

**DISSERTATION**

**INTEGRATED PEST MANAGEMENT OF TOMATO / POTATO PSYLLID,  
*PARATRIOZA COCKERELLI* (SULC) (HOMOPTERA: PSYLLIDAE) WITH  
EMPHASIS ON ITS IMPORTANCE IN GREENHOUSE GROWN TOMATOES**

**Submitted by**

**Ahmed Mohammad Al-Jabr**

**Department of Bioagricultural Sciences  
and Pest Management**

**In partial fulfillment of the requirements**

**for the Degree of Doctor of Philosophy**

**Colorado State University**

**Fort Collins, Colorado**

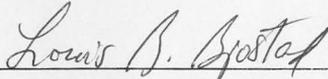
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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY AHMED MOHAMMAD AL-JABR ENTITLED INTEGRATED PEST MANAGEMENT OF TOMATO/POTATO PSYLLID, *PARATRIOZA COCKERELLI* (SULC) (HOMOPTERA: PSYLLIDAE), WITH EMPHASIS ON ITS IMPORTANCE IN GREENHOUSE GROWN TOMATOES BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

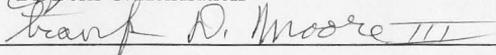
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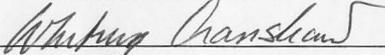
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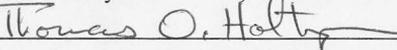
Dr. Boris C. Kondratieff



Dr. Frank D. Moore



Dr. Whitney S. Cranshaw, Advisor



Dr. Thomas O. Holtzer, Department Head

**ABSTRACT OF DISSERTATION**

**INTEGRATED PEST MANAGEMENT OF TOMATO / POTATO PSYLLID,  
*PARATRIOZA COCKERELLI* (SULC) (HOMOPTERA: PSYLLIDAE) WITH  
EMPHASIS ON ITS IMPORTANCE IN GREENHOUSE GROWN TOMATOES**

The tomato (potato) psyllid, *Paratrioza cockerelli* (Sulc), has developed into a key insect pest of greenhouse tomatoes of western North America and few options have been available for its management. A series of trials were thus conducted to identify techniques that could be used in establishing an integrated pest management (IPM) program for the insect.

In studies investigating improvements in sampling methods for tomato psyllid, the effects of trap color, trap orientation and height in respect to the crop were evaluated. Greatest captures of adult psyllids occurred on neon-green, neon-orange and standard yellow colored traps. Traps hung near the top of tomato plants captured significantly more psyllids than traps placed near the base of plants. Traps that were shaded or not orientated to receive direct sunlight caught fewer adult psyllids than did sun-exposed traps.

Screening of potential psyllid control products emphasized those that were efficacious but also compatible with other insects used in greenhouse tomato production (e.g., parasitoids for whitefly control, pollinators). Tested materials included insecticides

of microbial origin (*Beauveria bassiana*, *Verticillium lecanii*, *Metarhizium anisopliae*), microbial-derived insecticides (spinosad), selective synthetic insecticides (acetamiprid, pymetrozine) and selective botanical products (neem) for tomato psyllid control. The capability of *B. bassiana* to infect tomato psyllid and produce high mortality is reported for the first time. Acetamiprid, spinosad and formulations of *Beauveria bassiana* were particularly effective for control of tomato psyllid, consistently providing in excess of 80 percent control. As the latter two also are reportedly compatible with beneficial insects used in greenhouse tomato production they appear to show greatest potential for use in an integrated pest management program on the crop. Also, in trials of various repellents (Azatin XL, Trilogy, SunSpray and Garlic Barrier) for deterring oviposition, SunSpray and Trilogy did result in significant reductions in oviposition on treated leaves.

Two species of green lacewings, *Chrysoperla carnea* Stephens and *C. rufilabris* (Burmeister) were evaluated for potential use as biological controls of tomato psyllid. Both species were capable of completing their life cycle on tomato psyllid. *Chrysoperla carnea* larvae consumed approximately twice as many psyllids as did *C. rufilabris*, but development of the latter was 50% faster (8 days vs. 12 days). An outdoor field trial involving applications of *C. carnea* eggs to psyllid-infested potatoes did not produce reductions in psyllid numbers.

