AN ABSTRACT OF THE THESIS OF

Elif Barçin Dogan for the degree of Master of Science in Entomology presented on September 14, 1994.

Title: Development and Reproduction of Convergent Lady Beetle Feeding on Green Peach Aphid Exposed to Btt

Abstract approved: Redacted for Privacy

Philippe A. Rossignol

The impact of *Bacillus thuringiensis* on non-target organisms is of concern in agriculture. In order to assess any indirect effect of *B. t. tenebrionis* (Btt), which contains a coleopteran specific δ-endotoxin, on the convergent lady beetle (*Hippodamia convergens*), I determined the effect of Btt on the developmental biology and reproduction of the lady beetles when fed on green peach aphids (*Myzus persicae*) exposed to three different applications of Btt on potatoes.

I reared convergent lady beetles in the laboratory on potatoes sustaining colonies of green peach aphid. In the first set of experiments, plants and aphids were sprayed every week with Novodor, a commercial preparation of Btt. I found no changes in larval feeding behavior, or development period to maturity. Pupal weight, however, was reduced by 22% in the Btt treatment and emerging adults reared on the Btt treatment showed a lower ovipositional rate. In the second experiment, aphids reared on the transgenic potatoes carrying the Btt δ-endotoxin gene were fed to convergent lady beetles. There was no change in the feeding behavior, development period, or pupal weight. However, emerging adults in the transgenic treatment laid more eggs than those in the control treatment. A third experiment tested the toxicity of Btt on fourth instar

convergent lady beetles. An increase in development time of fourth instars was observed. Mortality occurred after the ninth day.

Overall, the results suggest that Btt may have a potential sublethal and negative direct effects on convergent lady beetles. Further studies are required to validate these results.

Development and Reproduction of Convergent Lady Beetle Feeding on Green Peach Aphid Exposed to Btt

by

Elif Barçin Dogan

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Completed September 14, 1994

Commencement June 1995

Master of Science thesis of Elif Barçin Dogan presented on September 14, 1994

APPROVED:

Redacted for Privacy

Co-Major Professor, representing Entomology

Redacted for Privacy

Co-Major Professor, representing Entomology

Redacted for Privacy

Chair of Department of Entomology

Redacted for Privacy

Dean of Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Redacted for Privacy

Elif Barçin Dogan

ACKNOWLEDGEMENT

A heartful thanks is owed to my co-major professors, Dr. Philippe A. Rossignol and Dr. Ralph E. Berry. Without their encouragement and constructive criticisms this thesis may never have come to be. I also thank Dr. Patrick M. Hayes and Dr. Ken Johnson for their review of my manuscripts and participating on my committee. My special appreciation goes to Dr. Berry and Dr. Rossignol for their enlightening discussions and moral support throughout my education in Oregon State University. I wish to thank Dr. Gary L. Reed who provided an endless supply of transgenic potatoes and Dr. John E. Dunley in UC, Berkeley, for sending us a non-commercial preparation of Btt. I would like to thank Greg Brenner and Ben Lyons for giving me ideas with the statistical analysis.

I would like to extend my sincere gratitude to my friends Jong-Neng Shieh, Armando Equihua-Martinez, Pete Oboyski, John Paustian and many others for their help and support.

Last, certainly not least, I would like to thank to my parents, to whom I owe everything, for their love and support in many ways.

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES TABLES

LIST OF APPENDICES TABLES (Continued)

DEVELOPMENT AND REPRODUCTION OF CONVERGENT LADY BEETLE FEEDING ON GREEN PEACH APHID EXPOSED TO BTT

Chapter 1

Introduction

Microbial pest control agents (MPCA) have been in commercial use since the 1950's. A variety of bacteria, protozoa, fungi, and viruses are known as microbial pest control agents. Like conventional chemical control agents, microbial insecticides are known to have side effects. It is important to understand selectivity of microbial pesticides and their effects on the natural enemies of target pests.

Bacillus thuringiensis Berliner (Bt) is a gram-positive, spore forming bacterium that has been used as an effective microbial insecticide for many years. *B. t.* ssp. *tenebrionis* (Btt) is Coleopteran-specific, and was originally isolated from the yellow mealworm, *Tenebrio molitor* L. Btt has been utilized in insecticidal formulations, and the genetic code for its toxin has been recently incorporated into the genome of potatoes. Btt is considered environmentally safe. The bacterium produces δ-endotoxin which is enclosed in a crystal protein polyhedron. Upon ingestion, the protein polyhedron is broken down in the insect midgut releasing the δ-endotoxin which acts on the apical membrane of the midgut epithelial cells. These cells either begin to swell or collapse causing gut paralysis, which inhibits further feeding. The result is death.

Btt is used commercially to control *Leptinotarsa decemlineata* (Say.), Colorado Potato Beetle in potato fields. Green peach aphid, *Myzus persicae* (Sulzer.) also is an important pest which damages the plants by transmitting viral diseases and by feeding on leaves. Coccinellids are one of the most common predators of aphids. In this study, *Hippodamia convergens* (Guerin and Manaville), the convergent lady beetle, was used as

a test predator. During Btt applications against Colorado potato beetle, green peach aphid also is exposed to the spraying. Therefore, the objective of the study was to determine the effect of Btt δ-endotoxin on *H. convergens*, feeding on *M. persicae* exposed to Btt. Three different applications of Btt were tested: first, the microbial insecticide Novodor was sprayed on aphid prey; second aphids feeding on transgenic potatoes were fed to lady beetles; and in a final experiment a non-commercial preparation of Btt crystals and spores were mixed with an artificial diet and fed to the convergent lady beetles to test for any direct or sublethal effects of Btt.

Chapter 2

Review of literature

2.1 Economic Importance of Potato

Potatoes are the most important root crop in terms of world production (Dalton, 1978; Horton, 1987). Originating in the Andes of South America, potato cultivation has spread throughout the world (Burton, 1985; Horton and Anderson, 1992). Among the world's food crops, potato is in the top five in tonnage (Horton, 1987). As a food energy source, it provides 1/6 as many calories as rice, wheat and maize (Horton, 1987). In terms of nutrients, potatoes are a high source of energy compared to other crops (Dalton, 1978; Horton, 1987).

World potato production was about 135 million tons at the turn of the 20th century, 250 million tons around 1950's, and 290 million tons in the late 1980's (Horton and Anderson, 1992). For the developing market economies, potato production has increased more than the production of most other food crops (Horton, 1987). During the first half of the century, Europe and Russia produced about 90% of the world's production. Since 1960, production in Europe has dropped. Currently, Eastern Europe and Russia produce just under half of the world's total production, and Western Europe produces 17%. Nearly 15% of the world's production comes from the Far East. The remainder comes from America, Oceania, Africa and the Middle East (Horton and Anderson, 1992).

The balance of the world potato production is gradually shifting from the developed to developing countries, and from the temperate zones to tropical and subtropical areas (Horton and Anderson, 1992). Developing countries also have started reducing potato production because incomes have risen and more processing is added to the potato value before it reaches the consumer (Dalton, 1978; Horton, 1987).

Potatoes are consumed by humans, fed to animals, and serve as a raw material for starch and alcohol. Potato starch is particularly suitable for sizing paper and textiles, and is used as starch for cotton goods. Alcohol is prepared by the fermentation of cooked potatoes with yeast, followed by distillation. The use of potato to produce alcohol is not economical without a large potato yield (Burton, 1985). The only flexibility of increased demand in potatoes is in the animal feeding market, which depends more on relative costs than the human food market. Relative price per unit of nutrient determines the feeding of fresh potatoes to animals (Dalton, 1978).

Most countries producing potatoes are self-sufficient and potatoes have a large amount of dietary energy per unit of land. Due to the standard of living in low-income countries, potato production is important because root crops provide a large part of the population's energy requirement and a good source of nitrogen, vitamin C and starch (Dalton, 1978; Burton, 1985). Unfortunately, potatoes also are subject to attack by a wide spectrum of pests and diseases. These diseases and pests limit productivity and affect the quality of plants (Gunn, 1990).

2.1.1 Colorado Potato Beetle

Colorado Potato Beetle (CPB), *Leptinotersa decemlineata* (Coleoptera: Chrysomelidae), was discovered in 1823, in the midwest of the USA, feeding on a solanaceous weed, *Solanum rostratum* L. When settlers started growing potatoes, the beetle found the potato plant to be a suitable host, and ate its way eastward through the settled regions where potatoes were cultivated. In 1875, the beetle reached the border of the Atlantic Ocean and was transported accidentally to Europe. In 1921, CPB was introduced to south-western France. By the beginning of World War II, the beetle had spread throughout Europe. By the end of the war, the beetle was established in Spain,

Belgium, Chezchoslovakia, Holland, Switzerland, Germany, Poland, Denmark, Portugal, Austria, Italy, Yugoslavia, Russia and Turkey (Burton, 1989, Ramen and Radcliffe, 1992).

CPB has two to three generations per year. The beetle overwinters as an adult in the soil. Emergence in spring is usually before potatoes have emerged. Females lay 300-500 eggs, in masses of 20. There are four larval instars. They are voracious feeders and complete defoliation can occur; 75% of total foliage consumption by immatures is caused by the fourth instar. Use of Paris green (cuprous acetoarsenite) was the first successful attempt of insecticidal control of CPB (Ramen and Radcliffe, 1992). Subsequently, DDT was used to control the beetle (Burton, 1989). Carbamates for soil treatment and organophosphates and pyrethroids also were used as foliar sprays to control the damage. The systemics, such as arsenicals and DDT, provided good overall control for several years, but as a result of continuous exposure, CPB developed resistance to the pesticides (Gauthier, *et al.*, 1981). At present, the most commonly used insecticides by growers are esfenvalerate, permethrin, azinphosmethyl, oxamyl, endosulfan, and cryolite (Ferro, *et al.*, 1993; Vencill and Zehnder, 1993).

CPB has been controlled experimentally by *B. t. thuringiensis* β-exotoxin, the bacteria, entomopathogenic fungi (especially *Beauveria bassiana*), and nematodes. *B. t. sandiego* and *B. t. tenebrionis* produce a polypeptide toxin that kills young beetle larvae. These have been commercially available as microbial insecticides since the 1980's. Recently, several companies have inserted the gene for producing Btt δ-endotoxin into the genome of potato plants. Availability of these cultivars may reduce insecticide usage, but researchers are concerned about the possible development of resistance in CPB to Btt δ-endotoxin (Ramen and Radcliffe, 1992).

2.1.2 Transgenic Potatoes

The first transgenic plants were engineered in the 1980's. Since then, it has been possible to transform a number of plant species. For example, β-phaseolin gene was transferred from bean to sunflower and tobacco plants (Peferoen, *et al.*, 1990). Most plant transformation research has focused on developing plants that are resistant to insects and diseases and that can tolerate certain herbicides. Insect-resistance research has centered on inserting a gene obtained from the bacteria *Bacillus thuringiensis* into plants (Dale and Flavell, 1988).

The mechanism utilized by one tumerous gall forming pathogen, *Agrobacterium tumefasciens*, has proven to be an efficient way to transform genes in certain species, including the potato (Walden, 1989). Plants regenerated from stems were highly aneuploid, therefore the leaf disc transformation method has been applied to potato. Regeneration and transformation have been achieved with the following varieties: Bintje, Berolina, Desiree, Russet Burbank, Kennebec and Kathadin potato cultivars (Peferoen, *et al.*, 1990). Many biological substances toxic to insects are mostly secondary metabolites. Engineering insect-resistance in a plant with a secondary metabolite requires the cloning of a complete metabolical pathway. Therefore, molecular breeders are focusing on toxins, which are the primary products of single genes (Walden, 1989; Gatehouse, *et al.*, 1991).

B. thuringiensis is a sporulating bacterium which produces crystal proteins (Cry) upon sporulation. Two forms of crystal proteins have been described. The first group of Cry is toxic to Lepidoptera and some Diptera, and synthesized as proteins of 130 kD, which are activated in the insect midgut to toxins of some 60 to 70 kD. The second group of Cry contains some of the Diptera and CPB toxins and proteins of 60 to 70 kD. Upon ingestion, swelling and bursting occur in the epithelial cells of the midgut (Höfte, *et al.*, 1989; Peferoen, *et al.*, 1990; Rhim, *et al.*, 1990; Qiang, 1993). Bt toxins bind to receptors

in the membrane of the insect midgut epithelial cells. The interaction between the binding sites and the toxin determines host specificity (Cheng, *et al.*, 1992; Peferoen, *et al.*, 1990).

For foreign gene expression in a plant cell, the gene has to be preceeded by a plant promoter. Two promoters, cauliflower mosaic virus 35S and the TR promoter from the Ti- plasmid of *Agrobacterium*, have been successfully used in engineering insect resistant plants. The TR promoter has been used for Bt toxin expression in potato. Potatoes have been transformed with a Btt gene -CryIIIA- highly toxic to CPB. Bt Cry genes are poorly expressed in transgenic plants, therefore the expression level is enhanced by truncating the gene, so that only the toxin encoding partial of the gene is transferred to the plant genome (Höfte, *et al.*, 1987; Peferoen, *et al.*, 1990; Gatehouse, *et al.*, 1991). Genetically engineered pest-resistant cultivars may help limit the use of chemicals which are not environmentally safe, but if these genes are widely used in homogenous commercial cultivars, pests may develop resistance and cause rapid loss of this pest management tool (Gould, 1988).

2.2 Importance of Aphids as Pests

Aphids are some of the most common and destructive pests of plants. About 4000 species of aphids have been described. Most of these species are abundant in temperate regions of the world (Mackauer and Way, 1978; Dixon, 1987). Aphid distribution also reflects their ability to survive the physical conditions and harsh environments of the tropics and subtropics. Aphids live on the plants which are common to these regions. However, aphids do not lose their pest status, even if they are newly introduced from the temperate regions to the tropical and subtropical regions of the world (Dixon, 1987).

Aphids affect plant growth and crop production in many ways such as:

1. Direct reduction of plant productivity,

2. Transmission of plant viruses which indirectly reduces the growth and productivity of plants. At least 10 potato viruses are aphid transmitted: Potato leaf roll virus, potato virus Y, potato virus A, SB29 virus, potato virus S, potato virus M, potato aucoba mosaic virus, potato calica virus, SB22 virus, and cucumber mosaic virus (Ramen and Radcliffe, 1992).
3. Leaf deformation or gall development due to the toxins in the aphid saliva, and
4. Attraction of saprophytic fungi to the excretion products of aphids deposited on the leaves which causes reduction of photosynthesis (Schepers, 1987).

2.2.1 Biology of Green Peach Aphid, *Myzus persicae*

The most important aphid involved in transmitting potato viruses is the green peach aphid (Burton, 1989). It was first identified as a pest of tobacco in the United States in the early 1940's. Since then, GPA has become one of the most important pests of crops (Semtner, 1984) throughout the world (Burton, 1989) and is a remarkably polyphagous species having more than 875 secondary hosts (Ramen and Radcliffe, 1992).

In regions with mild winters, GPA overwinter as apterous females on brassicas. They also overwinter as eggs in cold regions on *Prunus* spp. which includes peach and nectarine. Eggs hatch in the spring and larvae give rise to apterous, parthenogenetic, viviparous stem mothers known as fundatrices (Burton, 1989; Moran, 1992; Ramen and Radcliffe, 1992). Each fundatrix reproduces parthenogenetically (Miyazaki, 1987). These are wingless females that reproduce asexually. If the population density is high, the biochemical pathways change and the next generation becomes winged (alate). Alate females take off from the host and migrate to secondary hosts, often weeds (Miyazaki, 1987; Burton, 1989; Ramen and Radcliffe, 1992). GPA is an exceptionally dispersive species and has been collected at great heights in the atmosphere (Mackauer and Way, 1978).

Host alternation has enabled aphids to extend the period of time in which they can grow and reproduce (Dixon, 1987). Mate finding is not a primary factor in determining host specificity of aphids (Dixon, 1987). Emigration may be due to dislodgment by wind or rain, through taking protection against natural enemies or in response to other ecological factors such as population density. Location of a new host plant is by visual and olfactory means (Hodgson and Elbakheit, 1985). After landing on vegetation, the aphids probe by inserting their stylets of their mouthparts into the epidermis. If the response to the plant is positive, the stylets are inserted into the phloem, from which the contents are sucked (Burton, 1989).

