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EFFECT OF TRANSGENIC Bt CORN POLLEN AND GLYPHOSATE RESISTANT CANOLA POLLEN ON THE SURVIVAL OF THE HONEY BEE (*Apis mellifera* L.) WORKER LARVAE AND THE USE OF Bt CORN POLLEN AS A CONTROLLING METHOD FOR THE GREATER WAX MOTH (*Galleria mellonella* L.)
presented by

Anne Valdes Hanley

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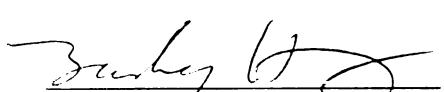

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By

Anne Valdes Hanley

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ABSTRACT

EFFECT OF TRANSGENIC Bt CORN POLLEN AND GLYPHOSATE RESISTANT CANOLA POLLEN ON THE SURVIVAL OF THE HONEY BEE (*Apis mellifera* L.) WORKER LARVAE AND THE USE OF Bt CORN POLLEN AS A CONTROLLING METHOD FOR THE GREATER WAX MOTH (*Galleria mellonella* L.)

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The honey bee, *Apis mellifera* L. (Hymenoptera: Apidae), a valuable pollinator, is one of the most important insects in the world's agriculture and food industry. With the recent concern about the effects of transgenic crops on non-target insects, the effects of transgenic crops on the honey bee must be evaluated. The effect of glyphosate resistant canola pollen and Bt transgenic corn pollen (Cry1F and Cry1Ab) on 4-5 d old honey bee worker larvae was tested in the field. Percent larval mortality, mean pupal weight, blood protein level of newly emerged adults, and percent adult emergence were determined for bees fed transgenic or non-transgenic pollen. There were no significant differences in the above parameters between larvae fed glyphosate resistant canola pollen and non-transformed canola pollen ($P>0.05$). There were no differences in the parameters between larvae fed transgenic corn pollen and non-transformed corn pollen. The use of Bt transgenic corn pollen as a control of the greater wax moth was tested in the laboratory. Mortality of larvae fed Cry1F corn pollen was significantly different from larvae fed Cry1Ab corn pollen and non-transformed corn pollen ($P<0.05$). Cry1F corn pollen appears to be a good control of wax moth larvae in the laboratory. These results suggest that there is a possible benefit of transgenic corn and canola pollen for bees.

To my grandfather, Clifford Burgess Godwin, Jr.

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INTRODUCTION

The honey bee, *Apis Mellifera* L. (Hymenoptera: Apidae), is one of the most important insects in the world's agriculture and food industry. Many of the world's crops including tree fruits, blueberries, and cucurbit vegetables require pollination for crop production. For nearly all of these crops, successful pollination depends either wholly or predominantly on the honey bee (Hoopingarner et al. 1992). It is clear that the world's agricultural industry depends on the honey bee for its very survival. It is essential to maintain an adequate pollinating force of honey bees for this important industry.

The use of transgenic crops in the United States is sizeable, making up 68% of soybean acreage, 26% of the corn acreage, and 69% of the cotton acreage (Commoner 2002). Published experiments have mainly tested the effects of purified transgene products on honey bees. There is a potential for pleiotropic effects due to the insertion of a foreign gene into the plants, which could produce other gene products besides the inserted gene. These products could also be toxic to bees or they could affect the longevity of foraging bees or decrease brood survival. Because of these reasons, it is important to determine the impacts of transgene plants by testing the effects of actual products like pollen and nectar from transgenic plants on the honey bee and other beneficial insects.

The greater wax moth, *Galleria mellonella* L., is a pest of weak honey bee colonies and stored honey combs. Current controls of the wax moth are chemical fumigants that can only be used on stored combs. Because Cry1Ab and Cry1F corns are designed for controlling Lepidopteran larvae, it is possible that they can be used as a

controlling method for wax moths in the field. It is with these two areas in mind that we formed the objectives of our study.

Research Objectives

The main research objectives of this study were 1) To determine the effect of transgenic glyphosate resistant canola pollen and transgenic Bt corn pollen on the survival of honey bee, *Apis mellifera* L., (Hymenoptera: Apidae) worker larvae and 2) To evaluate transgenic Bt corn pollen as a potential controlling method for the greater wax worm, *Galleria mellonella* L. (Lepidoptera: Pyralidae), a pest of the honey bee.

LITERATURE REVIEW

History of the honey bee

Mankind has valued honey since times of old, as evidenced in the book of Isaiah, 7:15: "He shall be living on curds and honey by the time he learns to reject the bad and choose the good" (New American Bible 1970). Also in Genesis 43:11, it states: "Their father Israel told them: 'If it must be so, then do this: Put some of the land's best products in your baggage and take them down to the man as gifts: some balm and honey, gum and resin, and pistachios and almonds'" (New American Bible 1970). In the Old Testament milk and honey usually mean excellent and abundant food (Orchard 1951).

Social bees have produced and stored honey for the last 10 to 20 million years. The earliest records of man's exploitation of honey are evidenced by rock paintings and by the findings of anthropological studies of both contemporary and pre-historic tribes, which hunted for honey in the wild (Crane 1983).

Honey bee biology

Honey bees are eusocial insects, meaning that they have a reproductive division of labor; multiple generations exist at the same time; and they care for their young cooperatively (Winston 1987). Three castes exist in the hive, a queen, workers and drones. All castes have four developmental stages: egg, larva, pupa, and adult (Winston 1987). The queen lays two types of eggs, fertilized and unfertilized. The unfertilized eggs develop into drones, while the fertilized eggs can develop into workers or queens, depending on what they are fed during development. The queen is fed royal jelly throughout her immature stage and for her entire life after emergence. Worker larvae are

fed royal jelly for the first three days of development and then are fed a mixture of worker jelly and pollen.

Each caste has different tasks to perform within the colony. The drones' only role in the hive is to mate with the queen in the spring or summer of the year. Those that do succeed in mating with the queen die soon after. The remaining drones are fed nectar and pollen by worker bees. In the fall, the drones are banished from the hive by workers, so that they will not use up the limited winter food reserves. The sole task of the honey bee queen is to lay eggs to populate the hive. In the first two weeks after she emerges in the summer, she goes on her first mating flight during which she usually mates with more than one drone. There is a record of a queen mating with 17 different drones on one mating flight (Winston 1987). After she is mated, she returns to the hive and begins to lay eggs. Queens have been known to lay up to 2,000 eggs a day in the summer. She is the only fertile female in the hive and usually lives for three to five years (Winston 1987).

Worker bees, which make up the majority of the hive's population, are nearly sterile females. Young workers have many tasks to perform in their lifetimes, which are completed in an order that is somewhat dependent on their age. Upon emergence, they begin cell cleaning, then they perform the tasks of capping brood, tending brood, attending the queen, receiving nectar, cleaning debris, packing pollen, comb building, ventilating the hive, guarding the hive, and finally the foraging flight (Winston 1987). There is some overlap in activities.

Honey bees as valuable pollinators

Apis mellifera L. is the most popular species of honey bees. It is highly valued today for its products such as honey, wax, pollen, and royal jelly, but more importantly

for its pollination of important fruits and vegetables. Honey bees have coevolved with angiosperms over millions of years (Crane 1989). Proof of this intricate relationship are the pollen baskets on their tibiae called *corbiculae* and the plumose hairs that cover bees' bodies, both of which help the bee collect pollen from angiosperms. On the plants' side, angiosperms have evolved colors, scents, and excess nectar and pollen to attract the bee to pollinate them (Caron 1999). Pollination is the transfer of pollen from the anther to the stigma. For most plants, pollination must be accomplished before fertilization and the eventual seed production can take place (Vansell and Griggs 1952). It has been estimated that approximately one third of people's diets in the United States comes directly or indirectly from bee pollinated crops (Hoopingarner 1992). In 1998, it was estimated that there were 2,500,000 colonies rented for pollination purposes in the United States (Morse and Calderone 2000). In 2000, the value of increased crop yield and quality gained through pollination by honey bees was $14.6 billion (Morse and Calderone 2000).

Pollen is the natural source of protein for bees, whereas nectar collected by bees contains rather low concentrations of amino acids (Baker and Baker 1977). The pollen consumption of newly emerged workers increases for the first week. However, when workers reach the nursing stage, their pollen consumption decreases (Crailsheim 1990). Large amounts of protein are required during larval development. One larva needs about 50mg royal jelly to reach a weight of 35mg (Crailsheim 1990). The protein content of the honey bee's hemolymph at emergence depends on the availability of food outside the hive during the nursing season (Kunert and Crailsheim 1987).

The nutritional value of pollen for bees is defined by the content of ten essential amino acids: arginine, histidine, lysine, tryptophan, phenylalanine, methionine, threonine,

leucine, isoleucine, and valine (Groot 1953). Honey bees are able to distinguish between different kinds of pollen. Corn pollen, for example, is not as attractive to bees but they will forage for it because of its great abundance and protein content (Crane 1984). Canola pollen is an excellent bee forage source that has a higher protein content than corn pollen.

A pest of the honey bee

Like other animals, honey bees are plagued by many diseases and pests. One of these pests is the greater wax moth, *Galleria mellonella* L., a pest of stored combs and weak colonies. Larvae of the greater wax moth cause considerable damage to beeswax combs left unattended by bees. Beeswax combs in weak or dead colonies and those placed in storage are subject to attack.

Treating infested comb with extreme temperatures is one of the current methods of wax moth control (Williams 1978). Other methods of controlling the wax moth used in the past are chemicals such as paradichlorobenzene, ethylene dibromide, methyl bromide, and ethylene oxide to fumigate stored equipment (Williams 1978). These chemical fumigants have several disadvantages. First, they may be toxic to humans and animals. Several applications are often required during an average four month storage period. After fumigation, a closed system in the storage area is needed for fumigation and this system is difficult and expensive to maintain (Cantwell 1981). These chemicals are no longer approved for use in the hive.