As soil applications of imidacloprid had previously been found to be highly effective for tomato psyllid, and had registration for use on field grown tomatoes, investigations were conducted on possible non-target effects on pollinators, specifically

the bumble bee *Bombus terricola occidentalis* (Greene). Indications of adverse effects from imidacloprid were observed during these trials, including reduced activity of bees within the hive, reduced visitation to flowers, and often, reduced survival.

Ahmed Mohammad Al-Jabr  
Department of Bioagricultural  
Sciences and Pest Management  
Colorado State University  
Fort Collins, Colorado 80523  
Fall 1999

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## **DEDICATION**

This work is dedicated to my other half, my wife Kholoud and to my Children, Hanan and Abdulelah.

## Table of Contents

Chapter I.....	1
Literature Review.....	1
The Tomato Psyllid.....	1
Description.....	1
Distribution.....	3
Host Plants.....	3
Overwintering and Migration.....	4
Tomato Psyllid and Psyllid Yellows.....	5
Tomato Psyllid Control.....	6
Green Lacewings as Biological Controls.....	7
Other Potential Tomato Psyllid Control Products.....	11
Microbial Insecticides.....	15
References Cited.....	20
Objectives.....	28
Chapter II.....	29
Detecting and Trapping Tomato Psyllid <i>Paratrioza cockerelli</i> (Sulc) (Homoptera: Psyllidae) in Tomato Greenhouses.....	29
Introduction.....	29

Results and Discussion .....	34
References Cited .....	43
Chapter III .....	44
Evaluations of Conventional and Non-conventional Insecticides and Repellents for Control of Tomato Psyllid, <i>Paratrioza cockerelli</i> (Sulc) (Homoptera: Psyllidae), on Greenhouse Tomato .....	44
Introduction .....	44
Results and Discussion .....	53
Literature Cited .....	60
Chapter IV .....	64
Evaluation of Green Lacewings (Neuroptera: Chrysopidae) as Biological Control Agents for <i>Paratrioza cockerelli</i> (Homoptera: Psyllidae) in Greenhouse Tomatoes .....	64
Introduction .....	64
Materials and Methods .....	66
Results and Discussion .....	68
Chapter V .....	74
Effect of Imidacloprid on <i>Bombus terricola occidentalis</i> (Hymenoptera: Apidae) Colonies .....	74
Introduction .....	74
Materials and Methods .....	80
Results and Discussion .....	82

References Cited ..... 90

## List of Tables

Table 2.1. Capture of adult <i>Paratrioza cockerelli</i> on different colored and patterned traps in a tomato greenhouse, Ft. Collins, CO 1997.....	34
Table 2.2. Capture of <i>Paratrioza cockerelli</i> on different colored traps in a tomato greenhouse, Ft. Collins, CO 1997.....	35
Table 2.3. Capture of <i>Paratrioza cockerelli</i> on different colored traps in a tomato greenhouse, Ft. Collins, CO 1997.....	36
Table 2.4. Capture of <i>Paratrioza cockerelli</i> on neon green and neon orange sticky traps in a tomato greenhouse, Ft. Collins, CO 1999.....	36
Table 2.5. Effects of shading on capture of <i>Paratrioza cockerelli</i> on colored traps in a tomato greenhouse, Ft. Collins, CO 1999.....	37
Table 2.6. Effect of different orientation of green and orange traps on capture of <i>Paratrioza cockerelli</i> in a tomato greenhouse, Ft. Collins, CO 1999.....	38
Table 2.7. Effect of different orientations of neon green traps on capture of <i>Paratrioza cockerelli</i> in a tomato greenhouse, Ft. Collins, CO 1999.....	39
Table 2.8. Capture of <i>Paratrioza cockerelli</i> on neon orange traps placed at two different heights adjacent to greenhouse tomatoes, Ft. Collins, CO 1999.....	40
Table 3.1. Mortality of tomato psyllid nymphs following exposure to various <i>Beauveria bassiana</i> formulations in spray tower applications, Fort Collins, CO 1998.....	53