The alate migratory females, like apterous females, are viviparous and reproduce asexually. When day lenghts are short, fall migrants are produced, followed by the production of males and females (sexuales). Fall migrants return to the primary hosts where the females, gynoparae, give birth to apterous female oviparae morphs. Oviparae mate with male fall migrants and lay fertilized overwintering eggs near buds, and in rough parts of the bark. In spring, the whole life cycle is repeated (Miyazaki, 1987; Burton, 1989; Moran, 1992; Ramen and Radcliffe, 1992).

GPA are more abundant, and populations peak sooner and decline earlier, on potato cultivars with a short growing season. On potatoes, apterae are almost always the dominant morph (Ramen and Radcliffe, 1992). Development of aphid population is related to the physiological age of leaves. GPA prefers older, lower leaves that are the first leaves to senesce. The total number of offspring is greater on the lower potato leaves than on the upper leaves (Jansson and Smilovitz, 1985). Under high temperatures and dryness, the turgor of old leaves decreases and this causes a decline in *M. persicae* fecundity (Mackauer and Way, 1978). A photoperiod of 16:8 h (light : dark) and constant temperature of 22^{o}C are optimum conditions to produce a large GPA colony (Xia and Tingey, 1986).

2.2.2 Control of GPA

2.2.2.1 Chemical Control of GPA

Before World War II, chemical control of aphids was mainly with the application of nicotine and arsenical products which did not have residual or systemic effects. After the war, the development of DDT and systemic aphicides such as the organophosphoric compounds progressed. Later, the carbamate insecticides and synthetic pyrethroids were developed. Some insecticides play an important role in controlling plant damage and virus transmission caused by aphids (Schepers, 1987). The economic threshold for GPA on potatoes is 30 apterae/105 leaves (Cancelado and Radcliffe, 1979).

Application of insecticides such as parathion may occasionally increase virus diseases. This has been observed on potato with potato virus Y. Greater incidence of virus infection is a result of longer initial feeding probes and a greater degree of movement of aphids on plants treated with insecticides. Local movement of apterous GPA increases with the application of deltamethrin, fenvalarate, primicarb and methamidophos (Lowery and Boiteau, 1988). Insecticides other than pyrethroids may reduce the ability of aphids to spread disease. Aldicarb reduces GPA flight and probing ability. Deltamethrin and fenvalarate have the same effect (Lowery and Boiteau, 1988).

2.2.2.2 Biological Control of GPA

The use of biological control of aphids in recent years has increased because aphids have infested new regions. Parthenogenesis, paedogenesis, viviparity and polymorphism together provide a high potential reproduction rate (Carver, 1987). The successful control agents, including predators, parasitoids and fungal pathogens include the following:

- Coccinellids are the most common predators of aphids. The effectiveness of lady beetles is enhanced by selecting coccinellid species which are not restricted by

temperature. Synchronized phenology of aphids and their host plants determines the time of coccinellids release and their effectiveness as biocontrol agents in the aphid populations (Frazer, 1987; Carver, 1987).

- Lacewings (Neuroptera) have been credited as the second most important predators of aphids (Carver, 1987). Lacewings are frequently found in association with aphids and have the potential to reduce aphid numbers significantly. They are important during periods when coccinellids are absent or inactive (New, 1987).

- The effectiveness of syrphid (Diptera) larvae as predators to limit aphid populations is important in field crops and orchards (Chambers, 1987). The third instar syrphids can affect aphid density and rate of increase (Carver, 1987). Their impact on aphid populations is attributed to the mobility and searching ability of adult females, habit of depositing eggs among the larval food, and the rapid increase in voracity as the larvae grow (Chambers, 1987).

- Aphidiids and Aphelinids (Hymenoptera) are the most common aphid parasitoids (Carver, 1987). Many aphid colonies include some mummified individuals, containing a fully-grown parasite larva or pupa (Mackauer and Way, 1978). The aphid dies gradually because a parasitoid needs some time to develop before consuming its food source completely (Stary, 1987). The percentages of parasitism in natural aphid populations may reach as high as 80-90%, but it is usually much lower. The potential control of GPA by parasitoids appears minimal. However, it is possible to increase parasitoid impact in the context of an IPM system (Mackauer and Way, 1978).

Aphid diseases have been recognized for more than a century. Bacterial and protozoan infections have not been demonstrated in aphids but baculoviruses and picornaviruses have been transmitted transovarially and decrease the longevity of aphids. Fungi are the most common pathogens because they are effective in natural and laboratory conditions. Many species are specialized on aphids, are harmless to beneficial fauna and can be produced *in vitro* easily (Latge and Papierok, 1987). *Entomophtora* spp. is one of

the most important fungi that significantly affect the aphid population. The mortality caused by *Entomophtora* spp. increases in rainy periods. This genus also should be considered an important element of IPM (Mackauer and Way, 1978).

2.3 Biology of Convergent Lady Beetle, *Hippodamia convergens*

Predaceous insects play an important role in maintaining the balance of natural populations, and this balance becomes especially important when species are closely related to crop pests. As an example, aphid outbreaks may occur yearly without the presence of predaceous insects, and this is certainly true in the case of most predaceous coccinellids (Rockwood, 1952; Frazer, 1988). Coccinellids are mostly polyphagous beetles with a wide range of accepted prey. Apart from aphids and other Homoptera (Psylloidea, Aleurodidae, Cicadoidea), they also prey on mites, small nematocerous Diptera and young instars of Lepidoptera and Coleoptera (Hödek, 1966).

The convergent lady beetle is an orange coccinellid with 12 black spots on the elytra, distinctive in the genus. It can be easily distinguished by the converging marks forming a broken "V" between the head and the body (Swan, 1964). After 48 hr of keeping isolated pairs of *H. convergens* in the laboratory, copulation occurs (Copp, 1983). Larvae of this species are black in color with orange strips across the dorsum, with a pointed abdomen. The deeply segmented body is covered with spines. The larvae are fully grown within 20 days and pupate after gluing the tip of their abdomen to a leaf or a branch. The pupa is not enclosed in a cocoon, but remains exposed on the surface of the plant (Swan, 1964). Orange yellowish eggs are laid by the females in clusters on the underside of leaves (Chedester, 1979; Wipperforth, *et al*, 1987), near colonies of aphids. This is a favorable condition for the newly hatched larvae to find prey (Bansch, 1966). Four larval instars are present in this species, and under laboratory conditions at 18-22 ^{o}C

and 20-30 percent relative humidity, the duration of the life cycle ranges from 20 to 39 days (Buttler and Dickerson,1972; Chedester 1979; Copp, 1983).

Coccinellid larvae do not have a complex sensory apparatus for locating prey. Instead, predation occurs when physical contact is made. When a beetle is searching for food, the aphids normally try to escape by dispersing on leaves to avoid predation (Frazer and Gilbert, 1976; Chedester, 1979). After eating the first prey, the larvae search intensively in the same area. Seeking behavior of the adults is different from that of the larvae because the adults can fly from uninfested plants to infested plants (Bansch, 1966).

Dispersion of beetles normally occurs to neighboring plants, but beetles also may search for prey on the ground. Distribution of prey normally affects the rate of predation in the laboratory; however, differences in predation rate were not observed in the field. Coccinellids are normally more active in the laboratory in comparison to those in the field, but temperature affects activity in both the laboratory and field (Frazer and Gilbert, 1976). CLB is found abundantly in meadows and agricultural crops. Geographically the species is widely distributed but is restricted to the places where its prey may be found. On a microhabitat scale, it is present on the upper parts of the plants (Chiang, 1966).

Convergent lady beetle has an unusual habit of migrating long distances in large masses for hibernation in the mountains. With the beginning of spring, the beetles warm up and migrate to valleys, responding to temperature and wind. When the time for hibernation comes, they migrate to mountains where they feed and presumably build up fat to support them for nine months of dormancy (Swan, 1964; Hagen, *et al.*, 1976)

First instars live in clusters, and after eclosion, competition for prey among them is present as well as cannibalism. Hunter (1978), studying behavior of CLB, found that females should be removed after eclosion of eggs to avoid cannibalism on the young larvae. According to New (1987), the effect of egg cannibalism on the voracity of first instars depends on the duration of the instar itself.

Lady beetles are sensitive to conventional insecticide applications in the field and the laboratory. Croft and Whalon (1982) have shown that pyrethroid insecticides may cause mortality in CLB varying from 50-100% on cotton, alfalfa, apple and cereals. *H. convergens*, *Adalia punctata*, and several species of *Stethorus* are highly susceptible to fenvalarate and permethrin, but low mortalities have been observed in *H. tredecimpunctata* and *Coleomegilla maculata* (Croft and Whalon, 1982). Mizell and Schifthauer (1990) stated that 100% mortality was observed when parathion, diazinon, chlorpyrifos, dimethoate, phosalone, azinphosmethyl, fluvalinate, methomyl and carbaryl were tested on CLB adults.

Microbial insecticides have become more popular in the last decade, especially because of resistance to conventional insecticides. Even though microbial insecticides are directed toward pest species, secondary effects have been observed on natural enemies. Haverty (1982) found that the mortality of *H. convergens* adults occurred 3-7 days after treatment with a dose of 18.7 l/ha of Dipel (*B. t. kurstaki*). James and Lighthart (1992) also noted that when the first 3 instars of lady beetles are maintained at 25°C, differences in susceptibility occurred with an application of *Pseudomonas florescens*. In contrast, the susceptibility of fourth instars was 2-7 times greater than younger instars.

2.4 Previous Studies on Microbial Insecticides

Use of microbial pest control agents (MPCA) has increased in recent years because insect pest species are showing resistance to chemical insecticides (Cantwell, *et al.*, 1983), and it is difficult to achieve selectivity of insecticides to predators and parasitoids (Croft, 1990). The potential for utilizing microorganisms as pest control agents was first recognized in the 1940's when the bacterium *Bacillus popillae* successfully controlled the Japanese beetle successfully (Mc Manus, 1990).

Bt is one of the microbial insecticides introduced in recent years as a control agent. Bt is a gram-positive, spore-forming bacterium that produces a parasporal crystal protein (Cheung, *et al.*, 1985; McIntosh, *et al.*, 1990). Bt was first observed in 1901 in Japan. In 1911, the first description of the bacterium was recorded. In 1950, the commercial potential of Bt was recognized. A short time later, the crystal was found to be toxic to lepidoptera. A preparation, Thuricide, was commercialized by Pacific Yeast Products, in 1957. Ten years later, the HD-1 kurstaki strain, which is used for caterpillar control, was identified. In 1970, Abbott Laboratories introduced Dipel. The activity of *B. t. israelensis* against mosquitoes and black flies was discovered in 1977. In 1983, *B. t. tenebrionis* which is active against some beetles including Colorado potato beetle and elm leaf beetle, was discovered. Recently, a biopesticide company, Mycogen, reported Bt strains that are toxic to nematodes and ants (Barton and Miller, 1993; Starnes, *et al.*, 1993).

Characterization of commercial formulations and strains of Bt are important because different strains have different selectivity features; different formulations have different carriers which may be toxic to natural enemies, different strains are formulated with different amounts of active ingredients, and every strain has different δ-endotoxins (Croft, 1990). For a complete characterization of a strain, it is necessary to determine its pathotype. The pathotypes of Bt differ in their toxicity. The pathotypes *finitimus* and *fowleri* produce crystals but are not entomocidal. The pathotypes *dakota, indiana, tohokuensis, kumanotensis, tochigiensis,* and *colmeri* have relatively weak insecticidal activities. Over 130 species of Lepidoptera, Diptera and Coleoptera are controlled by Bt pathotypes including the most serious crop pests such as cotton bollworm (*Heliothis virescens*), cabbage worm (*Pieris rapae*), European corn borer (*Ostrinia nubilalis*), and vectors of human diseases such as malaria, filariasis, yellow fever (Dean, 1984).

Thirteen Cry genes have been reported. These genes have been divided into four major classes and characterized by structural similarities and insecticidal spectra. The four

major classes are Lepidoptera specific (I), Lepidoptera and Diptera specific (II), Coleoptera specific (III), and Diptera specific (IV) genes (Carroll and Ellar, 1989; Höfte and Whiteley, 1989; Barton and Miller, 1993). The δ-endotoxins of Cry-III genes form rhomboidal crystal inclusions in spores (Herrnstadt, *et al.*, 1986). Three morphologically different Bt isolates have been discovered. These are known as *B. t. tenebrionis, B. t. sandiego*, and *B. t. EG2158*. The DNA sequences of the protoxin genes of each of the three strains are identical. They are commercially used to control Colorado potato beetle, elm leaf beetle and cottonwood leaf beetle (Barton and Miller, 1989; McIntosh, *et al.*, 1990). *B. t. tenebrionis,* named BI 256-82, is different from other pathotypes belonging to H-serotype 8a8b (*B. thuringiensis* strain), and isolated from *T. molitor.* Btt is known to be active only against Chrysomelidae larvae (Krieg, *et al.*, 1987).

Bt is characterized by the larvicidal properties of its proteinaceous parasporal crystals formed during the sporulation of the microorganism (Cheung, *et al.*, 1985). Crystals, upon ingestion, are quickly activated by a combination of the alkaline gut pH (9.0-10.5) and proteolytic enzymes present in the midgut of insects (Cheung, *et al.*, 1985; Croft, 1990; Martin, 1994). Swelling and vacuolization of the midgut ephitelial cells, separation of these cells from the basal membrane and disruption of the gut-haemocel barrier have been observed. These events in the gut cause insects to stop feeding, and this is followed by septicemia (Fast, 1981; Cheung, *et al.*, 1985, Himeno, *et al.*, 1985; Krieg, *et al.*, 1987; Baver and Pankratz, 1992). The feeding inhibition is a universal response to the toxin among susceptible insects and a major factor in the successful application of Bt for crop protection (Fast, 1981).

The side effects of microbial pesticides on natural enemies are difficult to generalize. Some are safer and have fewer side effects than conventional pesticides, but some may have greater impact on natural enemies. They may indirectly impact natural enemies by decreasing the food source or by reducing the host or prey quality. Miller (1990) stated that Lepidoptera serving as biocontrol agents of weeds could be affected by

B. t. kurstaki applications. He observed a reduction in abundance of non-target Lepidoptera, following Bt applications for gypsy-moth eradication. Birds in treated areas made significantly fewer nests. Bt applications increased emigration rates and caused dietary changes among shrews (Sample, *et al.*, 1993). Indirect effects of microbial pesticides may be greater for parasitoids when compared with predators. Direct mortality of *Coccinella septempunctata* has been reported when Bitoxibacillin, Entobakterin and Exotoxin were applied. However, other Bt formulations had no effect on other coccinellids (Croft, 1990).

Pest resistance to conventional insecticides is widespread; similar resistance to Bt is a potential risk. Resistance to Bt in Indian meal moth, *Plodia interpunctella*, tobacco budworm, *Heliothis virescens*, and almond moth, *Ephestia cautella* has been observed in laboratory studies by Johnson, *et al.*, (1990) and Tabashnik, *et al.*, (1990). Recently, resistance to Bt was documented in field populations of diamondback moth in Hawaii, and Asia (Tabashnik, 1994).

Chapter 3

Development and Reproduction of the Convergent Lady Beetle Feeding on Aphids Exposed to a Microbial Insecticide

3.1 Introduction

The use of microbial pest control agents (MPCA), or microbial insecticides, has increased as a result of insects developing resistance and of environmental pressures due to the residual and health effects of 'standard' chemical insecticides. MPCA differ from chemical insecticides in that they are 'biological' but differ from biocontrol agents proper in that they are not sustainable or self-reproducing in nature and frequent applications are required. This latter feature is comparable to conventional chemical control compounds. Most of the microbial insecticides have the advantage of being somewhat selective but they can affect closely related secondary and potentially beneficial non-target insects (Ferro, 1993).