An alternative method to chemical control of wax moth, Thuricide 90TS®, a *Bacillus thuringiensis* preparation that was incorporated into beeswax foundation, was studied in the early 1960's (Cantwell 1981). However, it did not prove to be very

successful. In the 1980's, a *Bacillus thuringiensis* bio-pesticide, Certan®, was developed to treat honey bee colonies in the field (Cantwell 1981). This new bio-pesticide was available for sale by bee supply companies but was not profitable so it is no longer manufactured (Caron 1999). At present the only available methods for wax moth control are extreme temperatures. This method is both time consuming and costly as it requires a large freezer or wax melter to be used efficiently. New natural controls that could be used in the field would be very beneficial for the beekeeping industry.

Bacillus thuringiensis

Since the first use of protection of plants against pests by transfer and expression of foreign genes coding for entomopathogenic proteins, transgenic crops have been the object of much concern and study (Jouanin et al. 1998). Genetically modified plants are plants that have genes inserted into them for several reasons including: herbicide resistance, insect resistance, and growth enhancement. One of the most popular genes that are currently being inserted into a wide variety of plants is a gene that expresses the *Bacillus thuringiensis* toxin. *Bacillus thuringiensis* (Bt) is a gram positive, facultative, anaerobic bacterium that forms characteristic crystals that are made of crystal (Cry) proteins, which are toxic to a wide variety of insects (Sonenshein 1993). Bt has been used in agriculture since the 1920's to control insect pests in the orders Lepidoptera, Coleoptera, and Diptera (Beegle and Yamamoto 1992). It is very specific in its toxicity, which makes it different from synthetic, broad-spectrum insecticides such as carbamates and organophosphates. Bt is harvested from soil, grain bins, and also from insects which have died from Bt infection (Harwood 1994).

Bt strains produce two types of toxins. The main types are Cry (crystal) toxins, encoded by different Cry genes, which is how different types of Bt are classified. The second types are the Cyt (cytolytic) toxins, which can augment the toxicity of the Cry toxins, adding to their effectiveness in insect control (Li 1991). These toxins can be expressed in plants' leaves and pollen.

History of transgenic crops

There were over 28 million ha of transgenic commercial crops grown worldwide in 1998 according to James (1998). These crops include corn, cotton, soybeans, rice, canola, tomatoes, and potatoes (James 1998). The categories of transgenic crops are as follows: glyphosate resistant, insect resistant, virus resistant, both insect and glyphosate resistant, and quality traits (James 1998). The use of worldwide (excluding China) glyphosate resistant crops increased by 6,232,159 ha from 1996-1997 (James 1998). The use of insect resistant crops worldwide (excluding China) also saw a significant increase of 2,954,205 ha from 1996-1997 (James 1998). One of the most used genes for insect resistance is from Bt. The major pest controlled by Bt corn is the European corn borer, *Ostrinia nubilalis* L (Anon. 2000). Grower satisfaction in1996 with Bt corn yield caused an increase from 1 percent of the national acreage to 9 percent of the 32 million ha of corn in 1997 in the United States (James 1998).

Canola as a forage crop

Canola (*Brassica napus*) is a plant that originated from rapeseed. Rapseed is not considered healthy for human consumption because of high levels of licosenic and erucic acids which are not essential to human growth. Canadian plant breeders produced canola by genetically altering rapeseed by reducing glucosinolates and licosenic and erucic

acids. Overtime canola has become a worldwide term not just a Canadian term. Today annual worldwide production of canola is 7.5 million tons on 1,618,743 ha (Oplinger 1989). Canola is an excellent bee forage crop and it is grown mainly in northern climates. Canada accounts for 15% of the world canola production, while the United States accounts for only 1% of canola production. The major states for canola production in the United States are North Dakota and Minnesota (Oplinger 1989). Recently farmers have begun to use glyphosate resistant or Roundup Ready Canola seeds instead of non-transformed canola seeds.

A study was done to determine if honey bees show a preference for genetically modified transgenic oilseed rape or non-transgenic oilseed rape. They found that if colonies are closer to a non-transgenic oilseed rape field than to a transgenic oilseed rape field, the bees will bring in a minimal amount of transgenic pollen as compared to non-transgenic pollen (Osborne et al. 2001).

In 1996, more than 121,410 ha of glyphosate resistant or Roundup Ready canola was grown in Canada (James 1998). The glyphosate resistant genes had no effect on yield but improved weed control which increased yield indirectly. The average yield of glyphosate resistant canola in Canada in 1996 was 9% higher than the yield of non-herbicide tolerant canola treated with herbicides (James 1998).

Effect of Bt-transgenic pollen on non-target Lepidoptera

A laboratory assay found that Monarch butterfly larvae fed Cry1Ab (Bt 11) transgenic pollen had a decreased survival rate compared to those larvae fed non-transformed corn pollen (Losey et al. 1999). This research caused concern about the impact of transgenic plants as well as criticism of the methods used in the study. In

response to the Losey study, five field studies were conducted in Iowa, Maryland, New York, and Ontario to determine the effect of exposure of monarch larvae in field conditions. The studies found that larvae did not suffer any acute effects under natural levels of Bt11 and Mon810 corn pollen (Sears et al 2001). In another study, two varieties, Pioneer variety 34RO7, Monsanto event 810, both of which express the Cry1Ab gene in their pollen were tested on black swallowtail larvae. Both field and laboratory studies in Illinois found no negative effects on the larvae by the corn pollen (Wraight et al 2000).

Possible effects of transgenic crops on the honey bee

With the recent concern about the effects of transgenic crops on non-target insects, the effects of transgenic crops on the honey bee must be evaluated in order to safeguard the future of the honey bee. There are two ways that transgenic plants can affect the honey bee: pollen collected by bees might prove to be toxic to them or pleiotropic effects could make the transgenic plant less attractive to the honey bee by producing less nectar (Burgess et al. 1996). Recently an article published in Harper's Magazine vigorously challenged current DNA theories. The results of the human genome project were not as expected, finding the number of human genes amounting to a mere 30,000, not the 100,000 genes the project scientists had predicted (Commoner 2002). A mustard-like weed has 26,000 genes, very close to the number found in humans. The central dogma of the project was that the molecular structure or DNA is the exclusive agent of inheritance in all living things (Commoner 2002). The results of the genome project cannot explain the difference between humans and other organisms having comparable numbers of genes. A possible reason for the differences is alternative

splicing. This is where the gene's original nucleotide sequence is split into fragments that are recombined to encode a multiplicity of proteins. These proteins are different in their amino acid sequence from one another and from what the gene encoded for originally. Thus, alternative splicing can be said to generate new genetic information (Commoner 2002).

The genetic engineering industry in the United States is guided under the same theory as the human genome project. They state that the transfer of a bacterial gene into a corn plant is expected to be very predictable. In a genetically engineered transgenic plant, the transplanted bacterial gene must interact with the plant whose system in most cases is entirely different from its own. This difference often leads to experimental failures that occur before a transgenic organism is produced and also to unexpected genetic changes that occur after the gene has been transferred (Commoner 2002). The possibility of these changes occurring in transgenic crops must be studied to ensure the safety of the honey bee and other pollinators that may be affected by the pleiotropic effects.

Laboratory Studies

Several studies examined the effects of transgenic products (proteins) on honey bees. Belzunces et al. (1994) fed honey bees doses of Soybean trypsin inhibitor (SBTI), Bowman-Birk soybean trypsin inhibitor (BBI), and orzacystatin mixed with sugar syrup. they found that trypsin activity was reduced after 3.5 days of feeding the 0.1 and 1 mg/g dose of BBI (Belzunces 1994). Honey bee larvae, ages 1-3 days old, were fed a purified Cry1Ac toxin as a rate of 20 µg/ml. Feeding on the toxin showed no significant effect on

the survival of these larvae. The rate of protein that they were fed was much higher than that actually found in the field (Sims 1995).

Picard-Nizou et al (1995) tested bee foraging activity on transgenic oilseed rape plants that had a bean chitinase gene inserted which was under the control of CaMV 35S promoter. These studies found that the transformation event did not appear to have an effect on foragers' choices .

The toxicity of cowpea trypsin inhibitor (CpTI), chitinase and *B*-1,3 proteins to honey bees were tested by feeding or injecting the proteins to adult worker bees. No toxic or behavioral effects were found between treatment and control bees except for CpTI which had a significant effect on adult bee learning behavior (Picard-Nizou et al. 1997).

The toxicity of two endopeptidase inhibitors, bovine pancreatic trypsin inhibitor, and soybean trypsin inhibitor was tested on honey bees. The study found that the two inhibitors fed in 15% (weight:volume) sugar solution killed honey bees within 8-16 days (Malone et al. 1995). The next step after this study was to determine whether or not the endopeptidase inhibitors were present in the pollen and nectar of the transgenic plants in which they were inserted.

Potato proteinase inhibitors, POT-1 and POT-2 were fed to newly emerged adult bees in either sugar syrup or pollen food. Honey bees fed POT-1 or POT-2 in pollen or syrup had significantly reduced lifespan. This adverse effect was stronger when applied through pollen than when applied though the sugar syrup treatment (Malone et al. 1998).

Newly emerged bees were fed Cry1Ba delta-endotoxin, Bt biopesticide preparations, Dipel 2A® and Foray 48B®, and Kunitz soybean trypsin inhibitor

(SBTI) in a pollen based food. The study showed that adults are unlikely to be harmed by transgenic plants expressing Cry1Ba or SBTI or by Bt biopesticides that are used as recommended (Malone et al. 1999). In a similar study, newly emerged bees were fed either Cry1Ba toxin or aprotinin proteinase inhibitor in pollen. Bees fed Cry1Ba did not differ significantly from control bees in their timing of first flight. However, bees fed aprotinin began to fly and also died three days sooner than bees fed Cry1Ba or control pollen (Malone et al. 2000).

Field Studies

Experiments using purified gene products mixed into bee food have the potential to provide useful information on the impacts of transgenic plants prior to release. However, because of possible alternative splicing, proteins other than the transgene product might be changed. Because of this, research must continue on testing actual plant parts/products (e.g. nectar, pollen) from transgenic plants that are already on the market to determine their possible effects on the honey bee.

Only a few field studies using transgenic pollen have been conducted. In Germany where honey bee colonies were placed inside gauze tents with Cry1Ab expressing Bt corn plants showed no effects of Bt pollen on bee survival, foraging frequency, behavior, or brood development during pollen tassling. Brood development was monitored for 30 days after pollen shed and no significant effects were shown (Schur et al. 1999 cited by Malone et al 2000).

