B) and standard error (lower, upper).
CHAPTER 1. General Introduction

Thesis Organization

This thesis is organized into four chapters. Chapter 1 includes a general introduction and literature review of *Agrotis ipsilon* ovipositional and feeding behaviors, Herculex™ I for control of *A. ipsilon*, pathogens of the *A. ipsilon*, and *Bacillus thuringiensis* and baculovirus combinations. At the end of the introduction is an explanation of the rationale behind this research. The literature review and rationale are followed by a list of the cited literature. Chapter 2 is a study of larval feeding behaviors of *A. ipsilon* on Herculex™ I, with and without the presence of an alternative food source. Chapter 3 describes a study of *A. ipsilon* larval susceptibility to *A. ipsilon* nucleopolyhedrovirus following feeding on Herculex™ I. The general conclusions of the thesis are presented in Chapter 4.

Literature Review

*Agrotis ipsilon* in corn

The black cutworm, *Agrotis ipsilon* (Hufnagel), is classified in the order Lepidoptera, family Noctuidae. The black cutworm feeds on a wide variety of cultivated plant and grass species, but is particularly damaging to corn, *Zea mays* L. (Busching and Turpin 1977). Prior to the 4th instar, black cutworm larvae feed on corn plant foliage. The damaging stage of the black cutworm is from the 4th-6th instar. Larvae at these stages cut or tunnel into the stem disrupting xylem or phloem systems, which causes serious damage to the young corn plant (Showers et al. 1983). When the plant is cut at or below the growing point, yield loss can result due to growth stunting or death of the plant (Whitford et al. 1989). Black cutworm infestations are sporadic and difficult to predict and population densities vary yearly (Archer and Musick 1977).
Oviposition

Adult black cutworm moths migrate to Iowa in the spring traveling on northbound airflows (Sherrod et al. 1979). Female moths oviposit on crop debris and weeds in or adjacent to crop fields. The following are characteristics of fields susceptible to black cutworm infestation: previous history of black cutworm infestations, permanent vegetation adjacent to the field (Sherrod and Luckmann 1979), weed cover prior to tillage in the spring (Busching and Turpin 1977) and poor drainage (Schuster and Boling 1973). Minimum tillage practices, allowing growth of annual and perennial weeds, provide necessary oviposition sites for female black cutworm (Busching and Turpin 1977). Busching and Turpin (1976) found that of 14 weed species tested, curled dock, *Rumex crispus* L. and yellow rocket, *Barbarea vulgaris* R. Br., were most attractive to black cutworm females for oviposition. Corn and soybeans, *Glycine max* (L.), were among the least attractive. Of debris evaluated, fence row pasture debris was most preferred.

Feeding preferences

Early instar black cutworms feed on many different hosts. Busching and Turpin (1977) tested black cutworms from neonate to pupation on 16 larval food types and found that survival was highest on bluegrass, *Poa pratensis* L., curled dock and wheat, *Triticum aestivum* L. None of the larvae survived on giant foxtail, *Setaria faberi* Herrm. or solely on debris. One of the 16 food types tested was corn, which black cutworms were able to survive on but with low rates of pupation (30%). Once eggs have hatched, larvae feed on these hosts at the ovipositional site (Showers et al. 1982). When alternative hosts are eliminated by tillage or herbicide, black cutworm larvae will migrate to nearby corn seedlings if adjacent to the alternative host (Mulder and Showers 1983).

The removal of weeds adjacent to a corn field can play an important role in prevention of black cutworm damage. Showers et al. (1985) showed that tillage 8-14 days prior to planting significantly reduces black cutworm damage. Weed removal 14 days before planting provides sufficient time for weeds to dry and become insignificant as a food source (Showers et al. 1982). Weed removal, by herbicide or tillage, 2 days before planting, on
planting day or 2 days after planting was not effective because larvae were able to survive in the debris (Showers et al. 1985). Engleken et al. (1990) showed that if weed removal is delayed until corn has at least reached the second leaf stage (V2), there will be significantly less damage than weed removal prior to this stage. Weed removal should be planned based on cutworm development. Removal at the improper time in the black cutworm lifecycle can actually be detrimental for corn if larvae switch hosts as a result of weed removal. In addition, Engelken et al. (1990) found that higher weed populations led to higher damage in corn when removed if corn was at the 2 leaf stage or smaller.

**Damage**

The black cutworm has multiple generations per year, but only the first generation is damaging to corn due to the coincidence of the insect and plant development. Later generations are not damaging because black cutworm larvae are not capable of cutting corn plants after the V4 stage (Whitford et al. 1989, Archer and Musick 1977). Black cutworm damage declines as the plant stage increases (Showers et al. 1983, Archer and Musick 1977, Clement and McCartney 1982, Levine et al. 1983). Studies have shown that coleoptile and 2 leaf-stage corn suffers far greater damage than 4 leaf stage (V4) (Mulder and Showers 1983). Levine et al. (1983) showed that plants cut lower on the stem and later in the growth stage (V1-V4 stage) will produce fewer ears and less grain than plants damaged higher up and in the coleoptile stage. In addition, the later instars cause a greater amount of damage to the plant (Clement and McCartney 1982). This study also showed that, in the greenhouse, 5th and 6th instar black cutworm cut and consume a seedling before another was cut. The larger the plant based on leaf stage, the more common the occurrence of this behavior.

**Herculex™ I for control of Agrotis ipsilon**

Transgenic corn plants modified to express insecticidal proteins from varieties of *Bacillus thuringiensis* have revolutionized Lepidopteran pest control in recent years. One commercially available insect control technology, Herculex™ I (Pioneer Hi-Bred International Inc., Johnston, IA) produces Cry1F protein from *Bacillus thuringiensis* var. *azawai* to protect against multiple Lepidopteran corn pests, including the black cutworm.
Host plant resistance mechanisms can be used to explain the plant ability to deter insect feeding. Antixenosis, also referred to as non-preference, is characterized by insect inability to colonize. Antibiosis is based on insect susceptibility to plant defenses, which can cause lower weights at all stages, increased time to maturity, reduced fertility and/or death (Panda and Khush 1995). These mechanisms will be used to explain the ability of Herculex™ I to resist feeding and cutting by black cutworms.