Bacillus thuringiensis (Bt) is one of the best known microbial insecticides. Bt produces, in culture, a parasporal crystal protein containing a δ-endotoxin. A particular strain, *B. t. tenebrionis,* is specifically toxic to Coleoptera : Chrysomelidae. A commercial preparation, Novodor ®, is a product of Novo-Nordisk Co. (Davis, CA) and is often used for controlling Colorado potato beetle, *Leptinotersa decemlineata.* Although this beetle has developed resistance to a wide range of insecticides including arsenicals, chlorinated hydrocarbons, organophosphates, carbamates and pyrethroids, Novodor continues to provide effective control (Ferro and Gelernter, 1989). Furthermore, Bt is not toxic to humans, domestic animals, most beneficial insects and other non-target organisms. Therefore, it does not raise the same environmental concerns as conventional insecticides (Tabashnik, *et al.,* 1990). Exposure of predators to microbial pesticides is mostly through ingestion. Non-target beneficials may be affected if the spores are ingested, as when

cleaning antennae and mouth parts; the spores also may be consumed in contaminated food or even directly penetrate the body cavities through a wound on the integument (Croft, 1990).

Concerns arise about the use of microbial insecticides on non-target populations, include; (i) effects on non-target populations important in food webs, (ii) adverse effects on population trends of endangered species and, (iii) conflicts with other biological control agents (Miller, 1990). The objective of this study addresses the first concern: the effects of Novodor sprayed green peach aphid, *Myzus persicae*, on the consumption, development, pupal weight, fecundity and longevity of a natural predator, the convergent lady beetle, *Hippodamia convergens*.

3.2 Materials and Methods

3.2.1 Study Area

All experiments were conducted in a temperature and humidity controlled laboratory. The laboratory was maintained at a constant temperature of 22 $\pm$ 2°C, 50 - 55% relative humidity, and 16:8 h light and dark photoperiod regime. The light intensity was provided by fluorescent and sodium lamps with an average output of 800 μE/sec/m^2.

3.2.2 Test Materials

The following materials were used in the experiments: Certified virus free Russet Burbank potatoes; a biological insecticide (Novodor) containing Btt δ-endotoxin crystals manufactured by Novo-Nordisk company; 40 ml plastic containers; and plastic pots (10 cm w, 10 cm h, 10 cm l).

3.2.3 Test Insects

3.2.3.1 Green Peach Aphid

Several pots of virus-free Russet Burbank potatoes were planted. After the potatoes reached 15 cm in height and had more than 10 leaves, 10 laboratory reared apterous green peach aphids were put on each plant. The density of GPA was allowed to increase until approximately 100 aphids were present on each plant prior to the beginning of experiments.

3.2.3.2 Convergent Lady Beetle

The initial colony of lady beetle adults was established from collections made in potato and peppermint fields at Hermiston and Corvallis, Oregon respectively. The adults

were put into wooden cages (45 cm h, 45 cm l, 30 cm w) containing one or two plants infested with green peach aphids. Adults were allowed to mate and lay eggs. Eggs were collected and removed prior to the beginning of experiments. The beetles were reared in the laboratory for the rest of the experiments. *H. convergens* was selected as the test predator because it is now occurs naturally, is easy to collect, and adults can be purchased commercially. It also is suitable for laboratory rearing, although it cannot be reared on artificial diets. A prey species must be cultured to maintain the colony.

3.2.4 Methods

Twenty pots of green peach aphid infested potato plants were selected for this experiment. Ten pots were sprayed weekly with Novodor using an atomizer delivering the equivalent of the suggested field application ratio (1.5-3 qts/acre in 20 gal of water). The remaining ten plants were not sprayed and served as a control. Potato leaflet petioles were wrapped in cotton and inserted individually through holes in the lids of twelve 40 ml plastic containers filled with water. A slightly larger cup was inverted over the container to keep the beetle larva and aphid prey inside. One first instar larva was placed on each leaf. Twelve randomly selected first instar beetle larvae were used in the experiment and each cups were treated individually. For the first instar, 10 aphids were supplied daily; 20 aphids were given to the second instar; 30 aphids to the third instar; and 40 aphids to the fourth instar. Second and third instar green peach aphids were used, to prevent aphid reproduction during the 24 h observation period. After 24 h, the number of aphids consumed was determined and the uneaten prey were removed. The amount of time elapsed from molting to the next stage of the beetle was recorded. Immediately after pupation, pupae were weighed. Following adult emergence, the beetles were sexed by observing the shape of the abdomen (males are smaller and the last abdominal segment curves toward the anterior; this suture is relatively straight in females). The mated females

were placed individually for oviposition in 40 ml plastic cups with cardboard lids. The eggs were collected every 12 h and removed, to minimize egg cannibalism. This experiment was replicated three times, each treatment was represented twelve times.

3.2.5 Statistical Analysis

Data were collected and graphics were drawn in a spreadsheet (EXCEL 4.0 and 5.0, Microsoft). Statistical analyses were done using Multifactor Analysis of Variance on StatGraphics 5.0 (Plus*Ware). Two procedures were done to test the treatment effects. Multifactor ANOVA was run and for a given response we fit the following ANOVA:

Source of Variation

Trial

Treatment

Trial * Treatment

Residual

When interaction term was found to be non significant (5% level), the interaction sums of squares was pooled with residual sums of squares to get a new residual error term for the *F*-test. ANOVA tables for these cases are reported in Appendix A and B. New ANOVA to fit was as follows:

Source of Variation

Trial

Treatment

Residual

When the interaction term was found to be significant, we inspected the plot of data. If interaction consisted of an opposite treatment effect for two different trials, treatment effects for individual trials were tested separately and Means Tables for these cases are reported in Appendix A.

3.3 Results

We tested whether or not Novodor sprayed on prey affected the following biological parameters of the convergent lady beetles: aphid consumption, development time, pupal weight, fecundity and adult longevity.

We observed no significant difference in the mean number of aphids consumed in each larval instar except in the first and third instars, although lady beetles in the Novodor treated group consumed less aphids than the control group (Fig. 3.1) (first through fourth instar *F*-ratio and *P*-value = 3.691, 0.0298; 0.459, 0.5075; 3.535, 0.0344; 0.036, 0.8531 respectively; d.f = 74) (Appendix A).

Next, we compared the larval and pupal development periods. We observed no statistical difference in developmental period except in the fourth instar. Minor differences were that Novodor treated lady beetles took longer time to develop in first and second instars, and pupa, but control groups took longer in the third and fourth instars (Fig. 3.2) (first instar through pupa *F*-ratio and *P*-value = 0.236, 0.6340; 1.700, 0.1963; 2.931, 0.0911; 6.807, 0.0020; 3.227, 0.0770, respectively; d.f = 74) (Appendix A).

When we compared the pupal weight of the two treatments, I observed the Novodor group was lighter by 22%, possibly as a result of lower aphid consumption (Table. 3.3) (*F*-ratio = 6.162; *P* = 0.0009; d.f = 66). However, this experiment showed an interaction between replicates (Fig 3.3; Appendix A), suggesting an experimental or technical error.

We measured the fecundity of lady beetle females over their life time. I did not observe any significant difference, although Novodor treated females on average laid fewer eggs than the control group (Table. 3.1) (*F*-ratio = 0.136; *P* = 0.8733; d.f = 27) (Fig 3.4; Appendix A).

Finally, we looked at female adult longevity. No significant difference was observed between the Novodor and the control treatments, although the life span of

females treated with Novodor was shorter on average than the control group (Table. 3.1) (F-ratio = 0.396; P = 0.6773; d.f = 27) (Fig 3.5; Appendix A).

3.4 Discussion

These experiments demonstrate that the effect of a commercial Btt preparation on an agricultural system, namely, potato-aphid-lady beetle, is not significant for the most part. I found that aphid consumption of first and third instar lady beetles,developmental period of fourth instar and pupal weight were the only significant factors (at a .05 level). The indirect or sublethal effects of insecticides may have an impact on the beneficial insects fecundity, longevity, development rate, behavior, progeny to reduce pest populations (Peckman and Wilde, 1993), but because of the specificity of microbial insecticides, any impact may be difficult to detect.

The development time for our control groups was similar to the results of Chedester (1979) and Simpson & Burkhardt (1960), suggesting that our experimental procedures were well carried out. The Novodor group was not significantly different than their results or our control, but did take longer to develop, suggesting the treatment may cause a short delay in the development period. More detailed experiments, particularly larger samples, may be required if these effects are judged important.

Aphid consumption levels were similar to those reported by Chedester (1979) and Simpson & Bukhardt (1960), again suggesting that our procedures were good. The Novodor group consumed fewer aphids than the control group, but our analysis did not show a statistical difference. The number of aphids consumed daily by coccinellids is influenced by species, sex of the beetle, density of the prey, temperature and food quality (Shands and Simpson, 1972). Aphid consumption by *Coccinella undecimpunctata* was reduced 30% when Bt 'contaminated' *Aphis durantae* were fed to the larvae (Croft, 1990).

Pupal weight was significantly affected in one of the three replicates. Given that our other results are biased in this direction, it would appear that this result is potentially important, but requires confirmation. It should be noted here that a new batch of Novodor was used after the second replicate, possibly introducing a new formulation

unbeknownst to us. The difference in the second replicate may indicate an experimental error. Differences in preparation must be taken into account in future experiments.

The maximum clutch size is dependent on the anatomical and physiological constraints of the beetle. Reproductive potential also depends on the environmental factors such as, temperature and food supply. Mean clutch size is 19.2 for CLB (Stewart, et al., 1991), and the maximum total number of eggs laid by a single female is 871 (Simpson and Burkhardt, 1960). When permethrin was applied, the longevity of CLB decreased compared to the control group, but the total number of eggs laid in control group was 360.6, and 431.9 in permethrin group (Peckman and Wilde, 1993).

Closely related studies showed the convergent lady beetle is susceptible to most insecticides as well as biological control agents such as the fungi, *Metarhizium anisopliae, Paecilomyces fumosoroseus, Beauveria bassiana* (James and Lighthart, 1994), and *B. t. kurstaki* (Dipel 4L) (Haverty, 1982). Mortality is observed when these agents were used. The bacterium *Pseudomonas fluorescens* did not cause septicemia if the beetles were well fed; susceptibility increased when the beetles were poorly fed (James and Lighthart, 1992).

Acute toxicology tests proved that *B. t. sandiego*, similar to *B. t. tenebrionis* genetically, is not toxic to birds, mammals and fish. In addition, hymenopterous parasitoids, coccinellid predators, lacewings, and honey bees are not affected by the ingestion or contact of *B. t sandiego*. Maximum mortality occured for the target organisms after 3-4 days of ingestion (Ferro and Gelernter, 1989).

Bt strains do kill non-target organisms in wide-field applications. Since the host range of Bt and field stability are limited, Bt must be applied frequently. The disruption of food webs, the fate of endangered species and conflicts with other biocontrol agents are a valid concern (Miller, 1992b). Reduction in the richness and abundance of non-target lepidoptera were observed after the applications of *B. t. kurstaki.* Regulating the applications of Bt according to the life cycle of the beneficials is suggested (James, *et al.*, 1993). The density of lepidoptera larvae dropped after 6-8 days of applications proving

Bt takes a longer time to show its toxicity. This application in turn may have caused a negative effect on populations of birds. The population level of the lepidoptera returned to the prespray levels only after 2 years (Miller, 1990).

Btt reduced honeybee longevity at a high concentration (10^8 spores/ml), but caused no visible pathology (Vandenberg, 1990). Bt formulations containing β-exotoxin caused mortality in *C. septempunctata*. Mortality has been reported when Bt formulation, Bitoxibacillin and Endobakterin, and exotoxin were used (Croft, 1990).

Our results indicate that convergent lady beetles preying on aphids reared on Btt sprayed potato may be adversely affected. However, further investigation, coupled with access to defined sources of Btt will be required to document these effects.

Table 3.1 Fecundity and adult longevity of convergent lady beetle exposed to Btt sprayed aphids.

Treatment	Fecundity (total eggs/ female ± S.E)	Longevity (days/ female ± S.E)
Control	288.87 ± 48.0	62.43 ± 8.59
Novodor	181.95 ± 51.46	54.58 ± 9.21

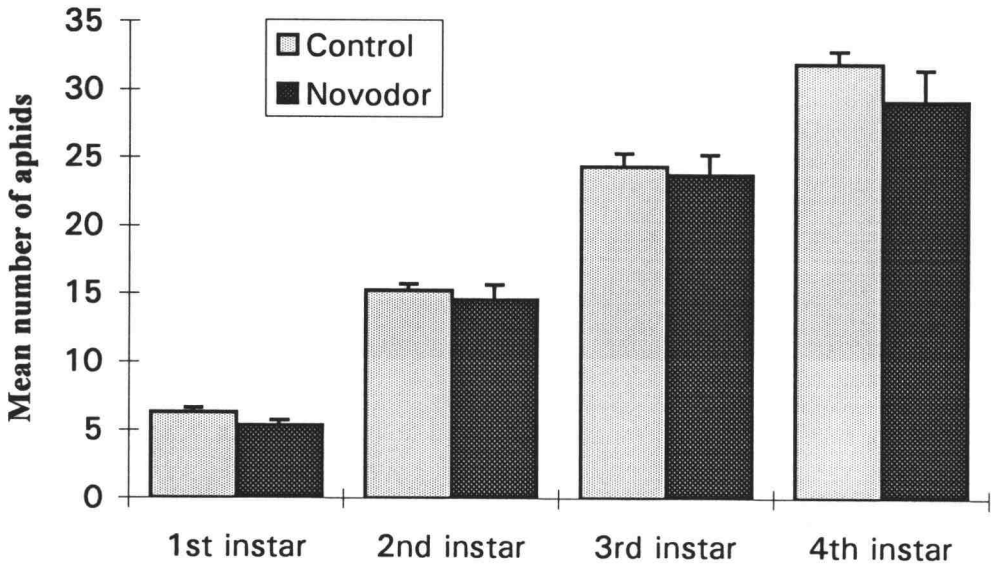

Fig 3.1 Mean aphid consumption per day ± S.E in Novodor treated and control convergent lady beetle larvae (n = 39).

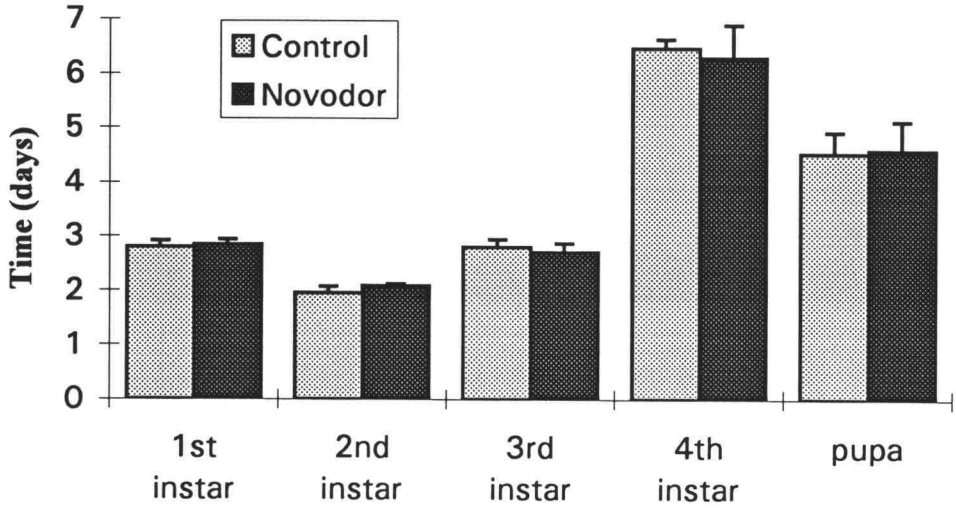

Fig 3.2 Comparison of mean development time ± S.E of immature convergent lady beetles in Novodor and control treatments (n = 39).