In another field conducted study, colonies were fed a sugar syrup solution of two concentrations of the coleopteran toxin, CryIIIB at a rate of .066% or .332% over a two-

month period. No effect on larvae survival or pupal dry weight was shown (Arpaia 1996).

A field trial conducted in Saskatoon, Saskatchewan used a method of allowing bees to forage freely in a Roundup Ready® canola field and in a regular canola field. Bees foraging for two pollen types did not differ significantly in larval survival, pupal weight, and adult survival (Huang unpublished). Further studies of this nature should be performed in order to test the effect of transgenic pollen on honey bees.

Honey bees play an important role in agriculture as valuable pollinators of fruits and vegetables. The latest estimate of increased yield and quality due to honey bee pollination is valued at $14.6 billion a year (Morse and Calderone 2001). Due to the importance of the honey bee in agriculture, toxicity of new pesticides on bees is assessed before registration. The genes inserted into transgenic crops that are designed to produce proteins to kill insect pests, must be tested for possible toxic effect to honey bees. The majority of published studies have dealt with feeding trans-gene protein directly to honey bees. Due to possible alternative splicing of the genes once they are inserted into the corn or canola plants, more realistic tests must be performed using actual pollen from transgenic plants containing the protein. This field study used pollen collected from corn and canola plants to feed larvae directly. The method is a better test of whether transgenic canola and Bt corn pollen have any negative effects on honey bee larvae.

Another aspect of this study tested pollen produced from transgenic Bt corn plants as a possible control for the greater wax moth, *Galleria mellonella* L. Current controls of the wax moth are chemical fumigants that can only be applied to stored combs.

Transgenic Bt corn pollen is a possible control method that could be applied in the field to existing colonies.

Research Objectives

The main research objectives of this study were 1) To determine the effect of transgenic glyphosate resistant canola pollen and transgenic Bt corn pollen on the survival of honey bee, *Apis mellifera* L., (Hymenoptera: Apidae) worker larvae and 2) To evaluate transgenic Bt corn pollen as a potential controlling method for the greater wax worm, *Galleria mellonella* L. (Lepidoptera: Pyralidae), a pest of the honey bee.

CHAPTER ONE

EFFECT OF TRANSGENIC GLYPHOSATE RESISTANT CANOLA POLLEN AND Bt CORN POLLEN ON THE SURVIVAL OF WORKER HONEY BEE LARVAE

ABSTRACT

The honey bee, *Apis mellifera* L. (Hymenoptera: Apidae), a valuable pollinator, is one of the most important insects in the world's agriculture and food industry. There were close to 28,329,000 ha of transgenic commercial crops grown worldwide in 1998, these crops include corn, cotton, soybeans, rice, canola, tomato, and potato (James 1998). With the recent concern about the effects of transgenic crops on non-target insects, the effects of transgenic crops on the honey bee must be evaluated to safeguard the future of the honey bee and crops dependent on bees. Transgenic crops could produce pollen toxic to adult bees or larvae or the expression of the foreign gene could result in pleiotropic effects in transgenic plants that would make them less attractive or nutritious to bees. The of feeding glyphosate resistant canola pollen and Bt transgenic corn pollen (Cry1F and Cry1Ab) to 5-6 d old honey bee worker larvae was determined. A positive control of a diazinon pesticide dose of 888ug/larvae gave a ~50% mortality rate. Percent larval mortality, mean pupal weight, blood protein level of newly emerged adults, and percent adult emergence were determined. There were no significant differences in the above parameters between larvae fed glyphosate resistant canola pollen and non-transformed canola pollen ($P>0.05$). There were no differences in the parameters between larvae fed transgenic corn pollen and non-transformed corn pollen. There were no significant differences between the parameters tested on larvae fed glyphosate resistant canola pollen and non-transformed canola pollen ($P>0.05$). In conclusion, glyphosate resistant canola pollen and Bt transgenic corn pollen (Cry1F and Cry1Ab) do not appear to have any significant effects on percent larval mortality, mean pupal weight, blood protein level of newly emerged adults, and percent adult emergence of larvae fed at age 4-5 d old.

INTRODUCTION

The honey bee, *Apis mellifera* L. (Hymenoptera: Apidae), is one of the most important insects in the world's agriculture and food industry. Many of the world's crops including tree fruits, blueberries, and cucurbit vegetables require pollination for crop production. For nearly all of these crops, successful pollination depends either wholly or predominantly on the honey bee (Hoopingarner 1992). It is clear that the world's agricultural industry depends on the honey bee for its very survival. It is essential to maintain an adequate pollinating force of honey bees for this important industry.

There were over 28 million ha of transgenic commercial crops grown worldwide in 1998 (James 1998). These crops include corn, cotton, soybeans, rice, canola, tomatoes, and potatoes. The categories of transgenic crops are as follows: glyphosate resistant, insect resistant, virus resistant, both insect and glyphosate resistant, and quality traits (James 1998). One of the most used genes for insect resistance is from *Bacillus thuringiensis*. The major pest controlled by Bt corn is the European corn borer, *Ostrinia nubilalis* L (Anon. 2000). Grower satisfaction in1996 with Bt corn yield caused an increase from 1 percent of the national acreage to 9 percent of the 32 million ha of corn in 1997 in the United States (James 1998).

Canola (*Brassica napus*) is a plant that originated from rapeseed. Canadian plant breeders produced canola by reducing glucosinolates and licosenic and erucic acids in rapeseed (which are not essential to human growth). Today annual worldwide production of canola is 7.5 million tons on 1,618,743 ha. Canola is an excellent bee forage crop and it is grown mainly in northern climates. Canada accounts for 15% of the world canola

production, while the United States accounts for only 1% of canola production. The major states for canola production in the United States are North Dakota and Minnesota (Oplinger 1989). Recently farmers have begun to use glyphosate resistant or Roundup Ready® canola seeds instead of non-transformed canola seeds.

With the recent increase of concern about the effects of transgenic crops on non-target insects, the effects of transgenic crops on the honey bee must be evaluated in order to safeguard the future of the honey bee. Transgenic crops could produce pollen toxic to adult bees or larvae or the expression of the foreign gene could result in pleiotropic effects in transgenic plants that would make them less attractive or nutritious to bees. (Malone et al. 1995, Burgess et al.1996).

Several studies examined the effects of transgenic products (proteins) on honey bees. Belzunces et al. (1994) conducted a study where bees were fed doses of Soybean trypsin inhibitor (SBTI), Bowman-Birk soybean trypsin in hibitor, and orzacystatin mixed with sugar syrup and showed no negative effects. Honey bee larvae, ages 1-3 days old, were fed a purified Cry1Ac toxin as a rate of 20 µg/ml. Feeding on the toxin showed no significant effect on the survival of these larvae. The rate of protein that they were fed was much higher than that actually found in the field (Sims 1995).

Bee foraging activity were observed on transgenic oilseed rape plants that had a bean chitinase gene inserted which was under the control of CaMV 35S promoter. These studies found that the transformation event did not appear to have an effect on foragers' choices (Picard-Nizou et al. 1995).

Toxicity of cowpea trypsin inhibitor (CpTI), chitinase and *B*-1,3 proteins on honrey bees was tested by feeding or injecting the proteins to adult worker bees. No

toxic or behavioral effects were found between treatment and control bees except for CpTI which had a significant effect on adult bee learning behavior (Picard-Nizou et al. 1997). The toxicity of two endopeptidase inhibitors, bovine pancreatic trypsin inhibitor, and soybean trypsin inhibitor was tested on honey bees. The study found that the two inhibitors fed in 15% (weight:volume) sugar solution killed honey bees within 8-16 days (Malone et al. 1995). Potato proteinase inhibitors, POT-1 and POT-2 were fed to newly emerged adult bees in either sugar syrup or pollen food. Honey bees fed POT-1 or POT-2 in pollen or syrup had significantly reduced lifespan. This adverse effect was stronger applied through pollen than if applied though the sugar syrup treatment (Malone et al. 1998).

Newly emerged bees were fed Cry1Ba delta-endotoxin, Bt biopesticide preparations, Dipel 2A® and Foray 48B®, and Kunitz soybean trypsin inhibitor (SBTI) in a pollen based food. The study showed that adults are unlikely to be harmed by transgenic plants expressing Cry1Ba or SBTI or by Bt biopesticides that are used as recommended (Malone et al. 1999). In a similar study, newly emerged bees were fed either Cry1Ba toxin or aprotinin proteinase inhibitor in pollen. Bees fed Cry1Ba did not differ significantly from control bees in their timing of first flight. However, bees fed aprotinin began to fly and also died three days sooner than bees fed Cry1Ba or control pollen (Malone et al. 2000).

Colonies were fed a sugar syrup solution of two concentrations of the coleopteran toxin, CryIIIB at a rate of .066% or .332% over a two-month period. No effect on larvae survival or pupal dry weight was shown (Arpaia, 1996). Effects of transgenic herbicide resistant oilseed rape on honey bees were tested in a greenhouse setting. No significant

differences were found in measurements of worker bee mortality, foraging activity and preferences, colony population, brood area, food stores, or diseases (Chaline et al. 1999).

In Germany, honey bee colonies were placed inside gauze tents with Cry1Ab expressing Bt corn plants showed no effects of Bt pollen on bee survival, foraging frequency, behavior, or brood development during pollen tassling. Brood development was monitored for 30 days after pollen shed and no significant effects were shown (Schur et al. cited by Malone et al.2000).

Honey bees play an important role in agriculture as valuable pollinators of fruits and vegetables. The latest estimate of increased yield and quality due to honey bee pollination is $14.6 billion a year (Morse and Calderone 2001). Due to the importance of the honey bee in agriculture, toxicity of new pesticides on bees is assessed before registration. The genes inserted into transgenic crops that are designed to produce proteins to kill insect pests, must be tested for possible toxic effect to honey bees. The majority of experiments have dealt with feeding transgene products (proteins) directly to honey bees. Due to possible splicing of the genes once they are inserted into the corn or canola plants, more realistic tests must be performed using actual pollen from transgenic plants containing the protein.