Pathogens for control of Agrotis ipsilon

Insect pathogens can offer alternatives to insecticides for insect control. Ignoffo and Garcia (1979) identified 7 viruses, 5 bacteria, 2 fungi and 1 protozoan of the 63 they tested that caused 30% or greater mortality in Agrotis ipsilon. Johnson and Lewis (1982) investigated Rachiplusia ou nucleopolyhedroviruses (RoMNPV) and Autographa californica nucleopolyhedroviruses (AcMNPV) for control of the black cutworm. RoMNPV and AcMNPV are classified in the family Baculoviridae in the genus Nucleopolyhedrovirus. Nucleopolyhedroviruses are highly specialized with restricted host ranges, usually only affecting a single species or a few species within a genus (Hunter-Fujita et al. 1998). Early instars of black cutworms infected by these viruses, through ingestion of bait formulation, caused less cutting damage in the greenhouse than the control. Black cutworms exposed to the high dose of virus did less damage to corn seedlings than those exposed to the low virus dose. In the field, significantly fewer plants were cut in plots treated with virus compared to untreated. The virus caused death or growth stunting, allowing the plants to grow large enough to avoid further cutworm damage (Johnson and Lewis 1982).

Agrotis ipsilon multicapsid nucleopolyhedrovirus (AgipMNPV) was identified in 1999. AgipMNPV is also classified in the family Baculoviridae in the genus Nucleopolyhedrovirus. The black cutworm is highly susceptible to infection by the virus, much more susceptible than to AcMNPV or RoMNPV (Boughton et al. 1999). The corn earworm, Helicoverpa zeae (Boddie), and the tobacco budworm, Heliothis virescens (Fabricius), also were shown to be somewhat susceptible to AgipMNPV but only at much
higher doses. For first instars, the LC$_{50}$ for _A. ipsilon_ is 269 POBs/µl, 797 POBs/µl for _H. virescens_ and 7083 POBs/µl for _H. zea_. (Boughton et al. 1999).

_AgipMNPV, AcMNPV_ and _RoMNPV_ affect the larval stage of the black cutworm. The virus must be ingested by the host for infection to occur. The high pH of the insect’s midgut degrades the polyhedron protein and releases virions which bind to the microvilli of the epithelial cells (Tanada and Kaya 1993). Virions travel to the cell nucleus where they will uncoat and release DNA for replication (Federici 1997). Newly produced virions spread to other cells and replicate. Infection causes cells to burst and release virus particles into the hemocoel and eventually into the environment (Blissard and Rohrmann 1990).

*Bacillus thuringiensis* (*Bt*) and baculoviruses have been evaluated for combined use to improve crop protection. Lipa et al. (1976) found a synergistic effect on _Spodoptera exigua_ (Hübner), the beet armyworm, when _Heliothis armigera_ (Hübner) nucleopolyhedrovirus (*HaNPV*) was combined with _Bt_ var. _kurstaki_ (*Dipel*). In 1981, Matter and Zohdy found the combination of _Bt_ var. _thuringiensis_ (bactospeine) and nucleopolyhedrovirus on _H. armigera_, the American bollworm, to be both synergistic, in some instars, and additive when the dosage for bactospeine was at the LC$_{50}$. An additive effect was observed when _Trichoplusia ni_ (Hübner), the cabbage looper, larvae were exposed to _T. ni_ nucleopolyhedrovirus and _Bt_ var. _kurstaki_ (*Dipel*) at high doses of LC$_{45}$ or greater (McVay et al. 1977, Young et al. 1980). Bell and Romaine (1980) found the highest cotton yield when the virus *AcMNPV* was combined with _Bt_ and an adjuvant as compared to the virus or virus + adjuvant alone for protection against _Heliothis virescens_, the tobacco budworm. Peters and Coaker (1993) found that _Pieris brassicae_ (L.) was more susceptible to _P. brassicae_ granulosis virus (*PbGV*) when combined with a microbial insecticide, _Bt_ var. _kurstaki_. When _Helicoverpa zea_ nucleopolyhedrovirus (*HzSNPV*) (*Gemstar* LC) was combined with _Bt_ cotton an additive mortality response was observed in _H. zea_ (Streett and Mulrooney 2000). Larvae of the fall armyworm, _Spodoptera frugiperda_ (J.E. Smith), that were reared on either a diet of sweet corn expressing Cry1A(b) toxin from _Bt_ var. _kurstaki_ or a non- _Bt_ diet and then exposed to equal dosages of _S. frugiperda_ nucleopolyhedrovirus
(SfMNPV), had mortality levels that were significantly higher for those fed the diet containing Bt (Farrar et al. 2004).

Some research has shown less effective results when Bacillus thuringiensis and baculoviruses are combined. Oatman et al. (1970) found that a combination Bt (BTB dust) and nucleopolyhedrovirus was less effective than the virus alone in H. zea. Lutrell et al. (1982) showed that the mortality of H. zea and H. virescens were greater when HzSNPV and AcMNPV were combined with Bt than mortality from Bt alone, but this combination was not greater than the virus alone. In Pingel and Lewis (1999), Bt from three different products (HD-1-S-1980, Javelin®, Xen-tari®) and Anagraphe falcifera multiple nucleopolyhedrovirus (AfMNPV) were tested against three corn ear pests: H. zea, Ostrinia nubilalis (Hübner) and S. frugiperda. Mixtures of the microbes showed either an equal or lower mortality rate than either pathogen alone.

Rationale

The following projects were designed to further research the feeding behaviors of Agrotis ipsilon on Herculex™ I. In preliminary research, larvae had lower larval weighs after exposure to Herculex™ I, but quickly recovered lost weight when placed on non-Herculex™ I corn. Larvae also spent more time wandering in Herculex™ I corn and less time feeding (Lefko 2003, personal communication). Due to the apparent lack of toxicity and also lack of feeding and infrequent cutting, the black cutworm/Herculex™ I interaction was classified as a combination of antixenosis and antibiosis. Antixenosis was the suspected mechanism of protection in the presence of an alternative food source. With no alternative, antibiosis was suspected to be the active mechanism of protection. Research reported in this thesis was conducted to assess the mechanism of protection and to determine how the mechanisms are affected by other variables, such as alternative food sources and pathogens.
Objectives

1. To determine if the combination of mechanisms observed in preliminary studies were effective enough to deter feeding on Herculex™ I after alternative host removal.