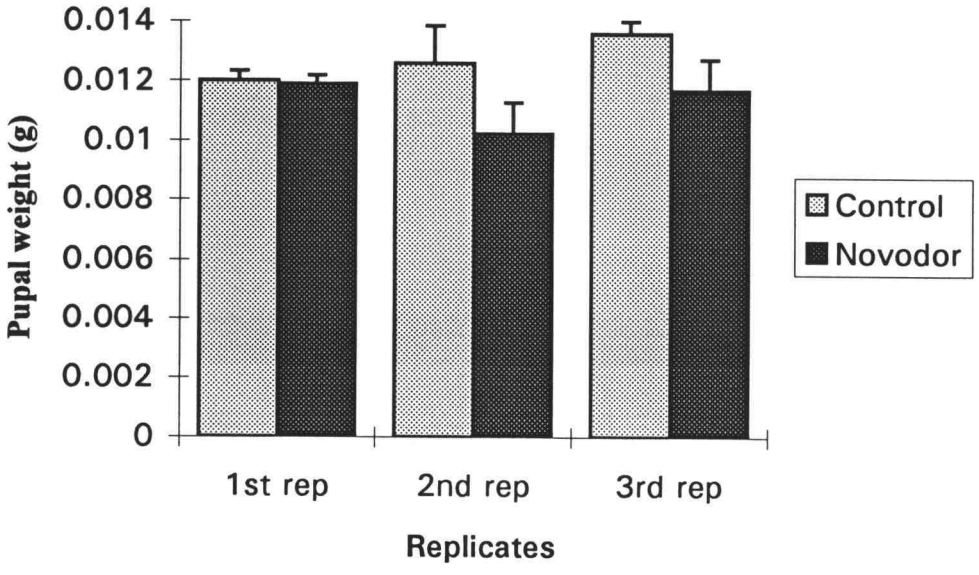

Fig 3.3 Comparison of pupal weight ± S.E in Novodor and control treated convergent lady beetles according to the replicates (n = 39).

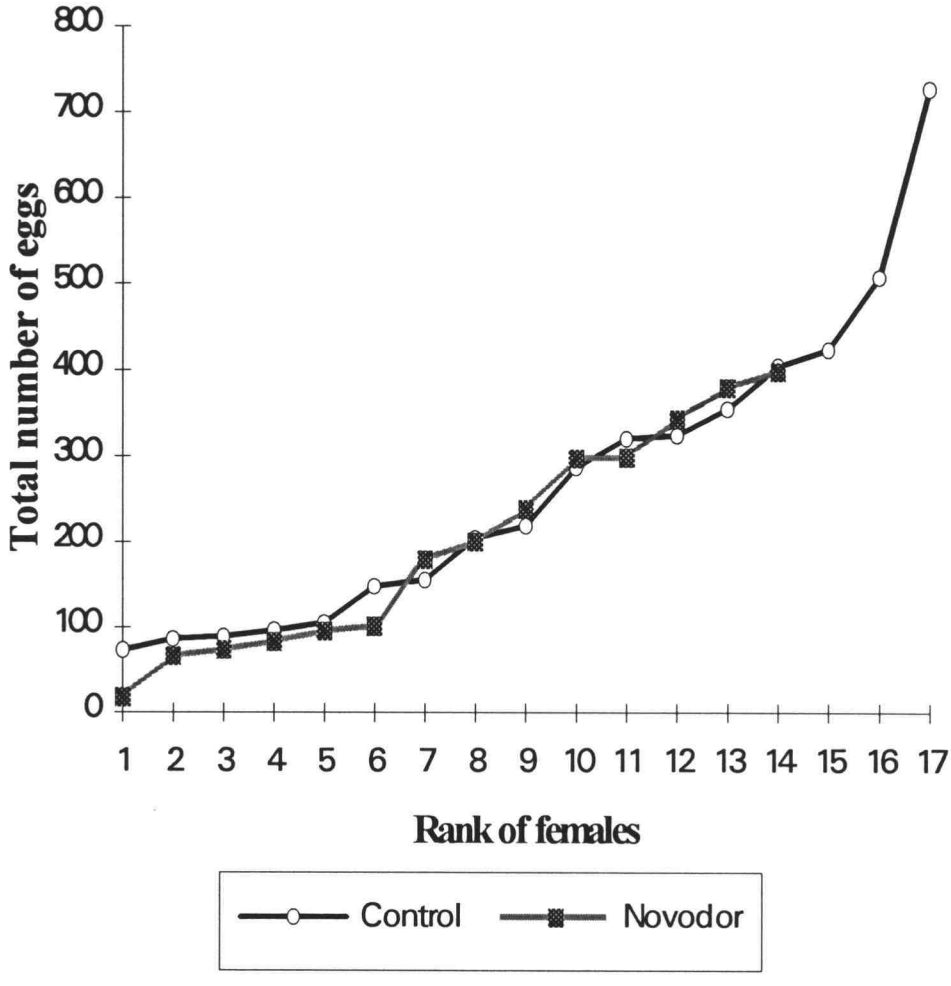

Fig 3.4 Comparison of total number of eggs per female laid by lady beetles in Novodor and control treatments. Each point represents a single female and each line ranks the points in order of increasing number of eggs.

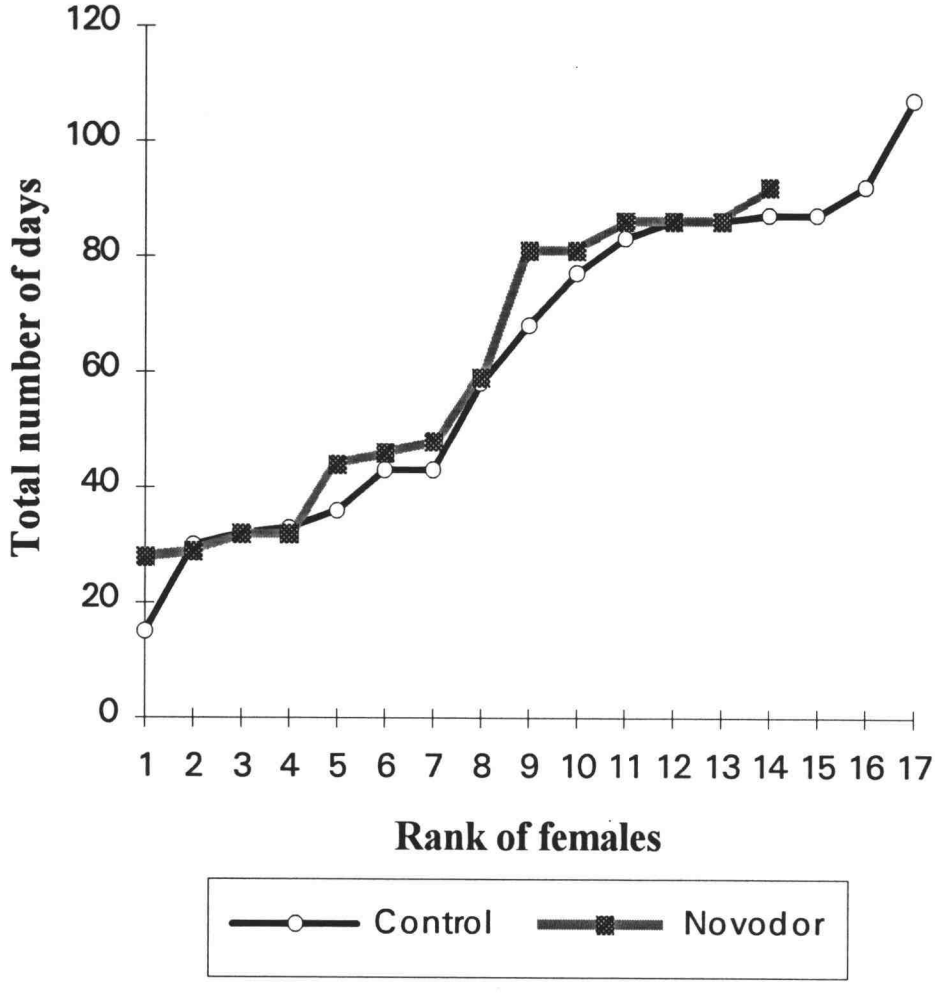

Fig 3.5 Comparison of adult longevity of lady beetle females in Novodor and control treatments. Each point represents a single female and each line ranks the points in order of increasing time (days).

References Cited

Chedester, L.D. 1979. Feeding habits, reproduction and sexual determination of the convergent lady beetle, *Hippodamia convergens* (Guer.). TAES, Oct 1979, MP-1437, The Texas A&M University System / College Station Texas.

Croft, B.A. 1990. Arthropod Biological Control Agents and Pesticides. Wiley and Sons, NY, NY.

Ferro, D.N., and W.D. Gelernter. 1989. Toxicity of a new strain *Bacillus thuringiensis* to Colorado potato beetle (Col: Chrysomelidae). J. Econ. Entomol. 82 : 750-55.

Ferro, D.N. 1993. Potential for resistance to *Bacillus thuringiensis*: Colorado potato beetle (Coleoptera: Chrysomelidae)- A model system. Amer. Entomol. 39 : 38-44.

Haverty, M.I. 1982. Sensitivity of selected non target insects to the carrier of Dipel4L in the laboratory. Environ. Entomol. 11 : 337-338.

James, R.R., and B. Lighthart. 1990. Bioassay for testing the lethal effects of bacterial pathogens on the predatory beetle *Hippodamia convergens* Gue. (Coleoptera: Coccinellidae). EPA National Technical Information Service. Publication no PB91-127795.

James, R.R., and B. Lighthart. 1992. The effect of temperature, diet, and larval instar on the susceptibility of an aphid predator, *Hippodamia convergens* (Coleoptera: Coccinellidae) to the weak bacterial pathogen *Pseudomonas florescens.* J. Invert. Path. 60 : 215-218.

Miller, J.C. 1992a. Temperature dependent development of the convergent lady beetle (Coleoptera: Coccinellidae). Environ. Entomol. 21 : 197-201.

Miller, J.C. 1992b. Effect of a microbial insecticide, *Bacillus thuringiensis kurstaki*, on nontarget Lepidoptera in a spruce budworm infested forest. J. Res. Lepid. 29 : 267-76.

Peckman, P.S., and G.E., Wilde. 1993. Sublethal effects of permethrin on fecundity and longevity of Hippodamia convergens (Coleoptera: Coccinellidae). J. Kansas Entomol. Soc. 66 : 361-64.

Shands, W.A., and G.W. Simpson. 1972. Insect predators for controlling aphids on potaotes. 8. Green peach aphid consumption by *Coccinella septempunctata* and *C. transversoguttata.* J. Econ. Entomol. 65 : 1388-92.

Simpson, R.G., and C.C. Burkhardt. 1960. Biology and evaluation of certain predators of *Therioaphis maculata* (Buckton). J. Econ. Entomol. 53 : 89-94.

Stewart, L.A., J.-L. Hemptinne, and A.F.G. Dixon. 1991. Reproductive tactics of ladybird beetles: relationships between egg size, ovariole number and development time. Funct. Ecol. 5 : 380-85.

Tabashnik, B.E., N.L. Cushing, N. Finson, and M.W. Johnson. 1990. Field development of resistance to *Bacillus thuringiensis* in diamondback moth (Lep : Pyralidae) J. Econ. Entomol. 83 : 1671-76.

Chapter 4

Biological Parameters of Convergent Lady Beetle Feeding on Aphids on Transgenic Potato

4.1 Introduction

Plant biotechnology has developed plants resistant to diseases, insects and tolerant to certain herbicides (Dale and Flavell, 1988). The 1980's witnessed transformation of plant cells with foreign genes and regeneration of fertile plants from these cells (Peferoen, *et al.*, 1990). The transfered genes are identified and isolated as follows. Transfer methods for the plant species is developed according to the suitable vector and tissue culture techniques for production of fertile plants. Expression of the gene in the transferred plant is monitored to determine if the suitable levels of resistance to the target pest are conferred in the desired tissues of the plant. The transformed plant is tested to determine whether or not it is toxic to humans or otherwise unsuitable for human use, and against possible adverse effects on the plant metabolism. The goal of transgenic technology is to develop plants with effective, stable heritable resistance to pests (Gatehouse, *et al.*, 1991).

Many solanaceous species are relatively easy to manipulate, in the context of transformation for insect resistance. Two approaches have been used. The procedure used in developing the test stocks we used, is to select plants that synthesize a polypeptide, most often *Bacillus thuringiensis* (Bt) δ-endotoxin, expressed in conjunction with photosynthesis, that is lethal to insects. A second approach is direct DNA insertion and selection techniques used in animal cells (Hilder, *et al.*, 1987; Walden, 1989; Houck, *et al.*, 1993). The same proteins have short half-lives when applied topically. The introduction of Bt genes into plants therefore provides long term control (Cheung, *et al.*, 1992).

Transgenic potatoes, expressing a *B. t. tenebrionis* δ-endotoxin specific to Coleoptera, are known to be resistant to Colorado potato beetles (Perlak, *et al.*, 1993). Shieh, *et al.*, (1994) reported that the feeding and probing behavior of the non-target pest, the green peach aphid, *Myzus persicae*, (GPA), is not affected when feeding on transgenic potatoes. This result however does not preclude that the toxin may be passed on to the aphid's beetle predators. The objective of this study was to determine the effects on convergent lady beetle, *Hippodamia convergens* preyed on aphids feeding on Btt δ-endotoxin expressing transgenic potatoes.

4.2 Materials and Methods

4.2.1 Study Area

All experiments were conducted in a temperature and humidity controlled laboratory. The laboratory was maintained at a constant temperature of $22 \pm 2^{o}C$, 50-55% relative humidity, and 16:8 h light and dark photoperiod. Light intensity was provided by fluorescent and sodium lamps with an average output of 800 $\mu E/sec/m^2$.

4.2.2 Test Materials

The following materials were used in the experiments: Certified virus free Russet Burbank potatoes; certified virus free, genetically engineered Russet Burbank potatoes expressing Btt δ-endotoxin (all transgenic potato tubers were obtained from Hermiston Agricultural Research and Extension Center, Hermiston, OR); 40 ml plastic containers; and plastic pots (10cm w, 10cm h, 10cm l).

4.2.3 Methods

Transgenic (provided by HybriTech Seed International) and non-transgenic Russet Burbank potatoes were planted and grown in the laboratory. After the potatoes reached 15 cm high and had more than 10 leaves, ten aphids were put on each plant. The density of GPA was allowed to increase until approximately 100 aphids were present per plant. Transgenic potato leaflet petioles were wrapped with cotton and inserted through a hole in the lids of twelve 40 ml plastic containers filled with water. A larger cup was inverted over the small container to keep the beetle larva and aphid prey inside. One first instar larva was placed on each leaf. Twelve randomly selected first instars were used in the experiment, and each cups were treated individually. Ten aphids from the transgenic potatoes were transferred daily to the leaves as prey for the first instar; 20 aphids were

given to the second instar; 30 aphids to the third instar; and 40 aphids to the forth instar. Second and third instar green peach aphids were used to prevent aphid reproduction during the 24 hour observation period. After 24 h, the number of aphids consumed was determined and uneaten prey were removed. The amount of time elapsed from molting to the next stage was recorded. Right after pupation, the pupa were weighed. Following adult emergence, the beetles were sexed (see Chapter 3) and allowed to mate. The mated females were placed individually for oviposition in 40 ml plastic cups with cardboard lids. The eggs were collected every 12 h to minimize egg cannibalism. Aphids reared on non-transgenic potatoes served as a control treatment. There were three replicates, each treatment was represented twelve times.

4.2.4 Statistical Analysis

Data were collected and graphics were drawn in a spreadsheet (EXCEL 4.0 and 5.0, Microsoft). Statistical analyses were done using Multifactor Analysis of Variance on StatGraphics 5.0 (Plus*Ware). Two procedures were done to test the treatment effects. Multifactor ANOVA was run and for a given response we fit the following ANOVA:

Source of Variation

Trial

Treatment

Trial * Treatment

Residual

When interaction term was found to be non significant (5% level), the interaction sums of squares was pooled with residual sums of squares to get a new residual error term for the *F*-test. ANOVA tables for these cases are reported in Appendix A and B. New ANOVA to fit was as follows:

Source of Variation

Trial

Treatment

Residual

When the interaction term was found to be significant, we inspected the plot of data. If interaction consisted of an opposite treatment effect for two different trials, treatment effects for individual trials were tested separately and Means Tables for these cases are reported in Appendix B.

4.3 Results

We tested for effects of transgenic potatoes on aphid consumption and larval development, pupal weight, fecundity and adult longevity.of convergent lady beetles.

No difference was observed in the aphid consumption between the two treatments except in the second and fourth instars. The control group consumed slightly more aphids in each instar, except the third (first through fourth instar *F*-ratio and *P*-value; 2.586, 0.08; 11.114, 0.002; 0.078, 0.7844; 8.435, 0.0005, respectively; d.f = 68) (Fig 4.1; Appendix B).