Considering the importance of the honey bee in agriculture and the possible negative effects of transgenic plants on the honey bee, our objectives were to determine the effect of glyphosate resistant canola pollen and transgenic Bt corn pollen on the survival of honey bee worker larvae.

MATERIALS AND METHODS

Canola pollen collection

The canola pollen was collected in Saskatchewan, Alberta, Canada. Four hives were placed directly in Roundup ready and non-transgenic canola fields with pollen traps on them to collect the pollen. The traps were checked daily for a two week period during the canola bloom. In total, 600 grams of regular canola pollen and 450 grams of Roundup Ready® canola pollen were collected.

Corn pollen collection

Corn pollen was collected during the week of corn tassling in late July or early August, depending on the variety of corn in 2000 and 2001. Lawson® waterproof bags were placed over the tassles and folded and then paper-clipped in place to ensure that the pollen would not leak out. Pollen bags were left in place for 24 hours and then collected. After the bags were collected the pollen was sifted using size 1, 2, and 5 sifters to remove any insects, tassles, and other debris. Once sorted the pollen was placed in a plastic container, labeled and stored in the freezer at –20° C. One corn plant yielded a very small amount of pollen so ~3000 plants were bagged over the course of the tassling season.

Larvae identification mapping

The larvae were mapped on day 4-6 of the larval stage with a transparency that was pinned to the frame and a permanent marker was used to circle the larvae that were to be used in the experiment. After the larvae were capped, on day 9, the tracing was placed over them again and percent mortality was measured. Former occupants of empty cells were assumed to be dead since workers remove dead larvae.

Experiment one - summer 2000

Field study setup

Miniature bee hives were established at the Michigan State University Apicultural Building on College Road in East Lansing, MI. The hives were a fourth of the size of a medium bee super (25 x 22.5 x 22 cm). The four frame Pettite hives were filled with ~ 3,000 bees using a cup measuring scale system in the July of 2000. Newly mated queens were placed in the hives. A pollen trap was put on the front of each hive to prevent returning foragers from bringing outside pollen into the hive. The queen was placed under a wire cage and allowed to lay eggs and then the cage and queen were moved to a new part of comb.

Pollen treatments

The pollen treatments for this experiment were as follows: Untreated corn pollen, corn pollen containing Cry1Ab (Event Bt 11), corn pollen containing Cry1F (Event TC1507 purchased from Mycogen- Dow Agrisciences Co.), and diazinon treated corn pollen. The Diazinon- 500AG dose used was 1.5 q to the acre, which is the dose a farmer would spray on his corn field. This application served as a positive control to ensure that the methodology of the experiment was valid.

Larval feeding

Approximately 2 g of pollen was placed in each hive directly on the comb around the newly laid brood. It was then sprayed with a 50% (weight: volume) sugar syrup solution to make it stick to the frame. A measurement was taken of pupal weight.

Experiment two - summer 2001

Field study setup

Eight four frame beehives (nuclei) were standardized using a package bee funnel method by which each package received approximately (6,000 bees) and a queen. Bees

were weighed using a cup measuring scale system as in 2000. The bees and queen were placed in each hive after the packages had been in a dark quiet garage for one evening so that they could settle down. A pollen trap was placed on the front of each hive. The trap had three layers of hardware wire cloth (5 sq. per 2.54 cm.) that the foragers had to pass through to enter and exit the hive. The goal of this trap was to prevent external pollen from being brought into the hive. A drawer was placed under the trap to catch any pollen that fell off the bees' hind legs. Queens were hived and kept in a plastic cage for three days in order for the hive to become accustomed to their pheromone. After three days she was released into a wire cage that was approximately 7.6 cm by 12.7 cm in size. The wire cage restricted the queen's egg laying to specific areas of the frame unit and the amount of brood in the hive. After approximately one week the queens would begin to lay eggs in the cages. After she had laid the entire area of the cage, the queen was moved to a second area in the hive and after laying there was moved to a third cage, until the available comb was all in use.

Pollen preparation

Canola pollen was mixed with a 75% (w/v) sugar syrup solution in a ration of 3:1. Pollen patties were placed in petri dishes, labeled, weighed, and then placed in the hives.

Pesticide test doses

A positive control was needed to prove that the method of the experiment was sound. Several pilot trials were run to try to determine which dose should be used in the final experiment.

Diazinon dose trial one

The doses used in this trial were fed to each hive mixed with 100 g of bee collected pollen. Two hives were fed each dose, with ten hives in total for the entire experiement. The experiement was replicated once. The diazinon doses were as follows: 0 dose, $0.39x10^{-6}$g, $1.95x10^{-6}$ g, $9.6x10^{-6}$ g, and $48.25x10^{-6}$ g per 100 g bee collected pollen.

Diazinon and chlorfluazuron (IGR) dose trial

Increased Diazinon doses and a dose of a new chemical called chlorfluazuron were tested. The treatment doses of diazinon which were fed to three hives each were as follows: 0 dose, 6 mg diazinon, 6.5 mg diazinon, 7mg diazinon per 100 g bee collected pollen. The chlorfluazuron dose was 1.56 mg per 100 g bee collected pollen. This gave a total of fifteen hives for the entire experiment.

Experiment three - summer 2002

Field study setup

Honey bees

In April of 2002, five three-pound packages were established at the Michigan State University Apicultural Building on College Road in East Lansing, Michigan. Each colony was medicated with Fumidil, Terramyacin, Apistan, and Cumophos strips to protect it against *Nosema*, American Foul Brood, and *Varroa* mites respectively. They were fed pollen patties, which were composed of a 3:1 mixture of mixed bee collected pollen and 50% sugar syrup solution. The pollen mixture was wrapped in wax paper and then given to the bees.

Treatment preparation

A positive control that is toxic to larvae but not toxic to adults was needed for the experiment to prove the method was sound. The chemical chosen was diazinon, a common agricultural pesticide. The diazinon dose was determined by performing a pilot trial that resulted in 50% larvae mortality. This dose was calculated to be 888 μg / g pollen. Untreated pollen was also treated with acetone to control for a possible effect of solvent. The acetone was allowed to evaporate and then 5 ml of 50% sugar syrup was added to the mixture to make it into a liquid form. There was 7.5 ug pollen per 6 ul sugar syrup solution. The mixture was then mixed using a vortexer and stored in a freezer (-20° C) until use. Before each trial it was thawed and then vortexed to remix it.

The treatments were as follows: untreated (larvae not fed), bee collected pollen, diazinon treated treated bee collected pollen (888 μg/g pollen), non-transformed corn pollen, corn pollen from Cry1A(b) (Event Bt11) corn, corn pollen from Cry1F (Event TC1507 purchased from Mycogen - Dow Agrisciences) corn, non-transformed canola pollen, and glyphosate resistant (Roundup ready) canola pollen.

Larvae identification and feeding

In five hives, groups of 4-5 d old larvae were selected and divided into eight sections on a transparency. The transparency was pinned to the frame so that it would stay in place. A tack was placed at each corner of the entire brood patch to delineate the treated area. With a permanent marker, the 4-5 d old larvae that were to be treated were circled on the transparency. They were then divided into the eight sections and randomly assigned a number that corresponded to one of the treatments. After mapping, the larvae were fed 6 μl of the designated treatment solution(7.5 ug pollen/ 6 ul sugar syrup) with a 20 μl Eppendorf repeat pipettor.

Larvae mortality, pupal weight, blood protein level, and adult emergence

When the larvae were capped in each of the five hives, we placed the transparency over the brood patch once again and checked for larvae mortality. Empty cells, indicating that the larvae had died and been carried out by a nurse bee, were counted as dead larvae. Larvae were considered alive, if the cell had been capped with wax by worker bees. In trial two, five pupae were collected at the blackeye stage from three hives to compare weights between treatments. The frame of pupae was left in the hive, until 3-4 days before emergence; then the worker bees were brushed off and the comb was placed in an incubator to monitor adult emergence. Blood protein levels of newly emerged bees from three hives were measured for each treatment. Adult emergence was measured in three hives for each treatment. This was done by placing the map over the previously capped cells and counting them as emerged if they were empty.

Midgut dissections

In order to ensure that there was no spread of pollen by nurse bees to other larvae, midgut dissections were made 24 hours after feeding on a subpopulation of larvae that was separate from the experiment. The pollen from the midguts was compared to slides made of each pollen type to make sure that it matched the original pollen that was fed to the larvae. The number of pollen grains in the non-transformed canola treatment was measured prior to feeding with a hemocytometer. After the midguts were dissected, the number of pollen grains in guts of larvae fed non-transformed canola were recorded, again using a hemocytometer.

STATISTICAL ANALYSIS

Experiment one – summer 2000

Pupal weights were analyzed using SAS general linear model (GLM) and the means were separated with Tukey's HSD test (SAS Institute 1999).

Experiment two – summer 2001

Percent honey bee larval mortality in the two canola pollen treatments was analyzed using SAS t test (SAS Institute 1999). The diazinon dose trial one and the diazinon and chlorfluazuron dose trial data was transformed using sqrt (x+.5) to stabilize the variance. Data was then analyzed with SAS general linear model. Means were separated using Tukey's HSD test (SAS Institute 1999).

Experiment three – summer 2002

All data was analyzed as a randomized complete block design, where each hive served as a block. Percent larval mortality, pupal weight, blood protein level, and percent adult emergence were analyzed using SAS general linear model procedure (GLM). Means were separated with Tukey's HSD test (SAS Institute 1999). Adult emergence data could not be analyzed because there was no variation in seven of the eight treatments (all but diazinon).

RESULTS

Experiment one – summer 2000

Results show a significant difference in the pupal weights of the Cry1F treatment and the diazionon treated corn pollen ($P<0.05$) (Figure 1).