2. To examine if the black cutworm is more susceptible to *Agrotis ipsilon* nucleopolyhedrovirus in Herculex™ I than in the isoline.
LITERATURE CITED


CHAPTER 2: Feeding of black cutworm *Agrotis ipsilon* (Lepidoptera: Noctuidae) larvae on Herculex™ I with and without the presence of an alternative host

Abstract

Research conducted by Pioneer Hi-Bred International Inc. showed significantly lower levels of black cutworm *Agrotis ipsilon* (Hufnagel) feeding and cutting on Herculex™ I (Cry1F) as compared to the isoline control. The mechanism of protection was described as a combination of black cutworm antibiosis and antixenosis. The objective of this study was to determine any effect that alternate host availability may have on the damage potential of black cutworms in Herculex™ I corn. The study was designed to determine if the combination of mechanisms observed in Pioneer’s studies were effective enough to deter feeding on Herculex™ I after alternative hosts were removed. The research was conducted in field and greenhouse experiments, and the alternative weed host was conventional corn (Pioneer 34M94). Treatments consisted of two corn types (Herculex™ I or its non-transformed isoline) with and without alternate host removal. Plants were infested with 3rd instar black cutworms at the V1 or V2 stage. Plants were sampled daily for cutting or tunneling above and below the soil surface. Weeds were removed at the V2 or V3 stage. The experiment concluded when all corn plants were cut or when plants reached the V4 stage. Analysis showed significant differences between isoline and Herculex™ I. No significant difference in Herculex™ I treatments with and without weed removal was recorded in the field experiments. In the greenhouse after weed removal, significantly more Herculex™ I plants without alternate hosts were cut than when the weed hosts remained.

Introduction

Black cutworm *Agrotis ipsilon* (Hufnagel) infestations can be very damaging to seedling corn in Iowa. Adult moths migrate to Iowa each spring by traveling on northbound airflows (Kaster and Shower 1982, Domino et al. 1983). No-tillage farming practices, allowing growth of annual and perennial weeds and presence of crop debris, provide necessary oviposition sites for female black cutworm (Sherrod et al. 1979). Damage done by black cutworm larvae consists of leaf and stem feeding before the 4th instar and cutting of
corn during the 4th-7th instars (Archer and Musick 1977). Stand reduction by black cutworm occurs when a corn plant is cut at or below the growing point (Whitford et al. 1989). Damage by the black cutworm declines as the plant stage increases (Showers et al. 1983, Archer and Musick 1977, Clement and McCartney 1982, Levine et al. 1983). Also the later the instar, the greater the amount of damage it can inflict on the plant (Clement and McCartney 1982).

When alternative weed hosts are removed, black cutworm larvae are more likely to feed on corn seedlings (Mulder and Showers 1983). Tillage of weeds 8-14 days prior to planting significantly reduces damage to seedling corn by eliminating food sources between weed removal and corn emergence (Showers et al. 1985). Weed removal by herbicide application or tillage 2 days before planting, on the day of planting or 2 days after planting was not effective because larvae were able to survive in the debris until corn emergence. Engleken et al. (1986) showed that if weed removal is delayed until corn has reached at least the V2 stage, there will be significantly less damage than if weed removal was done earlier.

Transgenic corn plants modified to express insecticidal proteins from Bacillus thuringiensis (Bt) have revolutionized Lepidopteran pest control in recent years. One commercial insect control trait, Herculex™ I (Pioneer Hi-Bred International Inc., Johnston, IA) produces Cry1F protein to protect against multiple Lepidopteran corn pests. In preliminary research conducted by Pioneer Hi-Bred International Inc., black cutworm showed significantly less feeding and cutting on Herculex™ I than on the isoline control without the Bt gene (Lefko 2003, personal communication).

A combination of antibiosis and antixenosis are the suspected mechanisms of protection in Herculex™ I. In a bioassay comparing weights of 3rd instar black cutworms after 7 days on isoline and Herculex™ I, weights of those on Herculex™ I were significantly lower than those on isoline (F= 194.93, df=7, 148, P <0.0001) (Chapter 3, Unpublished data). This difference in weight would suggest antibiosis as the active mechanism. In this bioassay, Herculex™ I appeared to be non-lethal to cutworms that fed on it. Although some cutworm mortality was observed during the bioassay, this was likely due to starvation/dehydration
rather than toxic effects of Herculex™ I since mortality was observed in both treatments. In preliminary research by Pioneer Hi-Bred International Inc., Herculex™ I appeared to be only moderately toxic to black cutworms that fed on it and larvae quickly recovered lost weight when placed on non-Herculex™ I corn (Lefko 2003, personal communication). Antixenosis may only be expressed in the presence of an alternative food source. With no alternative host available, antibiosis may become the more active mechanism. The objective of this study was to determine the effects of alternative weed host availability on the damage potential of black cutworms in Herculex™ I corn. The study was designed to investigate if the antixenosis observed in preliminary studies was sufficient to deter feeding on Herculex™ I even after removal of alternative weed hosts.

Materials and Methods

Field studies.

2004. Field studies were conducted at the Iowa State University (ISU) Johnson Farm near Ames, Iowa. Trials were run in late May to early June and late August to early September of 2004. The May to June time period would be the typical time for black cutworm infestations in Iowa. The August to September time period was chosen for the second repetition since the photoperiod would be similar to that of May to June.

The trial had a split plot design consisting of main treatments of two corn types, Herculex™ I (Pioneer® 34M93) and isoline (Pioneer® 34M92), and sub-treatments of weed removal and no weed removal. There were four repetitions of each treatment combination per trial. The alternative weed host used was conventional corn (Pioneer® 34M94). Corn was used as a weed host because it allowed for uniform emergence between isoline or unprotected corn, Herculex™ I corn and the weed host, density could be controlled and it is a recognized host for black cutworm for third instars and greater. Herculex™ I, isoline and weed seeds were obtained from Pioneer Hi-Bred International, Inc. (Johnston, IA). All seeds were hand planted on May 8 and August 31. Center row seeds were spaced 10 cm apart resulting in 18 seeds planted per row. One weed host row was planted on either side of the Herculex™ I or isoline corn rows in each plot. In May, side row weed seeds were planted 5
cm apart resulting in approximately 36 seeds per row. In August, the experiment was modified and seeds were planted 10 cm apart. Sixteen galvanized steel barriers (70 cm x 23 cm x 1.8m) were supplied by Dow AgroSciences, Indianapolis, IN (Huxley Research Station, Huxley, IA). Each barrier surrounded the three rows of plants per plot (Figure 1). The edges of the barriers were coated with Tanglefoot™ (The Tanglefoot Company, Grand Rapids, MI) to prevent black cutworm escape.

Third-instar black cutworms were obtained from the USDA-ARS Corn Insect and Crop Genetics Research Unit (Ames, IA). Plots were infested when corn reached V2 stage (Ritchie et al.1997), approximately 15 days after planting. Stand counts of corn and weeds were taken at the time of infestation. Each plot was infested with 2 black cutworms per center row plant (approximately 36 per plot) at 1 hour before sunset.