We compared the development time throughout the larval and pupal period. We observed no significant difference between two treatments, except in the fourth instar, although the control treatment took slightly longer in first and third instar, and pupal stages. The transgenic treatment took longer in second and fourth instars (first instar through pupa, *F*-ratio and *P*-value; 0.266, 0.6131; 1.644, 0.2042; 0.925, 0.3498; 13.39, 0.0001; 2.113, 0.1510, respectively; d.f = 68) (Fig 4.2; Appendix B).

We compared pupal weight between treatments. and found no significant difference. Pupae in control treatment weighed slightly more in two of the replicates, but pupae in the transgenic treatment weighed more in the second replicate (*F*-ratio = 0.00; *P* = 0.9854; d.f = 66) (Fig 4.3; Table 4.1; AppendixB).

When we measured fecundity of lady beetles in two treatments, we did not observe any significant difference, although females in the transgenic treatment laid slightly more eggs than the control group (*F*-ratio = 1.882; *P* = 0.1887; d.f = 26) (Fig 4.4; Appendix B).

Finally, we compared adult longevity in females. Adults in transgenic treatment lived slightly, but not significantly, longer than adults in the control treatment (*F*-ratio = 0.347; *P* = 0.5671; d.f = 26) (Fig 4.5; Appendix B).

4.4 Discussion

These experiments suggest that the impact of transgenic potatoes on the convergent lady beetles is not significant except in the second and fourth instar aphid consumption and fourth instar developmental period. We found no differences in the parameters tested. The potential transfer of toxic material from the transgenic plant through its aphid prey either does not occur or, if it does, is not significantly toxic to the convergent lady beetle. The biologically significant differences that we noticed are small and require further studies.

Development time and aphid consumption in our two treatments were similar to previous results of Chedester (1979), Obrycki and Tauber (1982), and Miller (1992), suggesting that experimental procedures followed in our experiments are well within other published studies and therefore not a source of error.

In target pests, sublethal effects of sprayed Bt includes reduced host consumption, reduced egg production, and reduced adult longevity (Flexner, *et al.*, 1986). We could not document such effects on CLB, leading us to suggest that they are not present. We did observe differences, but not significance. The possibility therefore exists that effects do occur but at such low levels that much higher sample numbers would be required to distinguish them. The biological relevance of such minor effects would be questionable.

Practical limitations of the present MPCA are high cost of preparations compared to the chemical pesticides, short field stability, and need for multiple applications (Barton and Miller, 1993). The need for cheap and stable pest control agents improved the idea of gene transformation. The main advantages of transgenic plants are reduction in the use of chemicals and improved residual activity. The disadvantages of engineering Bt toxin genes are possible resistance development and public acceptance of engineered foods (Holmes, 1993; Starnes, *et al.*, 1993).

Gene transfer technology in higher plants was developed using solanaceous species. Resistance to insect predation is an important objective of the process. The most successful strategy is Bt δ-endotoxin-expressing-transgenic plants such as transgenic tobacco toxic to *Manduca sexta* larvae and transgenic tomatoes resistant to *Heliothis zea* and *Keiferia lycopersicella* (Houck, *et al.*, 1993). The relationship between the amount of CryIA(b) (*B. t. kurstaki*) protein expressed in the plant and the level of protection from the pest damage was positive (Warren, *et al.*, 1992). Surprisingly, the parasitism activity increased on transgenic tobaccos against *Heliothis* larvae, but the difference was not significant (Warren, *et al.*, 1992). Transformed plants expressing Bt toxin contain low levels of toxin, but the presence of this gene causes adverse effects to test insects. Despite the low levels of toxin expression, most of the transformed plants were toxic to *M. sexta*, suggesting very high toxicity (Gatehouse, *et al.*, 1991). The CryIII gene which contains toxic polyhedron to Coleoptera showed toxicity to *Tenebrio* spp. and Colorado potato beetle. The toxicity was higher in the early larval stages (Barton and Miller, 1993).

When Bt is sprayed, a target insect must ingest Bt crystals to be affected. The protoxin is released from ingested crystals and protoxin is activated to the toxin. The toxin binds to the receptors on the midgut cell membranes. The insect stops feeding and dies (Martin, 1994). We do not know if the green peach aphid is exposed to the toxin while feeding on the transgenic potatoes or even if it is susceptible; therefore the results are not easy to interpret. Cysteine and serine proteinases are found in the midgut of herbivorous insects. The inhibitory action of these enzymes causes toxicity among animals such as chicks and insects. Transgenic tobacco and potato plants exhibited resistance to tobacco budworm, cowpea weevil, and Colorado potato beetle. Transformation of plants carrying genes coding for cysteine proteinase inhibitors are resistance to insect feeding damage (Ryan, 1989). Combination of these inhibitors with Bt toxin can prevent the resistance to transgenic plants.

Overall, our experiments suggest that transgenic potatoes will have little effect on non-target organisms such as CLB, although field tests clearly must be done in addition to laboratory experiments. An interesting area of investigation would be to measure the level of exposure of aphids to the toxin and whether or not aphids are susceptible at all.

Table 4.1 Pupal weight, fecundity and adult longevity of convergent lady beetles in transgenic and control treatments.

Treatment	Mean Pupal weight (g ± S.E)	Fecundity (total eggs/ female ± S.E)	Longevity (total days/ female ± S.E)
Control	$0.01255 \pm 6.49E^{-4}$	367.43 ± 61.55	70.32 ± 5.80
Transgenic	$0.01260 \pm 7.78E^{-3}$	421.14 ± 57.50	71.13 ± 5.42

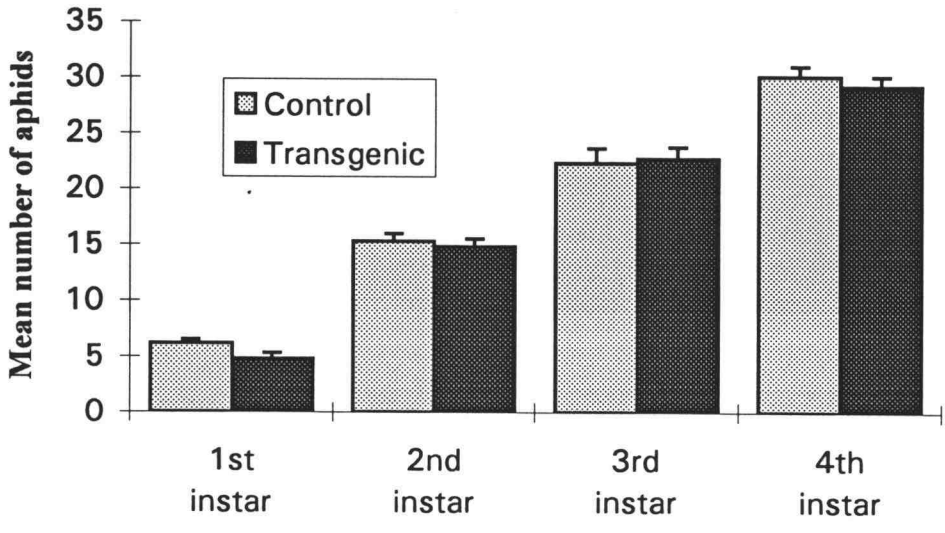

Fig 4.1. Mean aphid consumption ± S.E per day in transgenic treated and control convergent lady beetle larvae (n = 36)

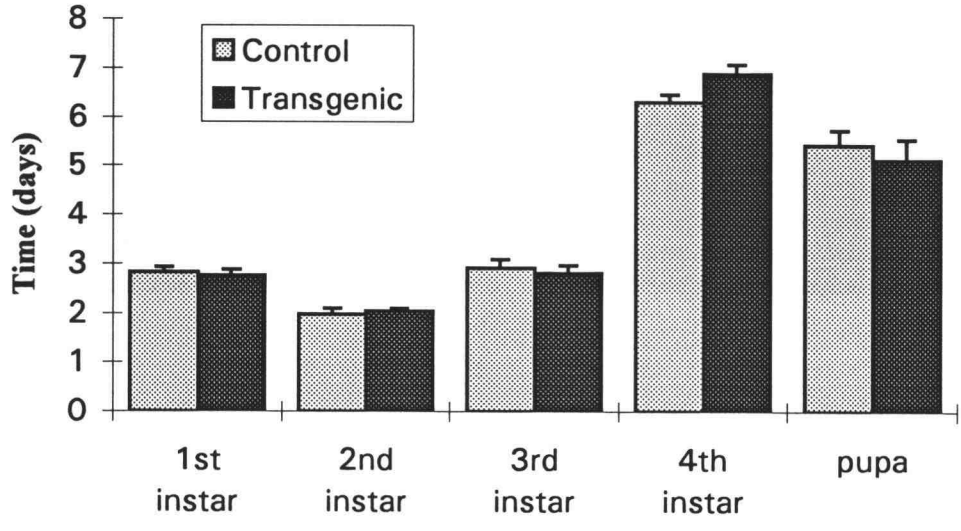

Fig 4.2. Comparison of mean development time ± S.E of immature convergent lady beetle in transgenic and control treatments (n = 36).

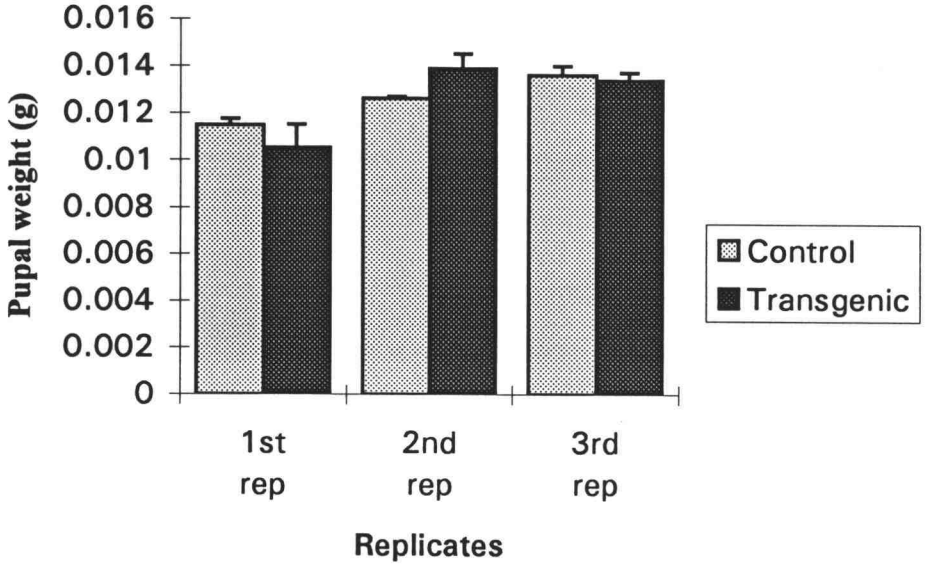

Fig 4.3. Comparison of pupal weight ± S.E in transgenic and control treated convergent lady beetles (n = 36).

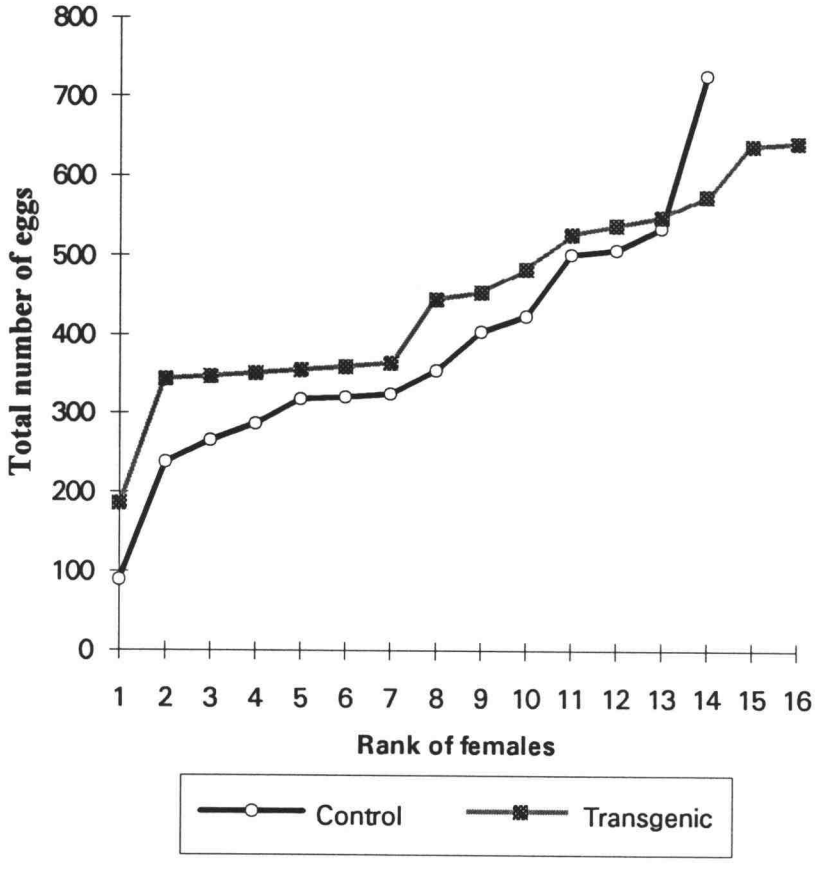

Fig 4.4. Comparison of total number of eggs per female laid by lady beetles until death in transgenic and control treatments. Each point represents a single female and each line ranks the points in order of increasing number of eggs.

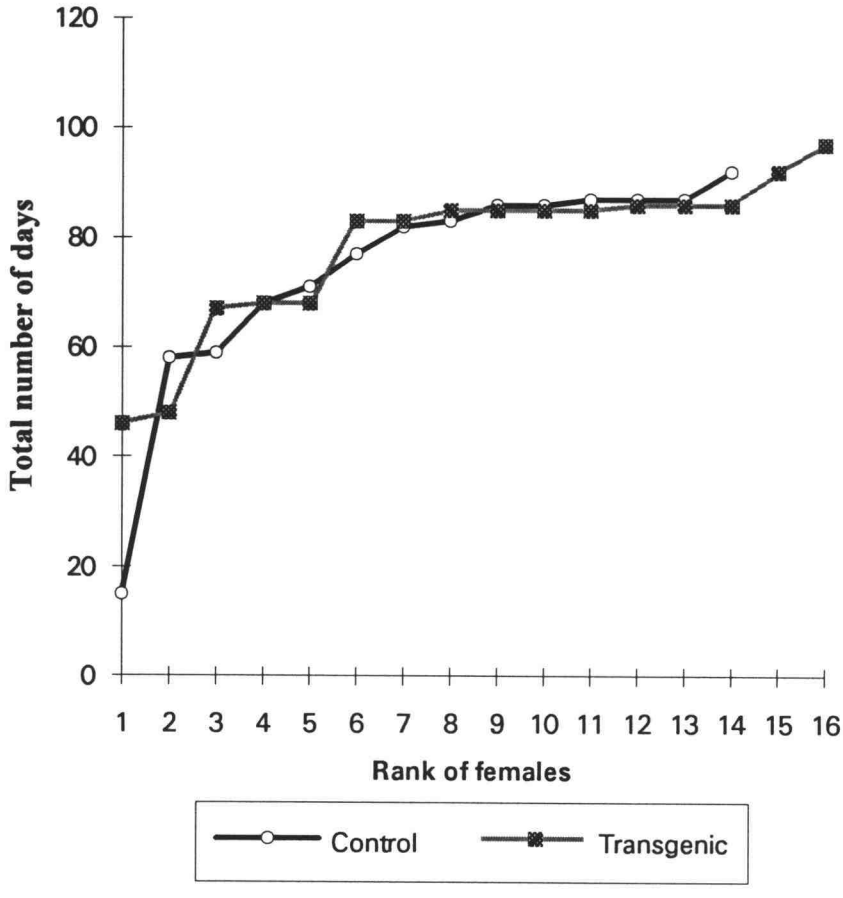

Fig. 4.5. Comparison of adult longevity of lady beetle females in transgenic and control treatments. Each point represents a single female and each line ranks the points in order of increasing time (days).