Experiment two – summer 2001

Results show no significant difference between treatments in the diazinon dose response trial (F=.47, df=4, 10, P=.7591) (Figure 3). There was no significant difference in the diazinon and chlorfluazuron dose response trial treatments (F=.72, df=4, 10, P>0.05) (Figure 4). These dose pilot trials proved to show puzzling results with the control treatments having the as high of mortality as the pesticide doses being tested. Results (Figure 5) show a significant difference between the percent mortality of the larvae fed regular canola pollen vs. larvae fed Roundup Ready® Canola pollen (t=2.57, df=1, P=0.0498). However, there is a large inter-hive variation in the Roundup Ready® canola pollen treated hives, yielding a treatment mean standard error of 9.29.

Experiment three – summer 2002

The results show a significant difference in the percent mortality between larvae fed diazinon treated pollen and the canola pollen fed larvae. However, there was not a significant difference between larvae fed transgenic glyphosate resistant canola pollen vs. larvae fed non-transgenic canola pollen (F= 35.94, df=4, 45, P=.3142) (Figure 6). There is a significant difference between the diazinon treated pollen and the corn pollen treatments. However, there is no significant difference in percent mortality between larvae fed non-transgenic corn pollen vs. those fed transgenic Cry1Ab and Cry1F pollen treatments (F= 45.45, df=5, 50, P=.0556) (Figure 7).

There is a significant difference in mean pupal weight between bees fed diazinon treated pollen and the canola pollen fed larvae. However, there was not a significant difference between larvae fed transgenic glyphosate resistant canola pollen vs. larvae fed non-transgenic canola pollen (F=23.99, df=4, 68, P<0.05)(Figure 8). Significant

differences in the mean pupal weight between larvae fed diazinon treated pollen and those fed the three corn pollen treatment were found. However, there is no significant difference between mean pupal weight of non-transgenic corn pollen fed larvae vs. those fed transgenic Cry1Ab and Cry1F pollen treatments ($F=26.98$, $df=5, 82$, $P<0.05$) (Figure 9).

There were no diazinon fed larvae that survived to adult emergence in the hives tested for blood protein concentration. Thus, only the other seven treatments were measured for blood protein concentration. There were no significant differences in blood protein concentrations between larvae fed glyphosate resistant canola pollen and those fed non-transformed canola pollen ($F=1.23$, $df=3, 56$, $P>0.05$) (Figure10). No significant differences were found in blood concentration (ug/ml) of larvae fed non-transformed corn pollen and those fed Cry1F corn pollen and Cry1Ab corn pollen ($F=1.78$, $df=4, 70$, $P>0.05$) (Figure 11). All treatments except for diazinon had a 100% emergence rate. Thus, the data could not be analyzed in SAS since there is no variation. Diazinon had a 28.25 ± 2.4 percent emergence rate.

Larvae dissection

Honey bee larvae midgut dissections were conducted a sub-sample of larvae not used in the experiment to monitor the type of pollen that were in the larvae's midgut. There was no spread of the pollen by worker bees; each larvae had the correct type of pollen in their midgut that had been given by pipette. Six ul of non-transformed canola pollen was mixed with 394 ul of 50% (weight/volume) sugar syrup. Pollen grains were counted on a hemocytometer. The average count of pollen grains in .1ul of this solution was 27.8 ± 1.01. After dissection the larvae midguts of larvae fed non-transformed

canola pollen were ground with a pestle and then diluted with 394 ul of 50% sugar syrup (weight/volume). Counts were taken from three larvae midguts. They had an average recovery count of 22.3 $\pm$ .68 pollen grains in .1ul of the solution. Only pollen of a size 50% or greater of the original was counted. Some of the pollen had been digested and was broken into small pieces and so was not counted. Thus, an average of 80% of the pollen fed to the larvae was found in the midguts.

DISCUSSION

Experiment one

Further trials must be done in order to test for the effect of transgenic Bt corn pollen on honey bee worker larvae and adults. Future studies should include larvae mortality as well as pupal weight and blood protein measurements.

Experiment two

The methodology in these experiments needs to be improved in many ways to avoid the inter-hive variation that was found in the pilot study. Normal hive larvae mortality is about 10% on average due to genetic abnormalities, weather, disturbances to the hive, or lack of food to feed the brood. This experiment placed many unnatural controls on the hives that were used in each treatment. The first of which is that the hives were made up of only 6,000 bees, a number that places them at a disadvantage for warding off diseases, predators, and pests. Secondly, the queen was caged and only allowed to lay a small amount of eggs and was moved every few days. Thus, the hive populations were not replenished. The pollen trap might have also been a factor in disrupting the colony and causing variation. I do not believe that the significant results of the canola trial can be taken at face value without considering the great variation. This

protocol must be modified to decrease control mortality before this experiment can be performed again.

Experiment three

The results suggest that transgenic Bt corn and glyphosate resistant canola pollen do not have a negative effect on worker larvae survival, pupal weight, blood protein level or adult emergence when fed on the 4th or 5th day of development. Further studies should be done in order to compare the foraging behaviors and longevity of workers fed transgenic Bt corn pollen and canola pollen with those fed regular non-transformed pollen. While the benefits of genetically engineered crops may be significant, including reduced pesticide use and increased yield, potential negative impacts must be carefully assessed before introducing them to the environment.

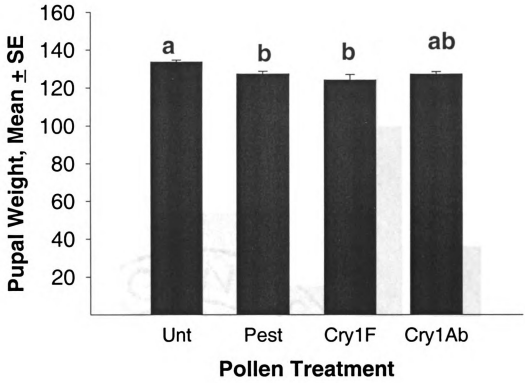

Figure 1. Mean pupal weight of bees fed pollen treatments.

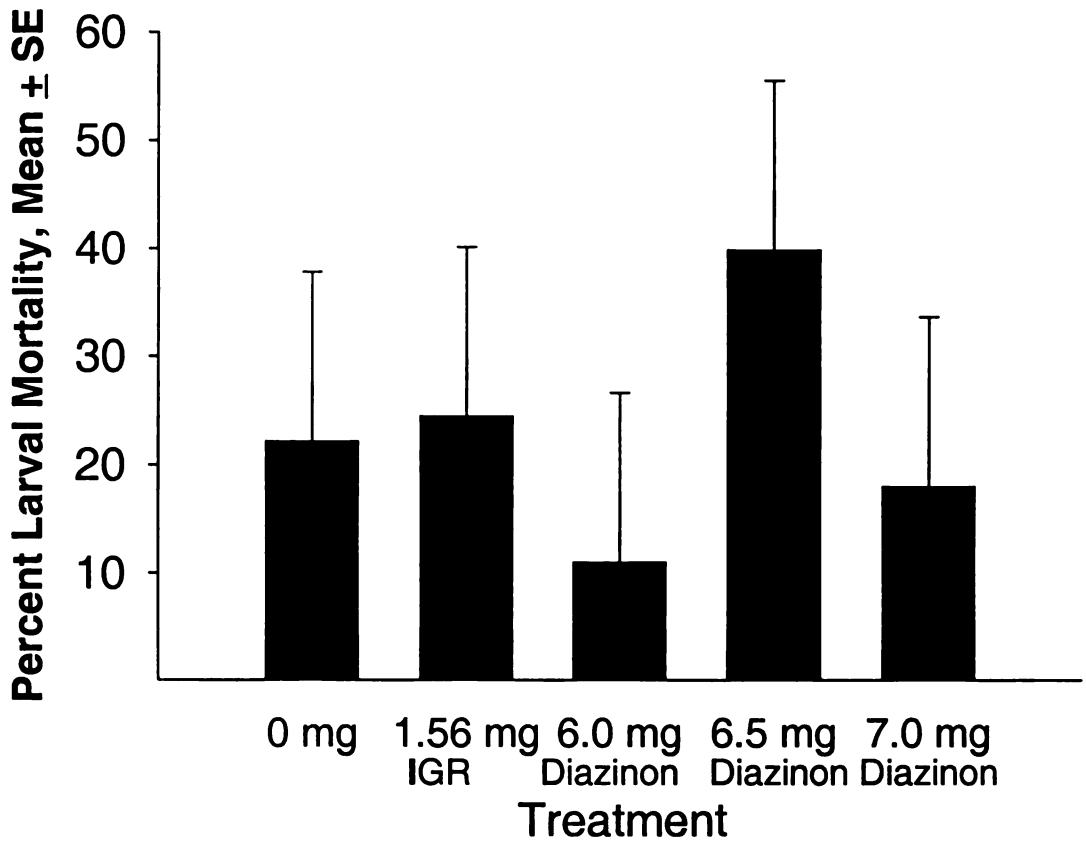

Figure 2. Percent larval mortality for diazinon and chlorfluazuron (IGR) dose response trial.