All plants were sampled daily for cutting. Cutting was classified as stem cutting above or below the soil surface or tunneling that affects xylem and phloem systems (Showers 1983). Any plant which had wilted leaves due to a visible cut stem, significant tunneling damage or below ground stem damage was classified as a cut plant. These plants were marked by a wood stake. The average vegetative stage for each plot also was recorded daily.

In May/June trials, glufosinate (Liberty®, Bayer Crop Science, Research Triangle Park, NC) was applied to all treatments requiring weed removal at the rate of 0.087 kg active ingredient per hectare to V3 corn using a hand sprayer. Herculex™ I and isolate treatments contained Liberty Link® providing resistance against plant damage by glufosinate. In August/September trials, the procedure was changed due to slow speed of kill by glufosinate. All weeds were removed by hand 7 days after infestation at approximately V3 corn stage. Plants were pulled out at the soil surface to remove the growing point. The experiment concluded when corn plants had reached the V4 stage.

A lack of cutting was seen in the August trials. This was likely due to the fast growth rate of the corn due to higher temperatures in August and September.
2005. Field studies were repeated in 2005 at ISU Johnson Farm in Ames, Iowa. Changes were made in the protocol due to absence of cutting and feeding after weed removal in 2004. Trials were run in May, June and July. August trials were eliminated in 2005 due to lack of cutting in 2004. The overall project design was identical to the design in 2004. Herculex™ I, isoline and weeds were planted on May 9, June 7 and July 6. Seed spacing and numbers were the same as August of 2004 trials. Barriers coated with Tanglefoot™ were used in all trials in 2005.

Late third-instar and early fourth-instar black cutworms were obtained from the USDA-ARS Corn Insect and Crop Genetics Research Unit (Ames, IA). In 2005, plots were infested when corn reached V1 stage, approximately 12 days after planting. This was changed from 2004 since the literature shows that larvae have higher rates of cutting in corn plants with a lower vegetative stage (Mulder and Showers 1983). Stand counts of corn and weeds were taken at the time of infestation. Plots were infested with 4 black cutworm larvae per center row plant at 1 hour before sunset. The rate was increased from 2004 in hopes of increasing overall percentage of cut plants. Weeds were removed earlier in 2005. Plants were pulled out by hand at the V2 stage. Experiments concluded when plants reached the V4 stage.

Sampling methods used in 2005 were the same as those in 2004. Damage counts from the time of weed removal and at the end of the trial were used for comparison in the analysis.

Greenhouse studies.

The field-experiment design was replicated in the greenhouse, using 30 gallon bins as arenas. Trials were conducted in May, June and July of 2005. There were three repetitions of each of the four treatment combinations per trial. A split plot design was used and treatments were the same as in the field trials. Treatments were randomly assigned to bins within the greenhouse. In each bin, five seeds of Herculex™ I or isoline were planted in the center row and 5 weed seeds were planted on either side. Plants were grown in professional growing mix (Sunshine; Sun Gro Horticulture, Vancouver, British Columbia). The edges of the bins were coated with Tanglefoot™ to prevent black cutworm escape.
At V1 stage (12-16 days after planting), two 3rd instar larvae per center-row plant were added to each bin. Stand counts for plants were recorded at the time of infestation. After 2-3 days, when plants were at approximately the V2 stage, all weeds were removed by hand from treatments with weed removal. The vegetative stage and total number of plants cut in the center and side rows were recorded daily. Trials concluded when all plants in the center row of a container were cut, which was at approximately the V3 stage. Damage counts from the time of weed removal and the conclusion of the experiment were used for analysis.

Analysis.

Field and greenhouse data were analyzed separately. The number of cut plants, from 3 replicates of each experiment, were pooled and analyzed using PROC logistic to determine differences between treatments (SAS v. 9.1). Logistic regression was used because the data were proportions and large variability in the number of plants in the center row stand count per experimental unit (11-19 plants for the field plots and 3-5 plants in the greenhouse) would make normal approximation unsatisfactory. Comparisons were made between treatments on the day of weed removal and at the end of the experiment. The difference in number of cut plants between observations, on the day of weed removal and the end of the experiment, were also compared.

Results

Cutting differences between isoline and Herculex™ I treatments were significant at the time of weed removal and the end of the experiment (P <0.0001 and P <0.0001) (Figure 2 & 3, Table 1). A comparison of cutting rates in Herculex™ I with and without weeds and before and after weed removal was used to determine the effects of alternative weed host availability on black cutworm damage to Herculex™ I. No differences in cutting were found in Herculex™ I with and without weeds before or after weed removal (P= 0.9585, P= 0.8708). Prior to weed removal, the mean percentage of cut Herculex™ I plants with and without weeds was the same at 2.7%. After weed removal, 5.3% of plants in the Herculex™ I plots without weed removal had been cut compared to 4.9% of plants in the Herculex™ I plots with weed removal (Figure 2 & 3, Table 1). To compare the percentage of cut plants
between Herculex™ I corn and unprotected corn between the time of weed removal and the end of the experiment, the mean proportion of cut plants for each treatment at the time of weed removal (Table 1: Mean proportion 1) must be subtracted from by the mean number at the end of the experiment (Table 1: Mean proportion 2) and then multiplied by 100 to make the proportion into a percent. After completion of this calculation, a significant difference can be seen between the Herculex™ I and isoline treatments, although there is no significant difference between the two Herculex™ I treatments (2.6% no weed removal and 2.2% with weed removal) or the two unprotected corn treatments (5.9% no weed removal and 5.9% with weed removal) (Figure 4).

In the greenhouse analysis, a significant difference between isoline and Herculex™ I treatments was seen at the time of weed removal (P <0.0001) and at the end of the experiment (P <0.0001) (Figure 5 & 6, Table 2). In comparing the isoline treatments, the effect of weed removal increased cutting by 22.9%. In the isoline with weed removal, the percentage of cut plants increased by 52.2% between the time of weed removal and the end of the experiment (Figure 7, Table 2: Mean proportion 2-Mean proportion 1 * 100). During the same interval, there was a 29.3% increase in cut plants in the isoline where no weeds had been removed (Figure 7, Table 2: Mean proportion 2-Mean proportion 1 * 100). These two percentages were significantly different (P=0.0332).