References Cited

Barton. K.A., and M.J. Miller. 1993. Production of *Bacillus thuringiensis* insecticidal proteins in plants. In S.Kung and R. Wu [eds.] Transgenic Plants, Engineering and Utilization. Vol 1. Academic Press, Inc. 383 pp.

Chedester, L.D. 1979. Feeding habits, reproduction and sexual determination of the convergent lady beetle, *Hippodamia convergens* (Guer.). TAES, Oct 1979, MP-1437, The Texas A&M University System / College Station Texas

Cheng J., M.G. Bolyard, R.C. Saxena, and M.B. Sticklen. 1992. Production of insect resistant potato by genetic transformation with a δ-endotoxin gene from *Bacillus thuringiensis* var. *kurstaki*. Science 81 : 83-91.

Dale, P.J., and R.B. Flavell. 1988. Evaluation of genetically engineered plants. In R.T. Fraley, N.M. Frey, J. Schell [eds], Current Communications in Molecular Biology: genetic Improvements of Agriculturally Important Crops, Progress and Issues. Cold Spring Harbor Lab, 116 pp.

Flexner, J.L., B. Lighthart, and B.A. Croft. 1986. The effects of microbial pesticides on non-target, beneficial arthropods. Agri., Ecol. and Envi. 16 : 203-254.

Gatehouse, J.A., V.A. Hilder, and A.M.R. Gatehouse. 1991. Genetic engineering of plants for insect resistance. In D.Grierson [ed.], Plant Genetic Engineering. Blackie and Sons Ltd. 267pp.

Hilder, V.A., A.M.R. Gatehouse, S.E. Sheerman, R.F. Barker, and D. Boulter. 1987. A novel mechanism of insect resistance engineered in tobacco. Nature 330 : 160-163.

Holmes, B. 1993. The perils of planting pesticides. New Scientist 139 : 34- 37.

Houck, C.M., D. Facciotti, and R.M. Goodman. 1993. Transgenic plants from Solanaceae. In S. Kung, and R. Wu [eds.] Transgenic Plants, Present Status and Social and Economic Impacts, Vol 2. Academic Press, Inc. 265 pp.

Martin, P.A.W. 1994. An iconoclastic view of *Bacillus thuringiensis* ecology. Amer. Entomol. 40 : 85-90.

Miller, J.C. 1992a. Temperature dependent development of the convergent lady beetle (Coleoptera : Coccinellidae). Environ. Entomol. 21 : 197-201.

Obrycki, J.J., and M.J. Tauber. 1982. Thermal requirements for development of *Hippodamia convergens* (Coleoptera : Coccinellidae). Ann. Entomol. Soc. Am. 75 : 678-83.

Peferoen, M., S. Jansens, A. Reynaerts, and J. Leemans. 1990. Potato plants with engineered resistance against insect attack. In M.E. Vayda and W.D. Park [eds.], The molecular and cellular biology of the potato. C.A.B International, Oxon, UK. 97-102p.

Perlak, F.J., T.B. Stone, Y.M. Muskopf, L.J. Petersen, G.B. Parker, S.A. McPherson, J. Wyman, S. Love, G. Reed, D. Biever and D.A. Fischhoff. 1993. Genetically improved potatoes: protection from damage by Colorado potato beetles. Plant Mol. Biol. 22 : 313-321.

Ryan, C.A. 1989. Proteinase inhibitor gene families: Strategies for transformation to improve plant defenses against herbivores. BioEssays 10 : 20-24.

Shieh, J.-N., R.E. Berry, G.L. Reed, and P.A. Rossignol. 1994. Feeding activity of green peach aphid (Homoptera: Aphididae) on transgenic potato expressing a *Bacillus thuringiensis* ssp. *tenebrionis* δ -endotoxin gene. J. Econ. Entomol. 87 : 618-22.

Starnes, R.L., C.L. Liu, and P.G. Marrone. 1993. History, use, and future of microbial insecticides. Amer. Entomol. 39 : 83-91.

Walden, R. 1989. Engineering plants to contain useful agronomic traits. In R.Walden [ed.], Genetic Transformation in Plants. Prentice Hall Advanced Reference Series. 138 pp.

Warren, G.W., N.B. Carozzi, N. Desai, and M.G. Koziel. 1992. Field evaluation of transgenic tobacco containing a *Bacillus thuringiensis* insecticidal protein gene. J. Econ. Entomol. 85 : 1651-59.

Chapter 5

Toxicity of *Bacillus thuringiensis tenebrionis* to Convergent Lady Beetle

5.1 Introduction

Predaceous coccinellids have a wide range of accepted prey. In addition to feeding on Homoptera and phytophagous mites, they often prey on young instars of Lepidoptera, Coleoptera, Hymenoptera, small nematocerous Diptera and Thysanoptera (Clausen 1940; Hödek, 1973). Some have been shown to feed at nectar glands of plants and on pollen, honeydew, fungi etc, during the times of natural prey scarcity (Clausen, 1940). Researchers have been trying to develop a suitable synthetic diet to rear the coccinellids as an alternative to maintaining a prey colony, which makes rearing time consuming and difficult (James and Lighthart, 1990). Artificial diets developed so far are not efficient in maintaining the complete life cycle (Smith, 1965; Baldwin, 1984).

The microbial agent, *Bacillus thuringiensis tenebrionis* (Btt) is used to control the Colorado potato beetle, *Leptinotarsa decemlineata*. The convergent lady beetle, *Hippodamia convergens*, is one of the predators of green peach aphid, *Myzus persicae*, a pest in potato. We have preliminary evidence that Novodor, a microbial insecticide containing Btt, may negatively affect the convergent lady beetle. In order to further document this observation, we proceeded to determine the toxicity of Btt. The mode of action of Btt is as follows: Parasporal crystals are formed during sporulation and after ingestion, crystals are activated by alkaline pH and the proteolytic enzymes of the midgut. The basal membrane of the midgut is disrupted, midgut epithelial cells swell, causing feeding inhibition (Krieg, *et al.*, 1987; Baver and Pankratz, 1992). Spores ingested by predators may occur via ingestion of a prey, cleaning the antennae and mouth parts or nectar feeding (Flexner, *et al.*, 1986). In the previous chapters, we have determined the indirect effects of Btt to convergent lady beetle feeding on either sprayed aphids or aphids

that fed on transgenic potatoes. In this experiment we tested the direct toxicity of Btt crystals and spores, mixed with an artificial diet on convergent lady beetle fourth instars.

5.2 Materials and Methods

5.2.1 Test Materials

The following materials were used in the experiments: Fourth instar convergent lady beetles; 40 ml plastic containers; banana; casein; wheat germ; Spectrum ® multivitamins and minerals; and a non-commercial preparation of Btt crystals and spores (Novo Nordisk, Davis, CA). The preparation contained no dispersant or suspension agent (courtesy of Dr. John E. Dunley, U. C. Berkeley).

5.2.2 Methods

One hundred and five first instars were randomly selected and fed aphids until they molted to the fourth instar. The fourth instars were separately put into 40 ml plastic containers. An artificial diet (Smith 1965) was prepared by using 90% banana, 9% casein, 0.9% wheat germ, 0.09% multivitamins and minerals and 0.4 ml water. Seven different concentrations of 0, 40, 70, 100, 130, 160, and 190 ppm Btt were added to the diet. Each concentration was fed to 15 fourth instars. Diet was made fresh every 72 h. Larval mortality was recorded daily. A consequence of the diet was that the fourth instar development was extended to twelve days in each treatment, in contrast to a maximum of 8 days when fed aphids.

5.2.3 Statistical Analysis

Analysis was performed with the procedures of POLO-PC (Probit or Logit analysis, LeOra Software, 1987). The data were organized and the graphics were produced in EXCEL 4.0 (Microsoft).

5.3 Results and Discussion

Larval mortality was not significant from the first to the ninth day. By the ninth day, Btt began to affect the fourth instar convergent lady beetles, resulting in mortality (Table 5.1). A probit analysis was conducted at the end of 12th day (Fig. 5.1). The results are as follows: Chi-square = 1.7162, d.f = 4. The .95 confidence intervals were LC_{10} = 41.658, 52.892; LC_{50} = 62.845, 74.088; LC_{90} = 93.527, 104.770; $P > 0.05$.

This preliminary study demonstrated that Btt causes mortality to fourth instar convergent lady beetles. Despite the inadequacy of the diet, a relatively clear dose response occurred. The high mortality in the control treatment and somewhat flat response following this initial mortality may be attributable to the variability introduced by the poor diet. Our results confirm indirect evidence of increased mortality due to spraying (See Chap. 3; Croft, 1990).

The convergent lady beetle is susceptible to many insecticides including parathion, diazinon, chlorpyrifos, malathion, dimethoate, phosalone, azinphosmethyl, fluvalinate, methomyl and carbaryl (Moffitt, *et al.*, 1972; Wilkinson, *et al.*, 1979; Mizell and Schiffhauer, 1990). The use of Btt may be less lethal, but mathematical models suggests that the actual quantity of an effect may not be as important as the quality of the actual effect (Puccia and Levins, 1985).

Artificial diet may not be adequate for maintaining the life cycle of the lady beetles. This is a general problem with coccinellids, other diets maintained the life cycle for longer periods, but egg to egg maintenance has yet to be achieved (Smith, 1965). The diet made from lyophilized potato tuberworm, yeast autolysate and honey mixture was accepted by *H. convergens* although no egg production occurred (Wipperfürth, *et al.*, 1987). *Aphelinus mali* lived over 1000 days and *Coleomegilla maculata* over 400 days on artificial diets, but still failed to reproduce (Smith, 1965). Banana was an acceptable diet for most of the coccinellids including *Adalia decempunctata*. This species was fed on

banana and the development period was extended suggesting banana lacks essential nutrients.

The results of our experiments showed an increase in the expected 5-8 day development period of fourth instar CLB, similar to the results of Cantwell, *et al.* (1986). Clearly, a better diet will be required for further studies. The β-exotoxin of Btt increased the development period of the Mexican bean beetle (*Epilachna varivestis*). Cantwell, *et al.* (1986) suggested that Btt may be affecting *corpus allatum* indirectly so that the release of juvenile hormone is increased and larvae maintained in that stage. However, the treatments did not produce extra larval instars but only extended the development time in each instar. The suppression of feeding may be another explanation. Thus, the mortality in the control group in our experiments may be due to the poor nutritional value of the artificial diet used throughout the experiment.

Table 5.1 Percent mortality of fourth instar convergent lady beetle fed with artificial diet mixed with Btt.

Dose (ppm)	9 days	10 days	11days	12 days
0	20	26.7	26.7	26.7
40	20	20	26.7	46.7
70	40	40	40	46.7
100	26.7	26.7	26.7	46.7
130	40	46.7	53.3	66.7
160	53.3	60	73.3	80
190	66.7	73.3	73.3	93.3

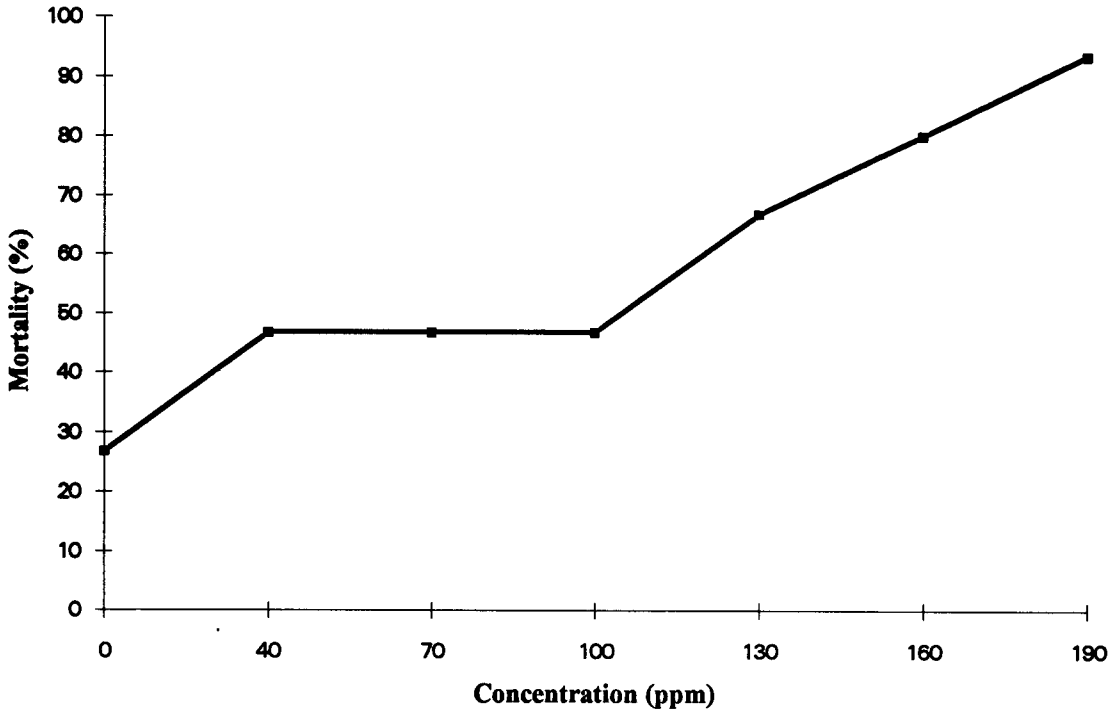

Fig 5.1. Lethal concentrations of Btt and percent mortality of fourth instar convergent lady beetles at the end of 12th day (n = 15).

References Cited

Baldwin, A.J. 1984. *Adalia decempunctata* (L.) (Col., Coccinellidae): Notes on diets, insolation and voltinism. Entomol. Mon. Mag. 120 : 237-38.

Baver, L.S., and H.S. Pankratz. 1992. Ultrastructural effects of *Bacillus thuringiensis* var. *sandiego* on midgut cells of the cottonwood leaf beetle. J. Invert. Path. 60 : 15-25.

Cantwell, G.E., W.W. Cantelo, and M.A. Cantwell. 1986. Effect of beta-exotoxin of Bacillus thuringiensis on development of the Mexican bean beetle (Coleoptera : Coccinellidae). Great Lakes Entomol. 19 : 77-80.

Clausen, C.P. 1940. Entomophagous Insects. McGraw-Hill Book Company, Inc. New York/ London. 688 pp.

Flexner, J.L., B. Lighthart, and B.A. Croft. 1986. The effects of microbial pesticides on non-target, beneficial arthropods. Agri., Ecol. and Env. 16 : 203-254.

James, R.R., and B. Lighthart. 1990. Bioassay for testing the lethal effects of bacterial pathogens on the predatory beetle *Hippodamia convergens* Gue. (Coleoptera : Coccinellidae). EPA National Technical Information Service. Publication no PB91-127795.

Krieg, A., W. Schnetter, A.M. Huger, and G.A. Langenbruch. 1987. *Bacillus thuringiensis subsp. tenebrionis,* strain BI 256-82: a third pathotype within the H-serotype 8a8b. System. Appl. Microbiol. 9 : 138-141.

Mizell III, R.F., and D.E. Shiffhauer. 1990. Effects of pesticides on pecan aphid predators *Chrysoperla rufilabris* (Neuroptera: Chrysopidae), *Hippodamia convergens, Cycloneda sanguinea* (L.), *Olla v-nigrum* (Coleoptera: Coccinellidae), and *Aphelinus perpallidus* (Hymenoptera: Encyrtidae). J. Econ. Entomol. 83 : 1806-12.

Moffitt, H.R., E.W. Anthon, and L.O. Smith. 1972. Toxicity of several commonly used orchard pesticides to adult *Hippodamia convergens*. Environ. Entomol. 1 : 20-23.

Puccia, C.J., and R. Levins. 1985. Qualitative modeling of complex systems,. An introduction to loop analysis and time averaging. Harvard University Press, Cambridge, Massachusetts, and London, England. 259 pp.

Smith, B.C. 1965. Effects of food on longevity, fecundity and development of adult coccinellids (Coleoptera : Coccinellidae). Can. Entomol. 97 : 910-19.

Wilkinson, J.D., K.D. Biever, and C.M. Ignoffo. 1979. Synthetic pyrethroid and organophosphate insecticides against the parasitoid *Apanteles marginiventris* and predators *Geocoris punctipes*, *Hippodamia convergens*, and *Podisus maculiventris*. J. Econ. Entomol. 72 : 473-75.