Table 3.2. Effect of different insecticides on <i>Paratrioza cockerelli</i> nymphs in a tomato greenhouse, Fort Collins, CO 1997.....	55
Table 3.3. Effect of different insecticides on <i>Paratrioza cockerelli</i> nymphs in a tomato greenhouse, Fort Collins, CO 1998.....	56
Table 3.4. Effect of different insecticides on <i>Paratrioza cockerelli</i> nymphs in a tomato greenhouse, Fort Collins, CO 1999.....	57
Table 3.5. Effect of different deterrents on oviposition of <i>Paratrioza cockerelli</i> on greenhouse grown tomatoes, Fort Collins, CO 1997.....	58
Table 4.1. Prey consumption and survival of <i>Chrysoperla carnea</i> and <i>C. rufilabris</i> reared on tomato psyllid or green peach aphid, Fort Collins, CO 1999 .....	68
Table 4.2. Consumption of green peach aphid and tomato psyllid <i>Chrysoperla carnea</i> and <i>C. rufilabris</i> in a choice test, Fort Collins, CO 1999 .....	69
Table 4.3. Effect of a <i>Chrysoperla carnea</i> application on numbers of tomato psyllids and green peach aphids in a potato planting, Fort Collins, CO 1999 .....	70
Table 5.1. Number of adult bees, honey pots, and pupae in bumble bee colonies placed in imidacloprid treated and untreated tomato greenhouses, Fort Collins, CO. 1997.....	82
Table 5.2. Number of adult bees, honey pots, and pupae in bumble bee colonies placed in imidacloprid treated and untreated tomato greenhouses for 25 days after imidacloprid application, Fort Collins, CO. Total of two replications (colonies). Trial One, 1998 .....	83

Table 5.3. Number of adult bees, honey pots, pupae and adult mortality percent in bumble bee colonies placed in imidacloprid treated and untreated tomato greenhouses for 20 days after imidacloprid application, Fort Collins, CO. Total of two replications (colonies). Trial Two, 1998 ..... 84

Table 5.4. Number of adult bees observed moving on top of bumble bee colonies (index of activity) placed in imidacloprid treated and untreated tomato greenhouses, Fort Collins, CO. Trial Two, 1998.....85

Table 5.5. Mean of honey pots and pupae per colony in imidacloprid treated and untreated tomato greenhouses, Fort Collins, CO. Combined data from trials two and three 1998 ..... 85

Table 5.6. Mean no. of adults, adult mortality, mean no. of pupae and mean no. of honey pots per colony in bumble bee colonies placed in imidacloprid treated and untreated tomato greenhouses, Fort Collins, CO. Total of three replications (colonies)\* 1999 ..... 86

Table 5.7. Numbers of visited flowers, foraging bumble bees and adults moving on top of the colony (index of activity) in imidacloprid treated and untreated tomato greenhouses, Fort Collins, CO. 1999, Means of three trial runs..... 87

## **Chapter I**

### **Literature Review**

#### **The Tomato Psyllid**

The tomato psyllid *Paratrioza cockerelli* (Sulc) belongs to the family Psyllidae (Homoptera). T. D. Cockerell collected the first specimen on cultivated peppers in Boulder, Colorado in 1909, which was subsequently described by Sulc as *Triozia cockerelli* (Pletsch, 1947). Crawford (1911) later assigned the species to the genus *Paratrioza* that he proposed in 1910. Three common names have been subsequently used for this insect: tomato psyllid, potato psyllid, and potato and tomato psyllid. Currently only the first two are formally recognized in the *Common Names of Insects & Related Organisms* produced by the Entomological Society of America (Stoetzel, 1989). The common name tomato psyllid will be adopted throughout this dissertation. There have been several reviews of the tomato psyllid (Carter, 1950; Cranshaw, 1994; Daniels, 1954; Knowlton and Janes, 1931; Pletsch, 1947).