In the greenhouse no significant difference was seen between Herculex™ I with and without weeds prior to any weed removal (P=0.1747). Numerical differences in cutting were observed; 4.8% of plants had been cut in Herculex™ I treatments that would be without weed removal, and 13.9% of the plants had been cut in the Herculex™ I treatment where weeds were scheduled to be removed. A significant difference was found between numbers of cut plants when comparing the Herculex™ I treatments at the end of the experiment (P=0.0523); however, this difference is confounded by cutting that occurred prior to weed removal (Figure 5 & 6, Table 2)
The effect of weed removal on cutting rates in Herculex™ I was estimated with the same formula that was used for isoline. In Herculex™ I, the effect of weed removal increased cutting by 12.2%; however, this effect was not significant (P=0.2580). An average of 48.8% of the plants were cut between the time of weed removal and the end of the experiment compared to 36.6% plants cut during the same interval in Herculex™ I without weed removal (Figure 7). These percentages are not significantly different from one another (P= 0.2580). Large differences in cutting rates at the time of weeding provides explanation for the significant difference between Herculex™ I with and without weeds at the end of the experiment.

Discussion

The data from the field and greenhouse studies show a significant difference in percentage of cut plants in unprotected isoline corn and Herculex™ I corn regardless of the presence of weeds, which supports the preliminary studies. In the field study, the effect of weeding in the isoline control did not have a significant effect on the percentage of cut plants. This absence of a significant difference after the weed removal shows that the black cutworm cutting pressure was not high enough in the field.

The rate of infestation was lower in the greenhouse than in the field (2 vs. 4 cutworms per center row plant), but the number of cut plants per black cutworm larva were much higher in both the isoline and the Herculex™ I treatments. In the greenhouse trials, a significant difference in cut isoline corn was found between the time of weeding and the end of the experiment. Since the cutworm pressure was high the expected difference in isoline treatments was measurable, in contrast to the field where no difference was measured.

One of the factors that may have resulted in the differences seen in the greenhouse versus the field was temperature. Temperatures (≥ 29° C) in the greenhouse were higher than the field temperatures would have been at an equivalent date. Higher temperatures would encourage faster larval growth and later instars would have greater cutting potential. Greenhouse corn and field-grown corn at identical leaf stages tend to be structurally
different; greenhouse plants are more spindly with thinner leaves. Due to this difference, larvae in the greenhouse may have had increased success in cutting plants because plants were less robust. Also, the greenhouse lacks external factors such as wind, rain, storms, and natural enemies, which likely increased black cutworm viability inside and explained the loss of larvae in the field. These factors could be the explanation of the higher rates of cutting in the greenhouse in spite of lower infestation rates.

Acknowledgements
This research was supported in part by a grant from Pioneer Hi-Bred International, Inc. (Johnston, IA) and the Iowa Agriculture Experiment Station.

References Cited


Figure 1. Field design, showing individual barrier size. Bold dashed lines in the center of the plots represent treatment rows of Herculex I or isoline. Outer dashed lines represent the rows of alternative host plants.
Table 1. Mean proportion of cut corn plants in the field at the time of weed removal (1) and at the end of the experiment (2) and standard error (lower, upper).

<table>
<thead>
<tr>
<th>Corn Type</th>
<th>Treatment</th>
<th>Mean proportion*</th>
<th>Standard Error (SEM)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Herculex™ I</td>
<td>No weed removal</td>
<td>0.027a</td>
<td>0.053A</td>
</tr>
<tr>
<td>Herculex™ I</td>
<td>Weed removal</td>
<td>0.027a</td>
<td>0.049A</td>
</tr>
<tr>
<td>Isoline</td>
<td>No weed removal</td>
<td>0.123b</td>
<td>0.182B</td>
</tr>
<tr>
<td>Isoline</td>
<td>Weed removal</td>
<td>0.107b</td>
<td>0.166B</td>
</tr>
</tbody>
</table>

* Data analyzed using Proc logistic (SAS v. 9.1).

Values in a column followed by a different letters are significantly different from one another (P≤0.05).
Figure 2. Mean percent of cut corn plants in the field with treatments of Herculex™ I (HX), Herculex™ I with weeds removed (HXWR), isoline (IS) and isoline with weeds removed (ISWR) at the time of weed removal. Bars indicate percentage of cut plants. Lines with each bar indicate standard errors.
Figure 3. Mean percent of cut corn plants in the field with treatments of Herculex™I (HX), Herculex™ I with weeds removed (HXWR), isole (IS) and isole with weeds removed (ISWR) at the time of weed removal and at the end of the experiment. Bars indicate percentage of cut plants. Lines with each bar indicate standard error at the end of the experiment.
Table 2. Mean proportion of cut corn plants in the greenhouse at the time of weed removal (1) and at the end of the experiment (2) and standard error (lower, upper).

<table>
<thead>
<tr>
<th>Corn Type</th>
<th>Treatment</th>
<th>Mean proportion *</th>
<th>Standard Error (SEM)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Herculex™ I</td>
<td>No weed removal</td>
<td>0.049a</td>
<td>0.415A</td>
</tr>
<tr>
<td>Herculex™ I</td>
<td>Weed removal</td>
<td>0.140a</td>
<td>0.628B</td>
</tr>
<tr>
<td>Isoline</td>
<td>No weed removal</td>
<td>0.561b</td>
<td>0.854C</td>
</tr>
<tr>
<td>Isoline</td>
<td>Weed removal</td>
<td>0.364b</td>
<td>0.886C</td>
</tr>
</tbody>
</table>

* Data analyzed using Proc logistic (SAS v. 9.1).

Values within a column followed by a different letter are significantly different from one another (P<0.05).
At time of weed removal

Figure 5. Mean percent of cut corn plants in the greenhouse with treatments of Herculex™ I (HX), Herculex™ I with weeds removed (HXWR), isoline (IS) and isolate with weeds removed (ISWR) at the time of weed removal. Bars indicate percentage of cut plants. Lines with each bar indicate standard error.
Figure 6. Mean percent of cut corn plants in the greenhouse with treatments of Herculex™ I (HX), Herculex™ I with weeds removed (HXWR), isoline (IS) and isoline with weeds removed (ISWR) at the time of weed removal and at the end of the experiment. Bars indicate percentage of cut plants. Lines with each bar indicate standard error at the end of the experiment.
Figure 7. Difference in percentage of cut corn plants in the greenhouse between the time of weed removal and the end of the experiment with treatments of Herculex™ I (HX), Herculex™ I with weeds removed (HXWR), isoline (IS) and isoline with weeds removed (ISWR). Bars indicate percentage of cut plants.
CHAPTER 3: Susceptibility of black cutworm (Lepidoptera: Noctuidae) to Herculex™ I and Agrotis ipsilon nucleopolyhedrovirus