Wipperfürth, T., K.S. Hagen, and T. E. Miller. 1987. Egg production by the coccinellid *Hippodamia convergens* fed on two morphs of the green peach aphid, *Myzus persicae*. Ent. Exp. Appl. 44 : 195-198.

Summary

Bacillus thuringiensis tenebrionis (Btt) is used commercially to control Colorado potato beetle, *Leptinotarsa decemlineata,* in potato fields. Green peach aphid, *Myzus persicae* is an additional pest occurring in potato fields. During Btt applications against Colorado potato beetle, green peach aphid also is exposed to the spraying. The objective of this study was to determine any indirect impact of Btt δ-endotoxin on an important aphid predator, convergent lady beetle, *Hippodamia convergens.*

Three different experiments were conducted. First, a microbial insecticide (Novodor) was sprayed on green peach aphid prey. The aphid consumption, development time of immature offspring, pupal weight, fecundity and adult longevity of convergent lady beetles in treatment and control groups were compared. We did not observe any significant differences in the number of aphids consumed or the development period of immature convergent lady beetles, although data suggested that fewer aphids were consumed in the treatment group. The pupal weight was 22% less in the Novodor treated lady beetles. Our data suggest that there was a lower ovipositional rate inNovodor treated lady beetles. Overall however, those differences were not statistically significant.

The second experiment consisted of feeding convergent lady beetles with aphids reared on transgenic potatoes. Aphid consumption, developmental period of immatures, pupal weight, fecundity and adult longevity were measured. No significant difference was observed in aphid consumption and development time of immature lady beetles or pupal weight. The transgenic treated lady beetles laid more eggs than the control group but the difference was not statistically significant. There was no difference in the adult longevity.

In the last experiment, different concentrations of a non-commercial preparation of Btt was fed to fourth instar convergent lady beetles by mixing in an artificial diet. Mortality of CLB was observed at high doses of Btt after the ninth day. The development period of fourth instar lady beetles was extended. Overall, Btt may have potential

negative direct and sublethal effects on *Hippodamia convergens*. However, further experiments involving larger sample sizes would be required to confirm this preliminary observation.

Bibliography

Baldwin, A.J. 1984. *Adalia decempunctata* (L.) (Col., Coccinellidae): Notes on diets, insolation and voltinism. Entomol. Mon. Mag. 120 : 237-38.

Barton, K.A., and M.J. Miller. 1993. Production of *Bacillus thuringiensis* insecticidal proteins in plants. In S.Kung and R. Wu [eds.] Transgenic Plants, Engineering and Utilization. Vol 1. Academic Press, Inc. 383 pp.

Baver, L.S., and H.S. Pankratz. 1992. Ultrastructural effects of *Bacillus thuringiensis* var. *sandiego* on midgut cells of the cottonwood leaf beetle. J. Invert. Path. 60: 15-25.

Burton, W.G. 1985. The industrial uses of potato. In R.N. Salaman [ed.] History and Social Influence of Potato. Cambridge University Press. Great Britain. 685p.

Burton, W.G. 1989. Colarado Potato Beetle, history of invasion. In The Potato. 3rd edition. Longman Scientific and Technical Ltd. UK. 742 p.

Buttler, G.D.Jr., and W.A. Dickerson. 1972. Life cycle of the convergent lady beetle in relation to temperature. J. Econ. Entomol. 65 : 1508-1509.

Cancelado, R.E., and E.B. Radcliffe. 1979. Action threshold for Green Peach Aphid on potatoes in Minnesota. J. Econ. Entomol. 72 : 606-609.

Cantwell, G.E., E. Dougherty, and W.W. Cantelo. 1983. Activity of the beta-toxin of *Bacillus thuringiensis var thuringiensis* against Colorado Potato Beetle (Coleoptera, Chrysomelidae) and bacterial mutagenic response as determined by the Amestest. Environ. Entomol. 12 : 1424-1427.

Cantwell, G.E., W.W. Cantelo, and M.A. Cantwell. 1986. Effect of beta-exotoxin of Bacillus thuringiensis on development of the Mexican bean beetle (Coleoptera: Coccinellidae). Great Lakes Entomol. 19 : 77-80.

Carroll, J., and D.L. Ellar. 1989. Proteolytic processing of a coleopteran specific δ-endotoxin produced by *Bacillus thuringiensis* var. *tenebrionis*. Biochem. 261 : 99-105.

Carver, M. 1987. Biological Control of Aphids. In A.K. Minks and P. Harrewijn [eds], Aphids, Their Biology, Natural enemies and Control. Elsevier. Amsterdam/ Oxford/ New York/ Tokyo.

Chambers, R.J. 1987. Syrphidae. In A.K. Minks and P. Harrewijn [eds], Aphids, Their Biology, Natural enemies and Control. Elsevier. Amsterdam/ Oxford/ New York/ Tokyo.

Chedester, L.D. 1979. Feeding habits, reproduction and sexual determination of the convergent lady beetle, *Hippodamia convergens* (Guer.). TAES, Oct 1979, MP-1437, The Texas A&M University System / College Station Texas.

Cheng, J., M.G. Bolyard, R.C. Saxena, and M.B. Sticklen. 1992. Production of insect resistant potato by genetic transformation with a δ-endotoxin gene from *Bacillus thuringiensis* var. *kurstaki*. Science 81 : 83-91.

Cheung, P.Y.K., R.M. Roe, B.D. Hammock, C.L. Judson, and M.A. Montague. 1985. The apparent in vivo neuromuscular in mice and insects of the delta-endotoxin of *Bacillus thuringiensis var. israelensis* in mice and insects of four orders. Pesticide Biochem. and Physiol. 23 : 85-94.

Clausen, C.P. 1916. Life-history and feeding records of a series of California Coccinellidae. U of California publications, Tech. Bull. 1 : 251-299.

Clausen, C.P. 1940. Entomophagous Insects. McGraw-Hill Book Company, Inc. New York/ London. 688 pp.

Copp, N.H. 1983. Temperature-dependent behaviors and cluster formation by aggregating ladybird beetles. Anim. Behav. 31 : 424-430.

Croft, B.A., and M.E. Whalon. 1982. Selective toxicity of pyrethroid insecticides to arthropod natural enemies and pests of agricultural crops. Entomophaga 27 : 3-21.

Croft, B.A. 1990. Arthropod Biological Control Agents and Pesticides. Wiley and Sons, New York, New York, USA.

Dale, P.J., and R.B. Flavell. 1988. Evaluation of genetically engineered plants. In R.T. Fraley, N.M. Frey, J. Schell [eds], Current Communications in Molecular Biology: genetic Improvements of Agriculturally Important Crops, Progress and Issues. Cold Spring Harbor Lab, 116 pp.

Dalton, G.E. 1978. Potato production in world and farm economy. In P.M. Harris [ed], The Potato Crop. Chapman and Hall, London.

Dean, D.H. 1984. Biochemical genetics of the bacterial insect-control agent *Bacillus thuringiensis*: Basic principles and prospects for genetic engineering. Biotech. Gen. Eng. Rev. 2 : 341-363.

Dixon, A.F.G. 1987. The way of life of aphids: Host specificity. In A.K. Minks and P. Harrewijn [eds], Aphids, Their Biology, Natural enemies and Control. Elsevier. Amsterdam/ Oxford/ New York/ Tokyo.

Fast, P.G. 1981. The crystal toxin of *Bacillus thuringiensis*. In H.D. Burges [ed.] Microbial Control of Pests and Diseases 1970-1980. Academic Press, pp 224-248.

Ferro, D.N., and W.D. Gelernter. 1989. Toxicity of a new strain *Bacillus thuringiensis* to Colorado potato beetle (Col: Chrysomelidae). J. Econ. Entomol. 82 : 750-55.

Ferro, D.N. 1993. Potential for resistance to *Bacillus thuringiensis*: Colorado potato beetle (Coleoptera: Chrysomelidae)- A model system. Amer. Entomol. 39 : 38-44.

Ferro, D.N., Q.-C. Yuan, A. Slocombe, and A.F. Tuttle. 1993. Residual activity of insecticides under field conditions for controlling the Colorado potato beetle (Coleoptera : Chrysomelidae). J. Econ. Entomol. 86 : 511-516.

Flexner, J.L., B. Lighthart, and B.A. Croft. 1986. The effects of microbial pesticides on non-target, beneficial arthropods. Agri., Eco. and Env. 16 : 203-254.

Frazer, B.D., and N. Gilbert. 1976. Coccinellids and Aphids: A quantitative study of the impact of adult ladybirds (Coleoptera:Coccinellidae) preying on field populations of pea aphids (Homoptera:Aphididae). J. Entomol. Soc. B.C. 73 : 33-56.

Frazer, B.D. 1987a. Coccinellidae. In A.K. Minks and P. Harrewijn [eds], Aphids, Their Biology, Natural enemies and Control. Elsevier. Amsterdam/ Oxford/ New York/ Tokyo.

Frazer, B.D. 1987b. Predators. In A.K. Minks and P. Harrewijn [eds], Aphids, Their Biology, Natural enemies and Control. Elsevier. Amsterdam/ Oxford/ New York/ Tokyo.

Gatehouse, J.A., V.A. Hilder, and A.M.R. Gatehouse. 1991. Genetic engineering of plants for insect resistance. In D.Grierson [ed.], Plant Genetic Engineering. Blackie and Sons Ltd. 267pp.

Gould, F. 1988. Evolutionary biology and genetically engineered crops. BioSci. 38 : 26-33.

Gunn, J.S. 1990. Crop Production handbook-Potatoes. BCPC, UK.

Haverty, M.I. 1982. Sensitivity of selected non target insects to the carrier of Dipel4L in the laboratory. Environ. Entomol. 11 : 337-338.

Herrnstadt, C., G.G. Soares, E.R. Wilcox, and D.L. Edwards. 1986. A new strain of *Bacillus thuringiensis* with activity against coleopteran insects. Bio/Technology 4 : 305-308.

Hilder, V.A., A.M.R. Gatehouse, S.E. Sheerman, R.F. Barker, and D. Boulter. 1987. A novel mechanism of insect resistance engineered in tobacco. Nature 330 : 160-163.

Himeno, M., N. Koyama, T. Funato, and T. Komano. 1985. Mechanism of action of *Bacillus thuringiensis* insecticidal delta-endotoxin on insect cells in vitro. Biol. Chem. 49 : 1461-1468.

Hodgson, C.J., and I.B. Elbakheit. 1985. Effect of color and shape of "target" hosts on the orientation of emigrating adult apterous *Myzus persicae* in the laboratory. Entomol. Exp. Appl. 38 : 267-272.

Höfte, H., and H.R. Whiteley. 1989. Insecticidal crystal proteins of *Bacillus thuringiensis.* Microbiol. Rev. 53 : 242-255.

Holmes, B. 1993. The perils of planting pesticides. New Scientist 139 : 34- 37.

Horton, D. 1987. Potatoes, Production, Marketing, and Programs for Developing Countries. Westview Press, I T Publ. London.

Horton, D.E., and J.L. Anderson. 1992. Potato production in the context of the world and farm economy. In P.M. Harris [ed.] The Potato Crop: The Scientific basis for Improvement. Chapman and Hall. 909p.

Houck, C.M., D. Facciotti, and R.M. Goodman. 1993. Transgenic plants from Solanaceae. In S. Kung, and R. Wu [eds.] Transgenic Plants, Present Status and Social and Economic Impacts. Vol 2. Academic Press, Inc. 256 pp.

Hunter, K.W.Jr. 1978. Searching behaviour of *Hippodamia convergens* larvae (Coccinellidae, Coleoptera). Psyche June-September,1978 : 2249-2253.

James, R.R., and B. Lighthart. 1990. Bioassay for testing the lethal effects of bacterial pathogens on the predatory beetle *Hippodamia convergens* Gue. (Coleoptera: Coccinellidae). EPA National Technical Information Service. Publication no PB91-127795.

James, R.R., and B. Lighthart. 1992. The effect of temperature, diet, and larval instar on the susceptibility of an aphid predator, *Hippodamia convergens* (Coleoptera: Coccinellidae) to the weak bacterial pathogen *Pseudomonas florescens.* J. Invert. Path. 60 : 215-218.

James, R.R., and B. Lighthart. 1994. Susceptibility of the convergent lady beetle (Coleoptera: Coccinellidae) to four entomopathogenous fungi. Environ. Entomol. 23 : 1-3.

Jannson, R.K., and Z. Smilowitz. 1985. Development and reproduction of the green peach aphid, Myzus persicae (Homoptera: Aphididae), on upper and lower leaves of three potato cultivars. Can. Entomol. 117 : 247- 252.

Krieg, A., W. Schnetter, A.M. Huger, and G.A. Langenbruch. 1987. *Bacillus thuringiensis subsp. tenebrionis*, strain BI 256-82: a third pathotype within the H-serotype 8a8b. System. Appl. Microbiol. 9 : 138-141.

Latge, J.P., and B. Papierok. 1987. Aphid pathogens. In A.K. Minks and P. Harrewijn [eds], Aphids, Their Biology, Natural enemies and Control. Elsevier. Amsterdam/ Oxford/ New York/ Tokyo.

Lowey, D.T., and G. Boiteau. 1988. Effects of five insecticides on the probing, walking and settling behavior of the Green peach aphid and the Buckthorn aphid (Homoptera: Aphididae) on potato. J. Econ. Entomol. 81 : 208-214.

Mackauer, M., and M.J. Way. 1976. *Myzus persicae* Sulz. an aphid of world importance. In Vittorio Delucchi [ed], Studies in Biological Control. Cambridge University Press, Cambridge, England. 304pp.

Martin, P.A.W. 1994. An iconoclastic view of *Bacillus thuringiensis* ecology. Amer. Entomol. 40 : 85-90.

McIntosh, S.C., T.B. Stone, S.R. Sims, P.L. Hunst, J.T. Greenplate, P.G. Marrone, F.J. Perlak, D.A. Fischhoff, and R.L. Fuchs. 1990. Specificity and efficacy of purified *Bacillus thuringiensis* proteins against agronomically important insects. J. Invert. Path. 56 : 258-256.

McManus, M.L. 1990. Microbial pesticides. 1990 USDA Gypsy Moth Research Review. 64-75.

Miller, J.C. 1990. Field assessment of the effects of a microbial pest control agent on non target Lepidoptera. Amer. Entomol. 36 : 135-139.

Miller, J.C. 1992a. Temperature dependent development of the convergent lady beetle (Coleoptera : Coccinellidae). Environ. Entomol. 21 : 197-201.

Miller, J.C. 1992b. Effect of a microbial insecticide, *Bacillus thuringiensis kurstaki*, on nontarget Lepidoptera in a spruce budworm infested forest. J. Res. Lepidop. 29 : 267-76.

Miyazaki, M. 1987. Forms and Morphs of Aphids. In A.K. Minks and P. Harrewijn [eds], Aphids, Their Biology, Natural enemies and Control. Elsevier. Amsterdam/ Oxford/ New York/ Tokyo.

Mizell III, R.F., and D.E. Shiffhauer. 1990. Effects of pesticides on pecan aphid predators *Chrysoperla rufilabris* (Neuroptera: Chrysopidae), *Hippodamia convergens*, *Cycloneda sanguinea* (L.), *Olla v-nigrum* (Coleoptera: Coccinellidae), and *Aphelinus perpallidus* (Hymenoptera: Encyrtidae). J. Econ. Entomol. 83 : 1806-1812.

Moffitt, H.R., E.W. Anthon, and L.O. Smith. 1972. Toxicity of several commonly used orchard pesticides to adult *Hippodamia convergens*. Environ. Entomol. 1 : 20-23.

Moran, N.A. 1992. The evolution of aphid life cycles. Annu. Rev. Entomol. 37 : 321-348.

New, T.R. 1987. Neuroptera. In A.K. Minks and P. Harrewijn [eds], Aphids, Their Biology, Natural enemies and Control. Elsevier. Amsterdam/ Oxford/ New York/ Tokyo.

Obrycki, J.J., and M.J. Tauber. 1982. Thermal requirements for development of *Hippodamia convergens* (Coleoptera: Coccinellidae). Ann. Entomol. Soc. Am. 75 : 678-83.