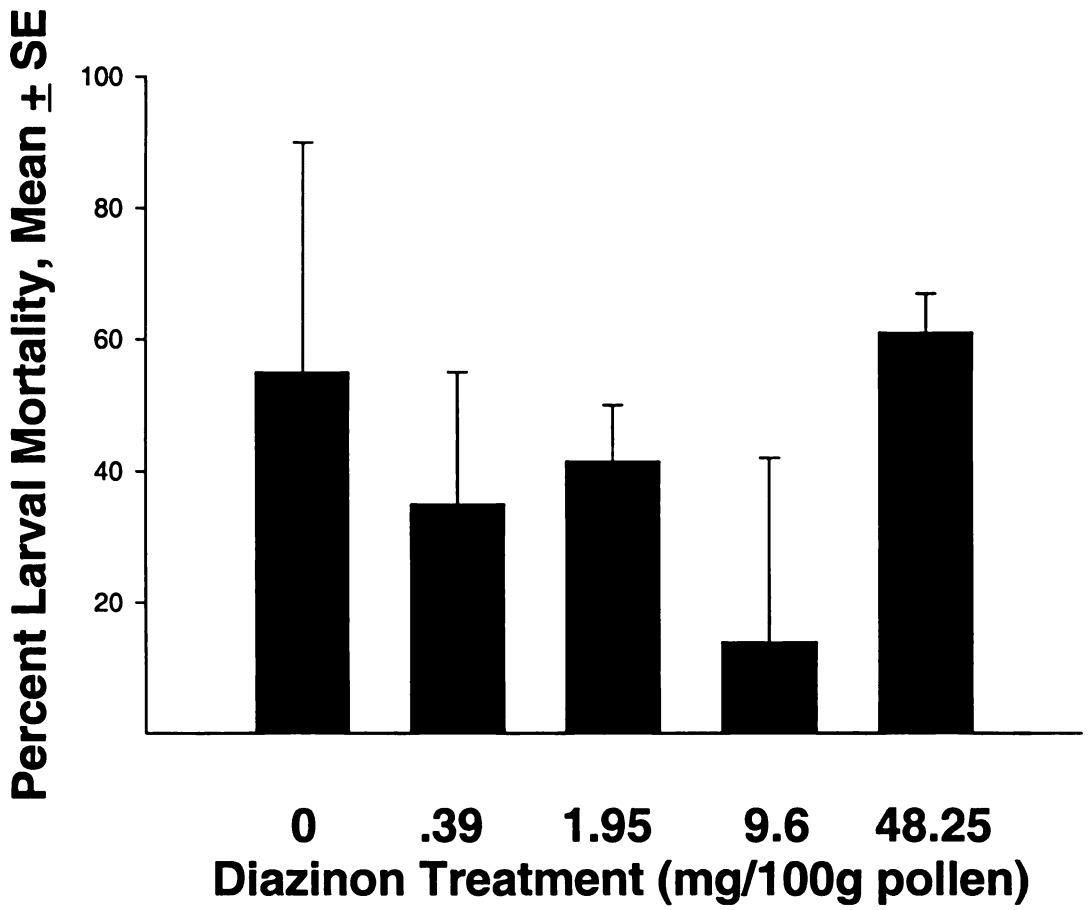

Figure 3. Percent larval mortality of larvae in diazinon dose trial.

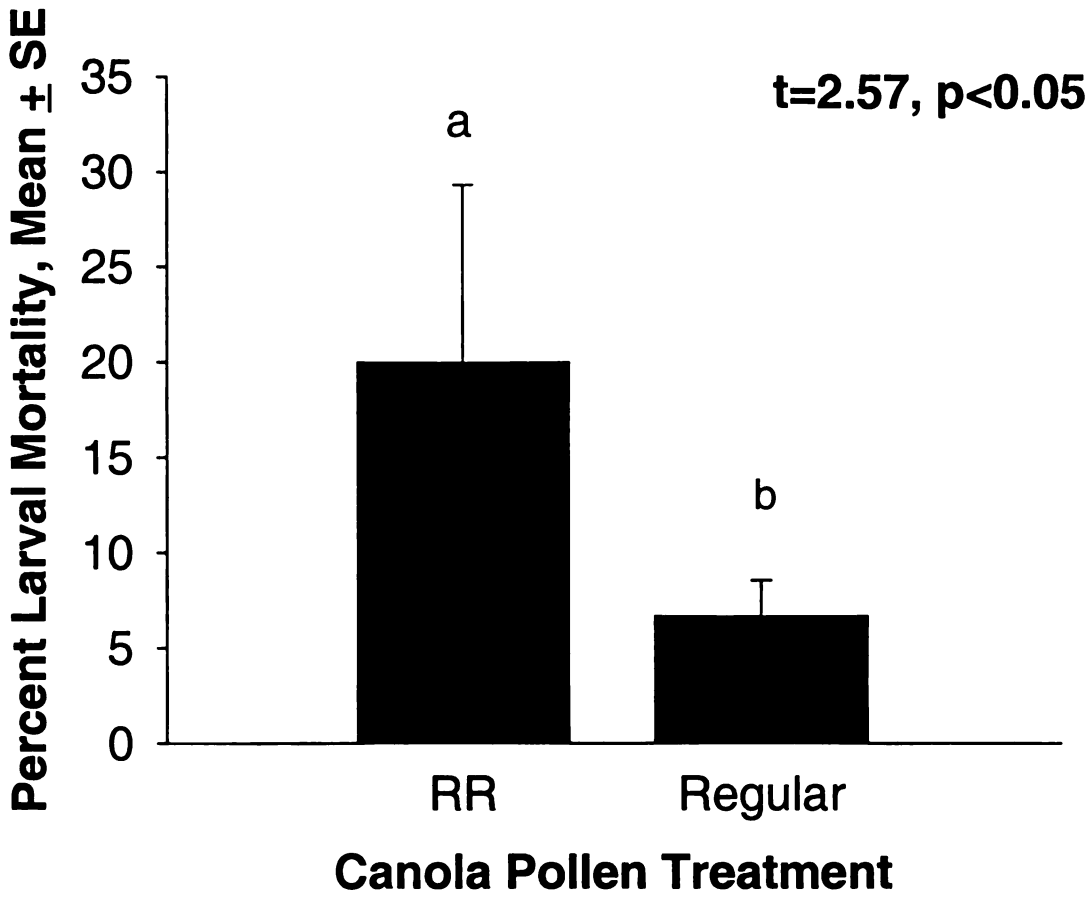

Figure 4. Honey bee larvae mortality measurement after being fed canola pollen treatment.

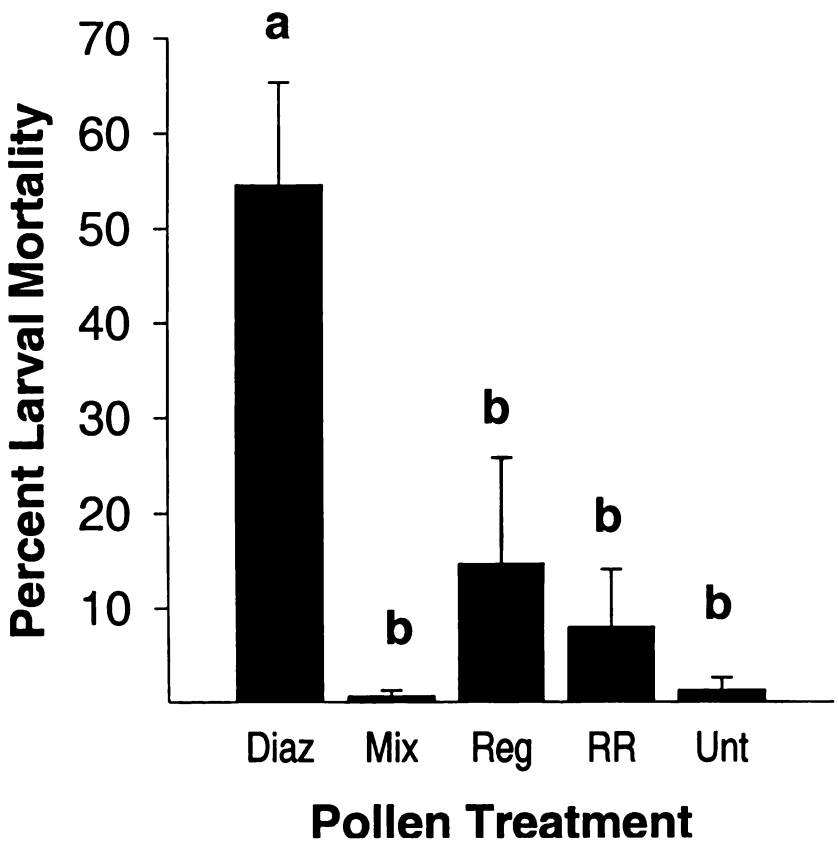

Figure 5. Percent larval mortality of bees fed canola pollen in trial one.

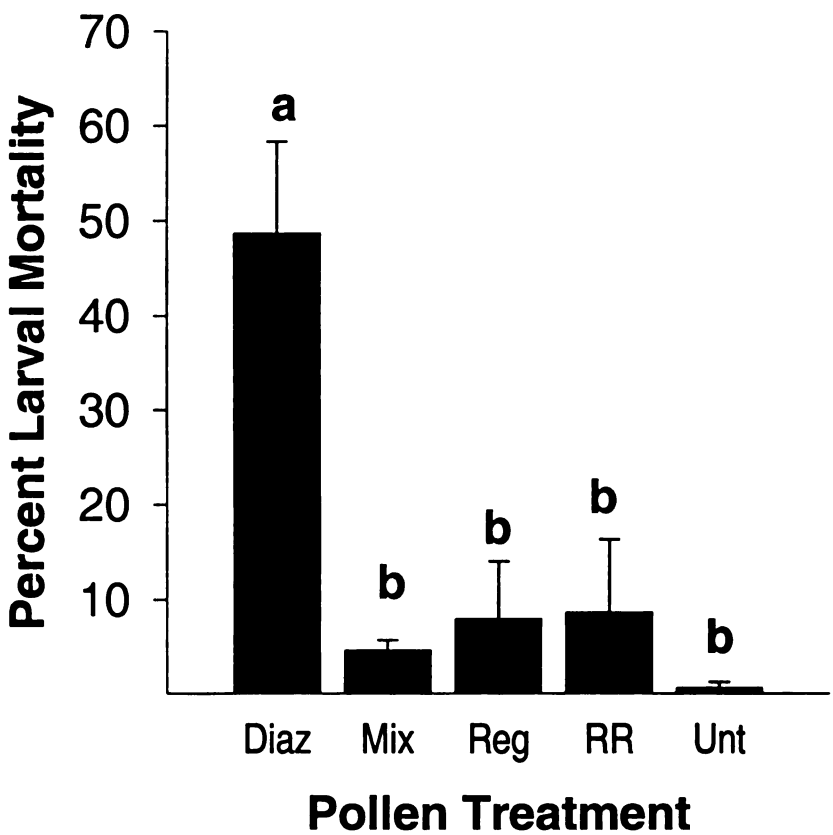

Figure 6. Percent larval mortality of bees fed canola pollen in trial two.