Abstract

In preliminary research black cutworm Agrotis ipsilon (Hufnagel) showed significantly lower levels of feeding and cutting on corn expressing the insecticidal protein Cry1F, commercially known as Herculex™ I corn, compared to unprotected isolate corn. The suspected mechanism of protection has been described as a combination of non-preference and susceptibility to the insecticidal protein. A 7 day bioassay was conducted to test the relative contribution of these host plant resistance mechanisms. Third instar black cutworms were fed Herculex™ I corn or isolate corn leaf and stem pieces for 7 days. Weights were taken on day 0 and day 7. Larvae fed isolate corn leaf pieces had a significantly greater weight gain over the course of 7 days than those on Herculex™ I corn. Pathogens, such as insect viruses, have been used for insect control as alternatives to chemical control. Black cutworm is highly susceptible to Agrotis ipsilon multicapsid nucleopolyhedrovirus (AgipMNPV). The objective of this study was to test black cutworm susceptibility to this pathogen after feeding on Herculex™ I corn. Third instar black cutworms were fed Herculex™ I and isolate for 24 hours. Larvae were then given the LD$_{50}$ dose of AgipMNPV on a small diet cube. After 24 hours, larvae that had consumed the entire cube were placed on diet and after 9 days were evaluated for virus symptoms. Larvae fed the Herculex™ I diet had significantly higher rates of virus infection than those fed the isolate diet.

Introduction

The black cutworm, Agrotis ipsilon (Hufnagel) is a sporadic pest of corn and other crops in the United States. Larvae cause damage to corn seedlings by cutting stems above and below the ground surface (Showers et al. 1983). Most commonly black cutworm control is based on larval monitoring, followed by chemical treatments when damaging populations are detected. Pathogen alternatives to chemical control have been identified by Ignoffo and
Garcia (1979) who found that of 63 pathogens tested, 7 viruses, 5 bacteria, 2 fungi and 1 protozoan caused 30% or greater mortality in *Agrotis ipsilon*.

Genetically modified crops have revolutionized the pest management industry specifically those expressing insecticidal proteins from *Bacillus thuringiensis* (Berliner). Corn, *Zea mays* L., has been modified by insertion of the *Bt* gene for protection against many Lepidopteran pests. One such modification can be seen in Herculex™ I corn, *Bacillus thuringiensis* var. azawai expressing Cry1F protein, which provides protection against multiple Lepidopteran pests including the black cutworm.

Nucleopolyhedroviruses have been used since the 1890s for insect management (Moscardi 1999). The black cutworm is highly susceptible to *Agrotis ipsilon* multicapsid nucleopolyhedrovirus (*AgipMNPV*) (Boughton et al. 1999). Although nucleopolyhedroviruses provide an attractive alternative to chemical control due to their high levels of host specificity, they show weaknesses such as slow speed of kill and low field stability due to UV-degradation (Moscardi 1999).

*Bacillus thuringiensis* and nucleopolyhedroviruses have been combined to increase crop protection. Many researchers have shown that the combination can result in additive or synergistic effects between the *Bt* (in spray form) and NPVs (Lipa et al. 1976, Matter and Zohdy 1981, McVay et al. 1977, Streett and Mulrooney 2000, Bell and Romaine 1980). Some research has shown less effective results with the combination (Oatman et al. 1970) and Lutrell (1982) found that a combination of the two pathogens was less effective than the virus alone in *Helicoverpa zea*. Bell and Romaine (1986) found that sub lethal concentrations of *Bt* and *Heliothis armigera* nucleopolyhedrovirus (*HaNPV*) had a synergistic effect on *H. virescens* and *H. zea*, but after a lethal dose *HaNPV* with the addition of *Bt*, larval mortality rates actually decreased significantly. Pingel and Lewis (1999) combined three *Bt* products with *Anagaphra falcifera* multiple nucleopolyhedrovirus (*AfMNPV*) and tested the combination against three corn ear pests: *H. zea*, *Ostrinia nubilalis* (Hübner) and *Spodoptera*
frugiperda (J.E. Smith). Mixtures of the microbes showed either an equal or lower mortality rate than either pathogen alone.

In an example of transgenic plants expressing Bt toxin, larvae of the fall armyworm, S. frugiperda, were reared on either a diet of Bt, expressing Cry1A(b), or non-Bt sweet corn. Larvae were then exposed to Spodoptera frugiperda nucleopolyhedrovirus (SfMNPV) which resulted in larval mortality levels which were significantly higher for larvae fed the diet containing Bt (Farrar et al. 2004).

In preliminary research conducted by Pioneer Hi-Bred International Inc. (Johnston, IA) Herculex™ provided good crop protection; however, black cutworm were not very susceptible to purified Cry1F insecticidal protein. In Herculex™ I corn tissue feeding assays, larvae quickly recovered lost weight when placed on unprotected corn. Observational studies suggested larvae spent a disproportionate amount of their time traveling from plant to plant sampling a small amount of stem or leaf tissue, but a low percentage of those larvae actually fed or cut the plants (Lefko 2003, personal communication). Because of these observed non-preference behaviors on Herculex™ I, black cutworms may actually be more susceptible to virus in a field situation due to increased wandering making them more likely to encounter virus bait and increased susceptibility due to virus/Bt interaction effects. The objective of this study was to examine susceptibility of the black cutworm to Cry1F in Herculex™ I and susceptibility to AgipMNPV after exposure to Herculex™ I corn.

Materials and Methods

Insects, plants and virus. Black cutworm larvae were provided by the USDA-ARS Corn Insect and Crop Genetics Research Unit (Ames, IA). Seeds of Herculex™ I corn (Pioneer® 34M93) and isoline corn (Pioneer® 34M92) were provided by Pioneer Hi-Bred International, Inc. (Johnston, IA). Seeds were planted in 113 liter bins in professional peat growing mix (Sunshine; Sun Gro Horticulture, Vancouver, British Columbia) with 15 seeds per bin. Plants were harvested and fed to larvae at the V2 stage (Ritchie et al.1997).
The original isolate of *Agip*MNPV was acquired from infected black cutworm larvae collected by Dr. J. Maddox (Illinois Natural History Survey, Champaign, IL) and characterized by Boughton et al. (1999). The virus amplification was performed using 5th instar larvae. Virus was applied to Lepidoptera larvae diet (Southland Co., Lake Village, AR) and fed to larvae. After death, infected larvae were homogenized and virus was purified as described by Boughton et al. (1999).