#### **Description**

Psyllid eggs usually are laid on leaf edges, on the underside on veins, and sometimes are scattered over the upper portions of the plant (List, 1939a). In field conditions the majority of eggs and nymphs are found on the lower side of the leaves (Pletsch, 1947). Knowlton and Janes (1931) reported that shade is the limiting factor of egg location and noted that psyllid eggs in greenhouses were found on all upper plant parts during the cooler seasons of the year. The eggs, which are deposited singly on a stalk two-thirds the length of the egg, are oblong-ovate, shiny yellow. The egg color changes to more orange with the development of the embryo during the incubation period, which ranged from three to nine days. In older eggs, the red nymphal eyes are observable near the apical end (Rowe and Knowlton, 1935). Knowlton and Janes (1931) reported the oviposition period averaged 21.45 days

There are five instars that are largely similar in their morphological features (Compere, 1916). The main structural changes in the later instars are the increase of body size, development of wings and an added number of marginal wax glands (Rowe and Knowlton, 1935). The first instar nymph is a light yellow color, oval and scale-like with red eyes. The nymphal color changes to light tan in the second instar, is tan with greenish hues in the third instar, and is green or greenish brown in fourth and fifth instars. Wing pad development is first noted in the third instar and becomes obvious in fourth and fifth instars (Pletsch, 1947). Knowlton and Janes (1931) reported the average nymphal development period ranged from 12 to 21 days.

The adults are described in great detail by Rowe and Knowlton (1935). They are minute with clear wings held roof-like over the abdomen and resemble miniature cicadas (Pletsch, 1947). Their color goes through gradual changes from light yellow to pale green when they first emerge, brown or green two or three days later, until they become gray or black when they are five days old (Pletsch, 1947). Mating, which may be repeated, first occurs two to three days after emergence when adults gain the dark color (Knowlton and Janes, 1931). Females lay up to 510 eggs over their lifetime and can live up to three times as long as males (Pletsch, 1947; Wallis, 1955). Males and females can be distinguished by the shape of the apex of the abdomen. The mature female abdomen, which terminates with a short ovipositor, is well rounded and more robust than the male abdomen. The male genitalia present a more blunt appearance at the tip (Pletsch, 1947).

Knowlton (1933c) designated the tomato psyllid as “temperature-zone insect” based on the correlation of summer temperatures and psyllid abundance. List (1939a; 1939b), investigating the effect of temperature on psyllid in the greenhouse, revealed that a temperature of 26.6<sup>o</sup>C seemed most favorable to psyllid development and survival. Development and survival

was low at temperatures below 15.5<sup>0</sup>C or above 32.2<sup>0</sup>C. Laboratory studies on effects of temperature on egg hatching and nymphal development indicated temperatures of 26.6<sup>0</sup>C are optimal for egg laying. Oviposition is reduced under 32.2<sup>0</sup>C, while 37.8<sup>0</sup>C is lethal to eggs and nymphs and females cease egg laying at this temperature.

### **Distribution**

Tomato psyllid distribution has been reviewed comprehensively by Pletsch (1947) and Tuthill (1943). Specimens of tomato psyllid have been collected from Arizona, California, Colorado, Idaho, Kansas, Minnesota, Nebraska, Nevada, New Mexico, North Dakota, Oklahoma, South Dakota, Texas, Utah, and Wyoming within the US; Alberta, Saskatchewan, and British Columbia in Canada; and Durango, Tamaulipas, D. F., and Michiocan in Mexico (Pletsch, 1947). Isolated collections from wild or cultivated hosts have been recorded in Nevada, Kansas, Oklahoma and four Mexican states. The level of psyllid abundance and damage vary from one location to another. Pletsch (1947), in mapping locations of greatest psyllid injury to potatoes, indicated that Utah, Colorado, Wyoming and Nebraska displayed most severe or frequent damage. Montana and New Mexico had moderate or occasional damage and light or rare damage occurred in California, Arizona, Texas, Idaho, North Dakota, South Dakota, Alberta and Saskatchewan. Texas, southern New Mexico, Arizona, California and northern Mexico are desert breeding areas. In recent years (notably 1999) severe damage to summer-grown potatoes has been reported in western Texas and eastern New Mexico (Cranshaw, personal communication).