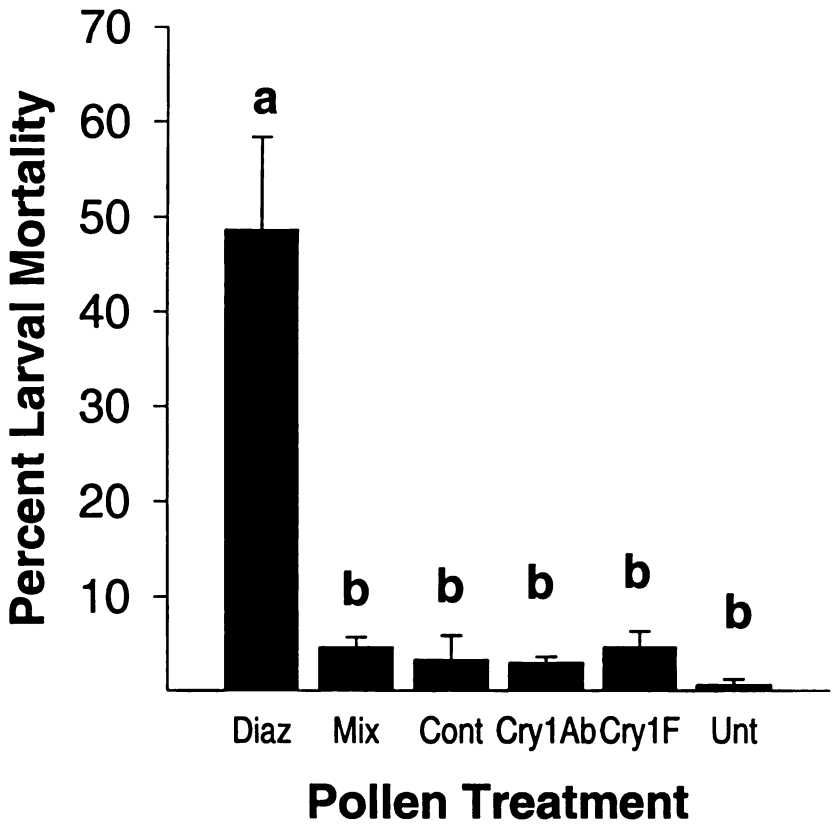

Figure 7. Percent larval mortality of bees fed corn pollen in trial one.

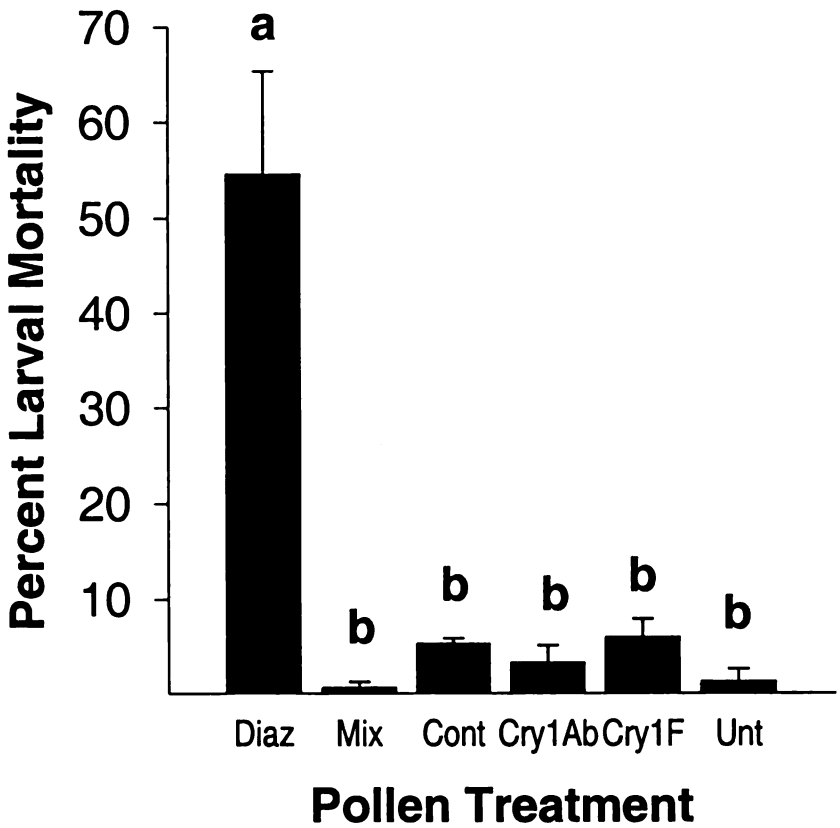

Figure 8. Percent larval mortality of bees fed corn pollen in trial two.

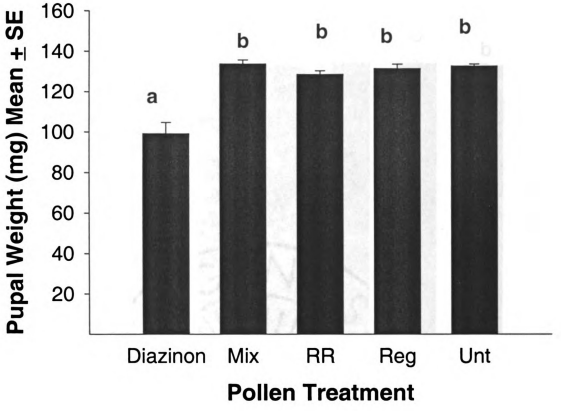

Figure 9. Pupal weight of bees fed canola pollen in trial one.

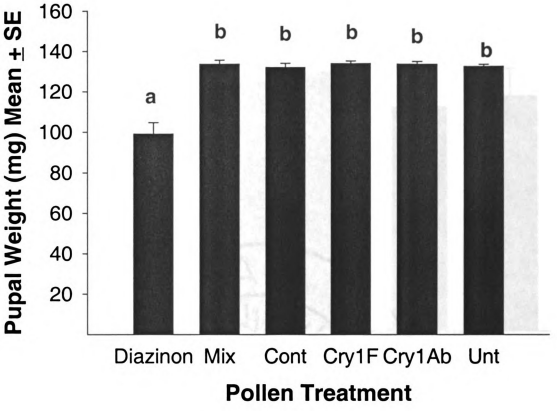

Figure 10. Pupal weight of bees fed corn pollen in trial one.

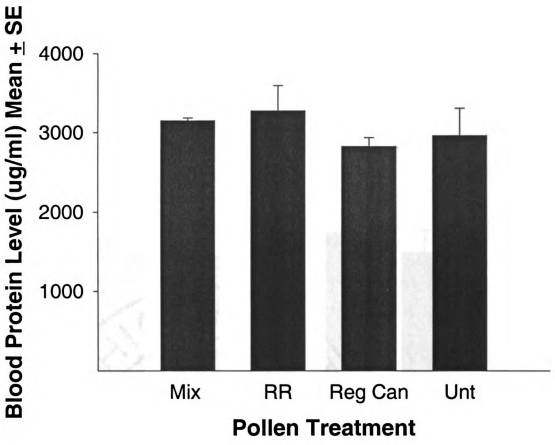

Figure 11. Blood protein concentration of bees fed canola pollen (ug/ml hemolymph) in trial one.

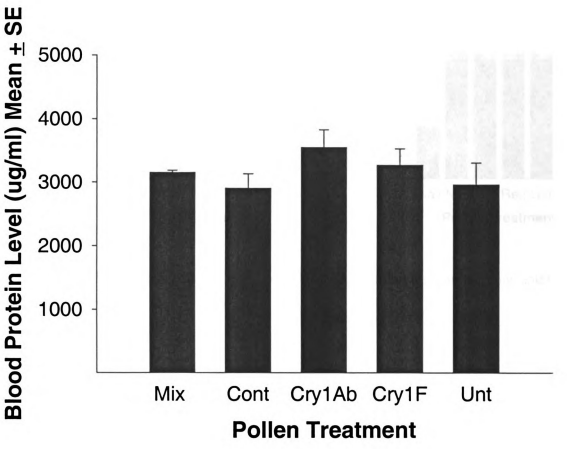

Figure 12. Blood protein concentration of bees fed corn pollen (ug/ml hemolymph) in trial one.

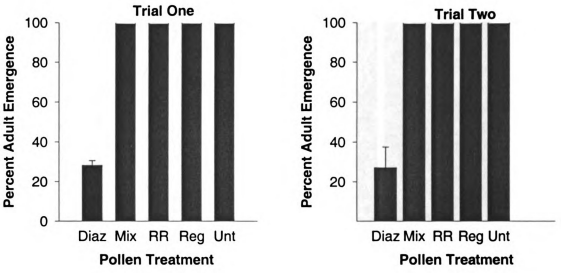

Figure 13. Percent adult emergence of bees fed canola pollen in trial one and two

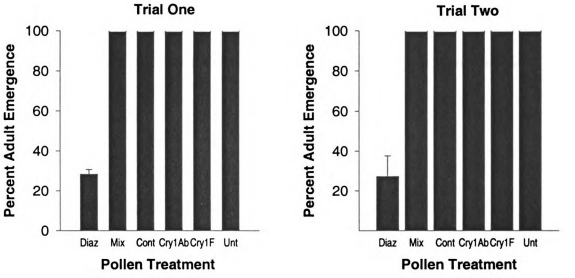

Figure 14. Percent adult emergence of bees fed corn pollen in trial one and two.

CHAPTER TWO

EVALUATION OF THE USE OF Bt CORN POLLEN AS CONTROLLING METHOD FOR THE GREATER WAX MOTH, (*Galleria mellonella* L.)

ABSTRACT

The greater wax moth, *Galleria mellonella* L. is a pest of the honey bee. The larvae feed on the pollen of a hive and in doing so make messy burrows and tunnels of webbing. This activity can destroy a weak hive or stored honeycomb in a short time. New natural controls that could be used in the field would be very beneficial for the beekeeping industry. Bt corn pollen is a potential method for controlling for the greater wax moth for several reasons. In a laboratory study, we fed first instar wax moth larvae three types of corn pollen: non-transformed corn pollen, Cry1Ab corn pollen, and Cry1F corn pollen. We found that the mortality of larvae fed Cry1F corn pollen was significantly greater than the mortality of larvae fed Cry1Ab or non-transformed corn pollen ($P < 0.05$). In each trial Cry1F fed larvae had a 100% mortality.