**Effects of Cry1F on larval weight.** A 7-day bioassay was conducted to test for physiological effects of Cry1F on black cutworm larvae. Third-instar black cutworms were placed individually into culture dishes (Fischer brand 150mm X 25mm). Thirty larvae were fed plant pieces from Herculex™ I and isoline corn plants with treatments assigned randomly. A 5 cm piece of stem and an 8 cm piece of leaf were provided per dish. Additionally, a 1 cm² piece of wetted cloth was provided as a water source. Every 24 hours uneaten plant material was removed and fresh pieces were added to the dishes. Larvae were weighed on Day 0 and again on Day 7 at the conclusion of the experiment. Culture dishes were kept in the dark in a growth chamber at 27°C and ~80% RH throughout the duration of the experiment. The test was replicated 4 times. Weight differences among treatments were analyzed using least squared means (PROC GLM, SAS version 9.1).

**Effects of Cry1F with virus exposure.** A factorial design was used with diets of either Herculex™ I or isoline followed by exposure to an equal dose of virus to test for predisposition to viral infection by Herculex™ I. This experiment included 4 reps for a total of 170 larvae exposed to the LD₅₀ dose of the virus in the Herculex™ I treatment and 149 in the isoline treatment.

Thirty-two third instar black cutworms were placed individually into a compartmental tray (Oliver Products Co., Grand Rapids, MI). Larvae were fed both 2 cm leaf and 2 cm stem piece from Herculex™ I or isoline plants for 24 hours. Diets were randomly assigned with half of larvae receiving each of the diets. After 24 hours any remaining food pieces were removed from trays and larvae were starved for 6 hours. The LD₅₀ virus dose for 3rd instars
(330 OBs/larva) (Boughton 2001) was added to \( \approx 2 \text{ mm}^3 \) pieces of diet. Larvae were transferred to new trays with one piece of the diet with virus and one larva in each compartment. After 24 hours, cutworms that had not eaten the entire cube of diet were discarded. The percentage of larvae that consumed the entire diet cube by treatment was analyzed (PROC GLM, SAS v. 9.1) Larvae that had consumed the entire diet cube were placed on diet in individual compartments in condo trays (27° C). Virus symptoms appear after approximately 7 days. Larvae were observed on day 2 for non-virus death and day 9 for classification as infected or non-infected by the virus. Larvae infected by the virus had a light colored, thin cuticle and were smaller in size than healthy larvae. Larvae showing these symptoms were classified as infected whether alive or dead. Percent infected larvae by treatment were analyzed. Data from separate trials were pooled and analyzed using PROC LOGISTIC to determine differences between the treatments (SAS v. 9.1).

Results

Effects of Cry1F on larval weight. A significant difference was found between weights of larvae after 7 days on Herculex™ I and isoline corn. Weights of larvae on Herculex™ I were significantly lower than those on isoline (\( F= 33.56; \text{df}= 7, 148; P <0.0001; \) Figures 1 & 2). The distribution of weight change from Day 0 to Day 7 was quite different in the Herculex™ I treatment versus the isoline. Of the insects given the Herculex™ I treatment, majority of the individuals (44/78 insects) lost weight throughout the course of the 7 days (Figure 2). In the isoline treatment the distribution of weight gained was fairly evenly distributed (Figure 2).

Effects of Cry1F with virus exposure. The number of larvae that consumed the entire diet cube by treatment was not statistically significant (\( F= 2.41; \text{df}= 3, 2; P= 0.3067 \)). About 68% of larvae with the Herculex™ I treatment consumed the diet cube compared to 71% of those fed the isoline.
A significant difference was found between Herculex™ I and isoline treatments for virus infection (P<0.0001). Of larvae on Herculex™ I, 76% became infected after exposure to the virus compared to 48% of larvae fed on the isoline diet (Figure 3).

**Discussion**

Herculex™ I was non-lethal to black cutworms that fed on it in this 7-day assay, although larvae on Herculex™ I diet had less weight gain than those on isoline. This bioassay suggests that antibiosis is the mechanism of protection for Herculex™ I against black cutworms. Additional studies need to be conducted to assess the time to pupation and adult weight because extended time to pupation and lower adult weights would be further indicators of antibiosis as the mechanism of protection.

The data from the experiment with AgipMNPV and Herculex™ I showed that black cutworms exposed to Herculex™ I were more susceptible to AgipMNPV than those on the isoline diet. The results may be explained by one or more factors. First larvae exposed to Herculex™ I may have had higher susceptibility due to reduced feeding causing stress on the insect. The results could also be due to a mechanistic interaction between the Bt and AgipMNPV in the midgut. Larvae may be more susceptible due to sub-lethal effects Cry1F insecticidal protein in Herculex™ I corn. Additional no-choice studies could be conducted to look specifically at these mechanisms and their internal interaction on black cutworm larvae. Infection rates of larvae could be observed after injection of a combination of purified protein or homogenized plant tissue and virus directly into the insect midgut of healthy insect using a microapplicator. This would ensure that all larvae were healthy at the beginning of the experiment and were injected with the same dose of Cry protein and virus. This would thus eliminate variables such as lower fitness due to lack of feeding. Also the gene encoding the Cry1F protein could be incorporated into the AgipMNPV genome to be used in comparison to separate pathogen exposure. Chang et al. (2003) have shown that Bt toxins and baculovirus can be combined to create a single more effective insecticide. By exposing black cutworm larvae to both pathogens simultaneously, compared to each individually or staggered as it
was in this research, we could better understand the effect of their combination when multiple variables are assessed.

In a field setting, larvae likely have a choice of host plants. Larvae found in fields of Herculex™ I will do less damage to plants than larvae in an unprotected field (See Chapter 2). In fields of Herculex™ I, larvae may do small amounts of feeding or cutting and then migrate elsewhere since they are capable of migrating long distances (50m in one hour) (W.B. Showers, personal communication). Mortality of black cutworm in a Herculex™ I corn field likely is not caused directly by Cry1F, or by Cry1F alone. When combined, Cry1F and AglpMNPV may increase black cutworm mortality. This combination could potentially prevent migration to other fields and, thus prevent further damage.
References Cited


Figure 1. Difference in black cutworm larval weight gain between those fed Herculex™ I and isoline for 7 days. Bars with different letters represent values that are significantly different from one another (P <0.0001).
Figure 2. Distribution of weight gained in black cutworm larvae fed for 7 days on Herculex™ I or isolate.
Figure 3. Percent mortality by AgipMNPV for black cutworm larvae on treatments of Herculex™ I or isoline diets. Lines with each bar indicate standard errors. Bars with different letters represent values that are significantly different from one another (P<0.001).
Chapter 4. General Conclusions

Three main conclusions can be made in completion of this thesis project:

1. Herculex™ I significantly reduced black cutworm cutting of corn in both the field and the greenhouse.
2. Herculex™ I is not acutely lethal to black cutworms, although it does significantly reduce larval weights.
3. Black cutworms feeding on Herculex™ I are significantly more susceptible to AgipMNPV than those feeding on an isoline diet.