### **Host Plants**

The tomato psyllid has a wide range host of cultivated and non-cultivated plants. Pletsch (1947) reported that potato plants were the favorite host for psyllid females to lay eggs on when

compared with other solanaceous hosts. However, the development period from egg to adult did not differ greatly on tomatoes and potatoes. In the other hand, nymphal life is considerably longer on non-solanaceous hosts such as bindweed (Pletsch, 1947).

Although tomato psyllid mostly is found on members of Solanaceae, they have been reported on Amaranthaceae, Asclepiadaceae, Asteraceae, Brassicaceae, Chenopodiaceae, Convolvulaceae, Fabaceae, Lamiaceae, Lycophyllaceae, Malvaceae, Menthaceae, Pinaceae, Poaceae, Polygonaceae, Ranunculaceae, Rosaceae, Salicaceae, Scrophulariaceae, Violaceae and Zygophyllaceae (Knowlton and Thomas, 1934; Pletsch, 1947; Wallis, 1955).

### **Overwintering and Migration**

Tomato psyllid migrates every summer to northerly locations desert breeding grounds (Pletsch, 1947). Evidence for this annual migration offered by Wallis (1946) was indicated by: 1) tomato psyllids never being found in considerable numbers in the northern areas; 2) airplane trapping of alates at up to 1524 m; 3) coincidental capture with sugar beet leafhoppers, *Circulifer tenellus* (Baker), a species with previously documented migration from southern areas; 4) a heavier pattern of infestations in areas closer to breeding sites; 5) intolerance of high temperature and disappearance from winter breeding sites during the summer; and 6) cage studies showing the species can not withstand sub-zero temperatures that exist in northern sites.

Romney (1939) reported that winter populations of tomato psyllid primarily breed on *Lycium andersonii* A. Gray and *L. macrodon* Gray. They occur on these plants from January to May with peak populations in April or May. In southwest Texas they can be found on winter crops of tomato and potato and on the native host plants *L. carolinianum* var. *quadrididum* (Dunal) C. Hitchcock, *Physalis mollis* (nutt. Waterfall), and *S. quetrum* Cav. (Janes, 1939). Following the northward migration in spring tomato psyllids are not found in the overwintering areas until late in October or early in November when an influx is observed (Romney, 1939).

## **Tomato Psyllid and Psyllid Yellows**

The importance of tomato psyllid to Solanaceae species is due to its association with the disease described as “psyllid yellows,” first reported by Richards (1928). Psyllid yellows is caused by a chlorotic toxemia that is produced by the feeding of the insect (Carter, 1950). Blood et al. (1933) reported that symptoms were not induced by inoculation with less than 30 psyllids and that if insects were removed plants would recover. Production of psyllid yellows symptoms is generally reported to be limited to feeding by the nymphal stage, although Carter (1950) found that not all nymphs have the ability to produce a toxic reaction. However, Daniels (1954) found a toxic reaction in tomato seedlings due to adult feeding, contradictory to other findings. Attempts to eliminate psyllid nymph’s inherited ability to produce toxic effects by rearing them on healthy plants has repeatedly failed (Carter, 1950; Daniels, 1954; Richards, 1928).

Physiological and histological changes within leaves at the psyllid feeding site have been reported. Eyer (1937) found protein breakdown to occur due to tomato psyllid feeding and healthy plants had higher nitrogen levels than diseased plants. Sucrose levels are abnormally high, starch levels low in damaged plants and carbohydrate metabolism is affected by the injury. The mechanical rupturing of cells leads to necrosis and localized inhibition of translocation. As a result, chloroplasts appear smaller, lighter in color, and distorted. Complete disruption of chloroplasts, lighter pigmentation and lower levels of chlorophyll and carotene are observed in heavily damaged tissues.

Under field conditions symptoms of psyllid yellows may appear within four to six days following feeding by large numbers of tomato psyllids (Daniels, 1954). However, Carter (1950) found psyllid yellows symptoms could be obtained on tomato seedlings in as little as two hours,

and within six hours with feeding of a single nymph. Daniels (1954) described psyllid yellows symptoms in tomato and further classified them as being primary or secondary symptoms. The primary symptoms in tomatoes are retarded growth, erectness of new growth, chlorosis, and purpling of the leaves. Secondary symptoms include malformation and distortion of foliage, widespread chlorosis, growth at a standstill for weeks to months, stimulated flower bloom, and production of numerous and small poor quality fruit. However, effects on fruit set can be variable. Abernathy (1991) found in field evaluation of eight varieties of tomatoes that yield loss from psyllid injury was caused by a combination of reduced fruit set and fruit size. These effects led to a reduction in yield for all varieties by more than 40 percent.