Peckman, P.S., and G.E. Wilde. 1993. Sublethal effects of permethrin on fecundity and longevity of Hippodamia convergens (Coleoptera: Coccinellidae). J. Kansas Entomol. Soc. 66 : 361-64.

Peferoen, M., S. Jansens, A. Reynaerts, and J. Leemans. 1990. Potato plants with engineered resistance against insect attack. In M.E. Vayda and W.D. Park [eds.], The molecular and cellular biology of the potato. C.A.B International, Oxon, UK. 97-102p.

Perlak, F.J., T.B. Stone, Y.M. Muskopf, L.J. Petersen, G.B. Parker, S.A. McPherson, J. Wyman, S. Love, G. Reed, D. Biever, and D.A. Fischhoff. 1993. Genetically improved potatoes: protection from damage by Colorado potato beetles. Plant Mol. Biol. 22 : 313-321.

Petiit, F.L., and Z. Smilowitz. 1982. Green peach aphid feeding damage to potato in various plant growth stages. J. Econ. Entomol. 75 : 431-435.

Puccia, C.J., and R. Levins. 1985. Qualitative modeling of complex systems,. An introduction to loop analysis and time averaging. Harvard University Press, Cambridge, Massachusetts, and London, England. 259 pp.

Qiang, M.K. 1993. Biological insect control and genetic engineering. In C. You, Z. Chen, Y. Ding [eds], Biotechnology in Agriculture. Kluwer Academic Publishers, Netherlands. 519 pp

Ramen, K.V., and E.B. Radcliffe. 1992. Pest aspects of potato production: Insect pests. In P.M. Harris [ed.], The Potato Crop: The Scientific Basis for Improvement. Chapman and Hall. 476-506 p.

Rhim, S.L., N. Jahn, W. Schnetter, and K. Geider. 1990. Heterologous expression of a mutated toxin gene from *Bacillus thuringiensis* subsp. *tenebrionis*. FEMS Microbiol. Letters 66 : 95-100.

Rockwood, L.P. 1952. Notes on the Coccinellids in the Pacific Northwest. Pan-Pac. Entomol. 28 : 139-147.

Ryan, C.A. 1989. Proteinase inhibitor gene families: Strategies for transformation to improve plant defenses against herbivores. BioEssays 10 : 20-24.

Salama, H.S., F.N. Zaki, and A.F. Sharaby. 1982. Effect of *Bacillus thuringiensis* Berl. on parasites and predators of the cotton leafworm *Spodoptera littoralis* (Boisd.). Z. ang. Ent. 94 : 498-504.

Salama, H.S., and F.N. Zaki. 1983. Interaction between *Bacillus thuringiensis* Berliner and the parasites and predators of *Spodoptera littoralis* in Egypt. Z. ang. Ent. 95 : 425-29.

Sample, B.E., L. Butler, C. Zivkovich, and R.C. Whitmore. 1993. Evaluation of Bacillus thuringiensis and defoliation effects on native Lepidoptera. USDA Forest Service, Northeastern Area Technical Paper 10-93 : 1-13.

Schepers, A. 1987. Chemical Control. In A.K. Minks and P. Harrewijn [eds], Aphids, Their Biology, Natural enemies and Control. Elsevier. Amsterdam/ Oxford/ New York/ Tokyo.

Semtner, P.J. 1984. Effect of transplantation date on seasonal abundance of the Green Peach Aphid (Homoptera: Aphididae) and two aphid predators on flue-cured tobacco. J. Econ. Entomol. 77 : 324-330.

Shands, W.A., and G.W. Simpson. 1972. Insect predators for controlling aphids on potaotes. 8. Green peach aphid consumption by *Coccinella septempunctata* and *C. transversoguttata*. J. Econ. Entomol. 65 : 1388-92.

Shieh, J.-N., R.E Berry, G.L. Reed, and P.A. Rossignol. 1994. Feeding activity of green peach aphid (Homoptera: Aphididae) on transgenic potato expressing a *Bacillus thuringiensis* ssp. *tenebrionis* δ -endotoxin gene. J. Econ. Entomol. 87 : 618-22.

Simpson, R.G., and C.C. Burkhardt. 1960. Biology and evaluation of certain predators of *Therioaphis maculata* (Buckton). J. Econ. Entomol. 53 : 89-94.

Smirnoff, W.A. 1958. An artificial diet for rearing coccinellid beetles. Can. Entomol. 90 : 563-65.

Smith, B.C. 1965. Effects of food on longevity, fecundity and development of adult coccinellids (Coleoptera: Coccinellidae). Can. Entomol. 97 : 910-19.

Starnes, R.L., C.L. Liu and P.G. Marrone. 1993. History, use, and future of microbial insecticides. Amer. Entomol. 39 : 83-91.

Stary, P. 1987. Aphelinidae. In A.K. Minks and P. Harrewijn [eds], Aphids, Their Biology, Natural enemies and Control. Elsevier. Amsterdam/ Oxford/ New York/ Tokyo.

Stary, P. 1987. Aphidiidae. In A.K. Minks and P. Harrewijn [eds], Aphids, Their Biology, Natural enemies and Control. Elsevier. Amsterdam/ Oxford/ New York/ Tokyo.

Stewart, L.A., J.-L. Hemptinne, and A.F.G. Dixon. 1991. Reproductive tactics of ladybird beetles: relationships between egg size, ovariole number and development time. Funct. Eco. 5 : 380-85.

Stewart, L.A., A.F.G. Dixon, Z. Ruzicka, and G. Iperti. 1991. Clutch and egg size in ladybird beetles. Entomophaga 36 : 329-33.

Swan, L.A. 1964. Beneficial Insects. Harper A. D Row, Publishers. New York/ Evanston/ London. 429 pp.

Tabashnik, B.E., N.L. Cushing, N. Finson, and M.W. Johnson. 1990. Field development of resistance to *Bacillus thuringiensis* in diamondback moth (Lep: Pyralidae). J. Econ. Entomol. 83 : 1671-76.

Tabashnik, B.E. 1994. Evolution of resistance to *Bacillus thuringiensis*. Annu. Rev. Entomol. 39 : 47-79.

Vandenberg, J.D. 1990. Safety of four entomopathogens for caged adult honeybees. (Hymenoptera: Apidae). J. Econ. Entomol. 83 : 755-59.

Vencill, A.M., and G.W. Zehnder. 1993. Within-field variation of susceptibility of Colorado potato beetle (Coleoptera : Chrysomelidae) to insecticides: Implications for sampling methods in an insecticide resistance bioassay. J. Econ. Entomol. 86 : 289-93.

Walden, R. 1989. Engineering plants to contain useful agronomic traits. In R.Walden [ed.], Genetic Transformation in Plants. Prentice Hall Advanced Reference Series. 138 pp.

Warren, G.W., N.B. Carozzi, N. Desai, and M.G. Koziel. 1992. Field evaluation of transgenic tobacco containing a *Bacillus thuringiensis* insecticidal protein gene. J. Econ. Entomol. 85 : 1651-59.

Wilkinson, J.D., K.D. Biever, and C.M. Ignoffo. 1979. Synthetic pyrethroid and organophosphate insecticides against the parasitoid *Apanteles marginiventris* and predators *Geocoris punctipes*, *Hippodamia convergens*, and *Podisus maculiventris*. J. Econ. Entomol. 72 : 473-75.

Wipperfürth, T., K.S. Hagen, and T.E. Miller. 1987. Egg production by the coccinellid *Hippodamia convergens* fed on two morphs of the green peach aphid, *Myzus persicae*. Entomol. Exp. Appl. 44 : 195-198.

Xia, J.K., and W.M. Tingey. 1986. Green peach aphid (Homoptera: Aphididae): Developmental and reproductive biology on a *Solanum tuberosum - S. berthaultii* hybrid potato. J. Econ. Entomol. 79 : 71-75.

Appendices

Appendix A

Analysis of Varience and means tables for data analysis.

A.1 Means table for aphid consumption of 1st instar lady beetles in Novodor and control treatments

Treatment	Replicate	Mean	Standard Error	Sample Size
Control	1st rep	14.53333	1.0748	15
	2nd rep	22.16666	1.2016	12
	3rd rep	18.41666	1.2016	12
Novodor	1st rep	15.53333	1.0748	15
	2nd rep	19.41666	1.2016	12
	3rd rep	13.33333	1.2016	12

A.2 ANOVA for aphid consumption of 2nd instar lady beetles in Novodor and control treatments

Source of Variation	Degrees of Freedom	Mean Squares	F-ratio	Significance Level
Trial	2	370.24776	5.751	.0048
Treatment	1	29.53846	.459	.5075
Residual	74	64.379998		

A.3 Means table for aphid consumption of 3rd instar lady beetles in Novodor and control treatments

Treatment	Replicate	Mean	Standard Error	Sample size
Control	1st rep	75.7373	4.2822	15
	2nd rep	60.6667	4.7877	12
	3rd rep	74.0000	4.7877	12
Novodor	1st rep	65.6000	4.2822	15
	2nd rep	72.8182	5.0006	11
	3rd rep	64.5833	4.7877	12

A.4 ANOVA for aphid consumption of 4th instar lady beetles in Novodor and control treatments

Source of Variation	Degrees of Freedom	Mean Squares	F-ratio	Significance Level
Trial	2	26917.603	27.800	.0000
Treatment	1	34.380	.036	.8531
Residual	72	968.26652		

A.5 ANOVA for development period of 1st instar lady beetles in Novodor and control treatments

Source of Variation	Degrees of Freedom	Mean Squares	F-ratio	Significance Level
Trial	2	.2798077	1.286	.2826
Treatment	1	.0512821	.236	.6340
Residual	74	.2176629		

A.6 ANOVA for development period of 2nd instar lady beetles in Novodor and control treatments

Source of Variation	Degrees of Freedom	Mean Squares	F-ratio	Significance Level
Trial	2	.3307692	2.742	.0710
Treatment	1	.2051282	1.700	.1963
Residual	74	.1206514		

A.7 ANOVA for development period of 3rd instar lady beetles in Novodor and control treatments

Source of Variation	Degrees of Freedom	Mean Squares	F-ratio	Significance Level
Trial	2	.9510987	3.973	.0230
Treatment	1	.7015721	2.931	.0911
Residual	73	.2393720		

A.8 Means table for development period of 4th instar lady beetles in Novodor and control treatments

Treatment	Replicate	Mean	Standard Error	Sample Size
Control	1st rep	7.1333	.2393	15
	2nd rep	6.0000	.2571	12
	3rd rep	6.4167	.2676	12
Novodor	1st rep	6.6667	..2393	15
	2nd rep	7.4000	.2931	11
	3rd rep	7.1000	.2931	11

A.9 ANOVA for development period of lady beetle pupas in Novodor and control treatments

Source of Variation	Degrees of Freedom	Mean Squares	F-ratio	Significance Level
Trial	2	2.7822178	7.132	.0016
Treatment	1	1.2590790	3.227	.0770
Residual	66	.3901199		

A.10 Means table for pupal weight of lady beetles in Novodor and control treatments

Treatment	Replicate	Mean	Standard Error	Sample Size
Control	1st rep	.01205	$3.65E^{-4}$	15
	2nd rep	.01374	$4.27E^{-4}$	11
	3rd rep	.01402	$4.08E^{-4}$	12
Novodor	1st rep	.01187	$3.65E^{-4}$	15
	2nd rep	.01118	$4.27E^{-4}$	11
	3rd rep	.01366	$4.48E^{-4}$	10

A.11 ANOVA for total number of eggs laid by lady beetles in Novodor and control treatments after square root transformation

Source of Variation	Degrees of Freedom	Mean Squares	F-ratio	Significance Level
Trial	2	48.70415	4.047	.0543
Treatment	1	174.76183	14.522	.0001
Residual	27	12.034437		

A.12 ANOVA for adult longevity of female lady beetles in Novodor and control treatments after log transformation

Source of Variation	Degrees of Freedom	Mean Squares	F-ratio	Significance Level
Trial	2	.0101355	.063	.8071
Treatment	1	.9173957	5.661	.0088
Residual	27	.1620551		

Appendix B

Analysis of Varience and means tables for data analysis.

B.1 ANOVA for aphid consumption of 1st instar lady beetles in transgenic and control treatments

Source of Variation	Degrees of Freedom	Mean Squares	F-ratio	Significance Level
Trial	2	172.05556	6.040	.0039
Treatment	1	249.38889	8.755	.0042
Residual	68	28.486928		

B.2 Means table for aphid consumption of 2nd instar lady beetles in transgenic and control treatments

Treatment	Replicate	Mean	Standard Error	Sample Size
Control	1st rep	23.1667	1.948	12
	2nd rep	37.5833	1.948	12
	3rd rep	28.5000	1.948	12
Transgenic	1st rep	32.000	1.948	12
	2nd rep	28.9167	1.948	12
	3rd rep	33.4167	1.948	12

B.3 ANOVA for aphid consumption of 3rd instar lady beetles in transgenic and control treatments

Source of Variation	Degrees of Freedom	Mean Squares	F-ratio	Significance Level
Trial	2	709.55556	2.358	.1023
Treatment	1	23.34722	.078	.7844
Residual	68	300.88521		

B.4 Means table for aphid consumption of 4th instar lady beetles in transgenic and control treatments

Treatment	Replicate	Mean	Standard Error	Sample Size
Control	1st rep	185.7500	7.6751	12
	2nd rep	173.9167	7.6751	12
	3rd rep	221.5000	7.6751	12
Transgenic	1st rep	224.5000	7.6751	12
	2nd rep	150.0000	7.6751	12
	3rd rep	222.9167	7.6751	12

B.5 ANOVA for development period of 1st instar lady beetles in transgenic and control treatments

Source of Variation	Degrees of Freedom	Mean Squares	F-ratio	Significance Level
Trial	2	.5138889	2.462	.0928
Treatment	1	.0555556	.266	.6131
Residual	68	.2087418		

B.6 ANOVA for development period of 2nd instar lady beetles in transgenic and control treatments

Source of Variation	Degrees of Freedom	Mean Squares	F-ratio	Significance Level
Trial	2	.1805556	1.335	.2699
Treatment	1	.2222222	1.644	.2042
Residual	68	.1352124		

B.7 ANOVA for development period of 3rd instar lady beetles in transgenic and control treatments

Source of Variation	Degrees of Freedom	Mean Squares	F-ratio	Significance Level
Trial	2	1.3888889	3.700	.0298
Treatment	1	.3472222	.925	.3498
Residual	68	.3754085		

B.8 Means table for development period of 4th instar lady beetles in transgenic and control treatments

Treatment	Replicate	Mean	Standard Error	Sample Size
Control	1st rep	6.6667	.1815	12
	2nd rep	5.9167	.1815	12
	3rd rep	6.4167	.1815	12
Transgenic	1st rep	8.3333	.1815	12
	2nd rep	6.0000	.1815	12
	3rd rep	6.4167	.1815	12

B.9 ANOVA for development period of lady beetle pupas in transgenic and control treatments

Source of Variation	Degrees of Freedom	Mean Squares	F-ratio	Significance Level
Trial	2	28.302749	67.615	.0000
Treatment	1	.884291	2.113	.1510
Residual	66	.4185878		

B.10 ANOVA for pupal weight of lady beetles in transgenic and control treatments

Source of Variation	Degrees of Freedom	Mean Squares	F-ratio	Significance Level
Trial	2	$3.92346E^{-5}$	17.047	.0000
Treatment	1	$8.03118E^{-10}$	.000	.9854
Residual	66	$2.30157E^{-6}$		

B.11 ANOVA for total number of eggs laid by lady beetles in transgenic and control treatments after log transformation

Source of Variation	Degrees of Freedom	Mean Squares	F-ratio	Significance Level
Trial	2	.0844136	.528	.5960
Treatment	1	.2912859	1.822	.1887
Residual	26	.1598746		

B.12 ANOVA for adult longevity of female lady beetles in transgenic and control treatments after log transformation

Source of Variation	Degrees of Freedom	Mean Squares	F-ratio	Significance Level
Trial	2	.4031668	4.544	.0203
Treatment	1	.0307996	.347	.5671
Residual	26	.0887326		