Future Research

While these studies provide information that has not been documented, further research is necessary to draw stronger conclusions about the mechanisms of Herculex™ I protection from black cutworm. Preliminary studies suggest antixenosis as the mechanism of protection and Chapter 3 bioassays suggest antibiosis as the mechanism of protection. Additional field trials should be conducted to draw stronger conclusions on the influence of the presence or absence of an alternative food source as a factor in black cutworm feeding behaviors and its injury potential in Herculex™ I. Black cutworm infestation rates should be significantly increased in an attempt to simulate feed pressure equivalent to that in the greenhouse trials.

As part of this project, a video tracking system with Ethovision software (Noddus Information Technology, Wageningen, Netherlands) was used to quantify the larval feeding behaviors (such as time spent wandering or time spent on the diet) of black cutworm exposed to the Cry1F protein in Herculex™ I (See Appendix). Results were not analyzed, but further work with this equipment could be used to determine this mechanism of protection. With a better understanding of this mechanism, we can better predict the factors that will influence
black cutworm feeding and cutting of Herculex™ I, and better understand the ecology of the corn system when Herculex™ I corn is used for pest management.

In addition, data from the virus study in Chapter 3 showed an interesting result, but the mechanisms behind the findings still need explanation. Are black cutworms more susceptible to AgipMNPV due to a decrease in overall fitness due to lack of feeding? Or is the susceptibility due to a mechanistic interaction between Bt and AgipMNPV in the midgut? More laboratory work to expose larvae to both pathogens simultaneously could provide insight into the reasoning behind the increased susceptibility. Additionally, field studies could also be incorporated into these concepts to make this technology more applicable to the grower. More work could be done to assess black cutworm migration to see if a chemical or biological insecticide would be necessary in Herculex™ I fields to prevent migration to adjacent non-Herculex™ I fields in order to prevent damage.
ACKNOWLEDGEMENTS

I thank my major professor, Dr. Jon Tollefson, for the opportunity to learn from him as a teacher and a mentor. I thank my committee members Drs. Bryony Bonning, Steve Lefko and Bob Hartzler. I appreciate their guidance throughout the course of my master’s work. Special thanks to those at Pioneer Hi-Bred, especially Steve Lefko and Rachel Binning, for their help with experiment design and creative problem solving. I thank Jean Dyer from USDA-ARS Corn Insect and Crop Genetics Research Unit for providing me with black cutworms and other supplies. Thank you to all the others that have helped me in small, but important ways, throughout the course of this project.
APPENDIX: Ethovision black cutworm trials

Materials and Methods

Ethovision 3.1 was used (Noddus Information Technology, Wageningen, Netherlands) to track larval movement in a laboratory setting. Trials were conducted at Pioneer Hi-Bred International, Inc. (Johnston, IA) and at USDA-ARS Corn Insect and Crop Genetics Research Unit (Ames, IA).

In trials at Pioneer Hi-Bred International, 9 cm petri dishes were filled with 5 mm of agar. Filter paper was placed on top of the agar. The edge of the dish was coated with Tanglefoot™ (The Tanglefoot Company, Grand Rapids, MI) to prevent black cutworm escape. A diet plug (1 cm² approx.) of Southland Lepidopteran diet (Southland Co., Lake Village, AR) with leaf tissue of Herculex™ I or isoline (1ppm) and purified protein of Cry1F (4 ppm) was placed in the center of the dish. Trials were also run with 1 cm leaf disks (harvested from greenhouse plants at V2) in place of a diet plug. Six dishes were set up under the camera at one time. Two dishes contained diet with Herculex™ I leaf tissue or Cry1F protein or a Herculex™ I leaf disk. Two contained diet with isoline leaf tissue or no protein or an isoline leaf disk. The remaining two contained no food as a negative control.

A series of trials were run using different variables to determine the protocol that would show the most consistency in larval behavior within the same treatments. Larvae were starved 12 hours, and in later trials for 24 hours, and then placed individually into the petri dish arena. Larvae were initially tracked for 4 hours in the arena and the tracking time was later decreased to 2 hours. Trials were conducted with 3rd, 4th and 5th instars. Larvae were tracked at a rate of 6 observations per second.

In trials at USDA-ARS Corn Insect and Crop Genetics Research Unit (Ames, IA), 9 cm petri dishes were also used. Trials were conducted with leaf pieces (Herculex™ I or isoline) that were grown in the greenhouse and harvested at V2. Pieces were cut and laid in a
strip across the diameter of the dish. Agar was placed on either side of the leaf to hold it down. The edges of the dishes were coated with Tanglefoot™. Set up was the same as above with 6 dishes under the camera for tracking at one time. Fourth and fifth instars were used for different reps of the trials. Larvae were starved for 12 hours and then placed individually into the arenas. The duration of tracking was 2 hours. Distance traveled and time spent on the diet cube/leaf disk was recorded.

In another variation of this experiment, stem pieces from Herculex™ I and isoline were harvested from plants (V2) grown in the greenhouse. Stems were cut into 4 cm pieces and 3 pieces were hot glued vertically onto the bottom of the dish in a triangle formation about 2 cm from each other. About 5mm of agar was poured into the dishes after stems were glued in. Trials were run without filter paper, but larvae fed on the agar so filter paper was added in later trials. The edges of the dishes were coated with Tanglefoot™. Larvae were starved for 12, and in later trials 24 hours, before being placed in the arena. Set up was the same as the other experiments with 6 dishes under the camera at one time. Fourth and fifth instars were used. Larvae were tracked for 2 hours. Distance traveled and time spent in the areas adjacent to the stems was recorded.

Results

The experiment was not analyzed due to inconsistency in results. Experimental protocols were continually changed in an attempt to find a variation that yielded better consistency in movement within the same treatment. Although the raw data has not been analyzed, some interesting trends were observed based on the video tracks of larval movement. Larvae traveled the greatest distances in the Petri dishes with no food. Larvae traveled greater distances in the Herculex™ I/Cry1F treatments than in the isoline. On the Herculex™ I/Cry1F treatments, larvae encountered the diet plug/leaf disk but then continued to wander within the arena suggesting that it may have been in search of a different food source. On the isoline, larvae encountered the diet plug/leaf disk and stayed on it to feed. These observations were not consistent among all individuals within a treatment, but they were seen in many of the tracks.
**Figure 1.** Ethovision tracks of larval movement on 3 treatments: No food, Herculex™ I, isoline.