INTRODUCTION

Wax moth biology

Like other animals, honey bees are plagued by many diseases and pests. One of those pests is the greater wax moth, *Galleria mellonella* L., a pest of stored comb and weak colonies. Larvae of the greater wax moth cause considerable damage to beeswax combs left unattended by bees. The larvae feed on the pollen of a hive and in doing so make messy burrows and tunnels of webbing. This activity can destroy a weak hive or stored honeycomb in a short time (Williams 1978). The wax moth is a holometabolous insect having four stages in its life cycle: egg, larvae, pupae and adult. The moth thrives at a temperature of 30 degrees Celsius and at a humidity of 70% with total darkness (Williams 1978).

Control methods

Methods of controlling the wax moth in the past have used chemicals such as paradichlorobenzene, ethylene dibromide, methyl bromide, and ethylene oxide to fumigate stored equipment. These chemical fumigants have several disadvantages. They may be toxic to humans and animals. During an average four month storage period, several applications are often required. After fumigation, a closed system in the storage area is needed and this system is difficult and expensive to maintain (Cantwell 1981). Many of the fumigants are not legal for use in controlling wax moth.

An alternative method to chemical control of wax moth, Thuricide 90TS®, a *Bacillus thuringiensis* preparation that was incorporated into beeswax foundation was studied in the early 1960's. However, it did not prove to be very successful. In the 1980's, a *Bacillus thuringiensis* bio-pesticide, Certan®, was developed to treat honey bee

colonies in the field (Cantwell 1981). This new bio-pesticide was available for sale by bee supply companies but was not profitable to make so it is no longer manufactured (Caron 1999). Current control methods that are approved are extreme temperatures, which are very time consuming for the beekeeper and requires a large freezer or heating device. New natural controls that could be used in the field would be very beneficial for the beekeeping industry. Bt corn pollen is a potential control method for the greater wax moth for several reasons. First of all, Bt Cry1Ab and Cry1F toxins are specific for Lepidoptera, so it might be toxic to the wax moth. Secondly, Bt corn is already grown so the pollen is available to bees. Thirdly, this method of control, should it prove successful, is economical, non-chemical, and does not cause honey contamination.

MATERIALS AND METHODS

Laboratory biological assays were conducted in the winter of 2000 at Michigan State University. Later instar wax worm larvae were purchased at a local shop (Grand River Bait and Tackle Shop, Lansing, Michigan). They were then raised to adulthood on an artificial diet. The wax moth diet consisted of the following ingredients: 8 parts wheat bran, 6 parts corn meal, 6 parts whole wheat flour, 2 parts brewer's yeast, 10 parts glycerin, and 5 parts distilled water (Hoopingarner 1990).

After the larvae pupated, they were placed in 2.2 liter plastic containers (12x 30cm), at a density of about 25 pupae per container. Strips of wax paper were cut and folded accordion style and then paper-clipped and placed into the containers. The wax paper strips served as oviposition sites for the adult gravid female moths.

Once the females had laid eggs on the strips, they were removed and the eggs were placed in a plastic container to hatch. After five days, newly hatched larvae were

transferred into small plastic containers holding the pollen being tested. The larvae were transferred using small pieces of wax paper to pick them up being careful not to damage them in the process.

This experiment was performed using a completely randomized design. There were three treatments: non-transformed corn pollen, corn pollen containing the toxin Cry1Ab, and corn pollen containing the toxin Cry F. There were three replicates for each experimental unit, which consisted of small plastic Solo cups that had 2.5 g of the pollen in each. Five larvae were placed in each cup and then placed in the growth chamber at 70% relative humidity, 30 ° C in complete darkness. After nine days, each cup was inspected for larvae mortality. A microscope was used to locate the larvae due to their small size. This study was repeated four times.

STATISTICAL ANALYSIS

Wax moth percent mortality in the pollen treatments Cry1Ab and untreated was analyzed using logistic regression to determine the least square means assuming a binomial distribution. Data were analyzed with SAS GLM (general linear model) procedure. Means were separated using Tukey's HSD test (SAS Institute 1999).

RESULTS

Wax moth larvae fed Cry1Ab corn pollen and untreated pollen do not show a significantly different mortality from one another. Cry1F was statistically significant from both the untreated pollen and Cry1Ab corn pollen ($p< 0.05$). With an unfailing 100% mortality rate, Cry1F provides excellent control for the greater wax moth in a laboratory setting.

DISCUSSION

Cry1Ab does not appear to be an effective control of wax moth, as it shows no significant difference from the untreated pollen. Although Cry1Ab is specific for Lepidoptera, it did not have an effect on this specific moth.

Cry1F corn pollen is an effective control for wax moth in a laboratory. We did not collect enough Cry1F pollen to perform any further tests. I would recommend that a laboratory choice test and a field trial be done to solidify our conclusions. The field test could be done by placing honey bee hives in the middle of a Cry1F corn field during tassling and submitting them to wax moth infestation.

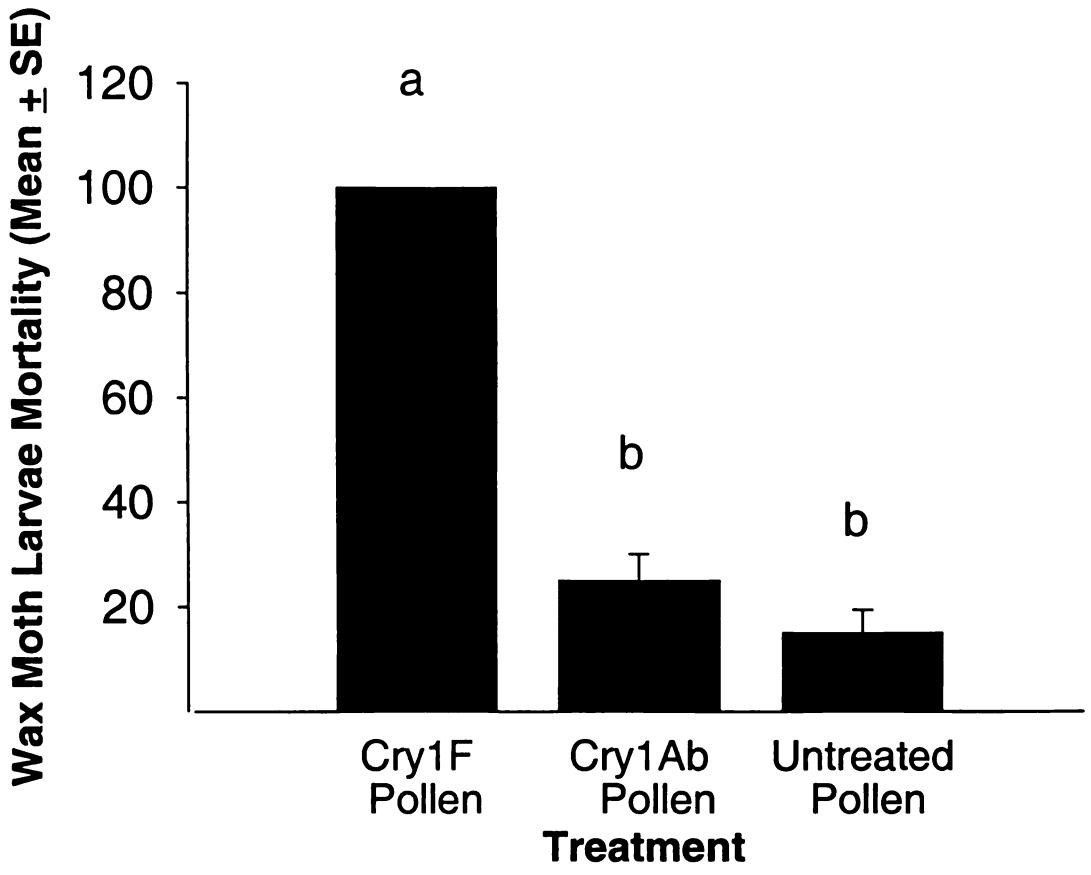

Figure 15. Wax moth larvae mortality measurements from four trials of a laboratory study.

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LITERATURE CITED

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APPENDIX

Appendix 1

Record of Deposition of Voucher Specimens*

The specimens listed on the following sheet(s) have been deposited in the named museum(s) as samples of those species or other taxa, which were used in this research. Voucher recognition labels bearing the Voucher No. have been attached or included in fluid-preserved specimens.

Voucher No.: 2002-06

Title of thesis or dissertation (or other research projects):

Effect of transgenic Bt Corn pollen and Roundup Ready canola pollen on honey bee (*Apis mellifera* L.) worker larvae and the use of transgenic Bt corn pollen as a controlling method for the greater wax moth (*Galleria mellonella* (L))

Museum(s) where deposited and abbreviations for table on following sheets:

Entomology Museum, Michigan State University (MSU)

Other Museums:

Investigator's Name(s) (typed)
Anne Valdes Hanley

Date June 27, 2002

*Reference: Yoshimoto, C. M. 1978. Voucher Specimens for Entomology in North America.
Bull. Entomol. Soc. Amer. 24: 141-42.

Deposit as follows:

Original: Include as Appendix 1 in ribbon copy of thesis or dissertation.

Copies: Include as Appendix 1 in copies of thesis or dissertation.
Museum(s) files.
Research project files.

This form is available from and the Voucher number is assigned by the Curator, Michigan State University Entomology Museum.

Appendix 1.1

Table 1: Voucher Specimen Data

Page 1 of 1 Pages

Species or other taxon	Label data for specimens collected or used and deposited	Number of: Eggs	Larvae	Nymphs	Pupae	Adults ♀	Adults ♂	Other	Museum where deposited
Galleria mellonella (L)	Michigan, Ingham Co., Lansing e.x. Bait shop		13			8			MSU
Galleria mellonella (L)	Michigan, Ingham Co., East Lansing MSU Campus ex. Honey bee hive		10		4	4			MSU
Apis mellifera L.	Michigan, Ingham Co., East Lansing MSU Campus, Apiary ex. Honey bee hive		11			14			MSU

(Use additional sheets if necessary)

Investigator's Name(s) (typed)

Anne Valdes Hanley

Date 27-Jun-02

Voucher No. 2002-06

Received the above listed specimens for deposit in the Michigan State University Entomology Museum.

Curator 6 July 2002

Date