### **Tomato Psyllid Control**

**Cultural Control.** Hartman (1937) suggested that early potato plantings are more severely affected by psyllids than late plantings. In Montana, during the 1938 outbreak, this seemed generally true, although, in lighter psyllid infestations no differences in disease prevalence were observed (Pletsch, 1947). Soil characteristics and mineral and fertilizer richness can help to minimize the effect of infestation. Schall (1938) stated that on plants grown on highly alkaline soils, plants with fungal disease, and plants with injured stems and root systems psyllid yellows symptoms developed from feeding by relatively fewer nymphs. Eyer and Enzie (1939) pointed out the possible value of fertilizers and supplementary minerals in correcting the lack of chlorophyll and nitrate nitrogen of plants affected with psyllid yellows.

**Biological Control.** Romney (1939) observed parasitism of *P. cockerelli* on uncultivated hosts and described as a hymenopterous parasite (Eulophidae), *Tetrastichus* sp., which was later described by B. D. Burks as *T. triozae* (Pletsch, 1947). Johnson (1971) later made numerous field observations on this species. He concluded that it was generally ineffective for biological

control of tomato psyllid because of: 1) poor synchronization between the psyllid and the parasite, with the latter appearing late in the season; and 2) high parasite pupal mortality in the field which ranged from 38-100%. In addition, studies in the laboratory revealed that female wasps only deposited eggs on fourth and fifth instar psyllid nymphs (Pletsch, 1947) allowing considerable feeding and injury to occur before the insect was killed.

Compere (1943) described a parasitic wasp, *Metaphycus psyllidus* Compere (Encyrtidae), which he reared from *P. cockerelli* in California. There is no further discussion regarding this parasite or its biology in the literature.

There are no reported field experiments or field observations of tomato psyllid predators (Pletsch, 1947). Knowlton (1933a; 1933b; 1933d; 1934) reported laboratory feeding of several predators when confined with tomato psyllid nymphs or adults in the same vial. The list of predators includes several species of lady beetles: larvae and adults of *Hippodamia convergens* Guerin-Meneville, adults of *H. americana* Crotch, *H. lecontei* Mulsant, *H. quinquesignata* Kirby, and *H. tredecimpunctata* (L.). Also, noted as feeding on tomato psyllid were larvae of a green lacewing, *Chrysopa* (= *Chrysoperla*?) spp., adult nabids, *Nabis ferus* (L.), an unidentified syrphid fly larva and adult big-eyed bugs, *Geocoris decoratus* Uhler. Knowlton and Allen (1936) reported three hemipterous predators feeding on caged psyllid adults: *Anthocoris melanocerus* Reuter, *Deraeocoris brevis* Uhler, and *Orius tristicolor* B. White. Among the predators, Pletsch (1947) argued that the lady beetles, big-eyed bugs and lacewings warranted further research attention in regards to psyllid management.

### **Green Lacewings as Biological Controls**

The potential use of *Chrysoperla carnea* Stephens and other lacewing species to control greenhouse species is substantial (New, 1975). Two species of green lacewing are commonly

used in biological control of insect pests in North America, *C. carnea* and *C. rufilabris* (Burmeister). Both are widely distributed (Hydorn, 1971) and are among the seven species of chrysopids reported in the United States (Burke and Martin, 1956; Van den Bosch and Hagen, 1966; Whitcomb and Bell, 1964).

*Chrysoperla carnea* and *C. rufilabris* are predaceous in the larval stage and attack a wide variety of pest insects, including aphids, chinch bugs, mealybugs, scales, whiteflies, leafhoppers, lepidopterous eggs and larvae, and mites (Hydorn, 1971). Compared to *C. carnea*, *C. rufilabris* tends to occur in greater numbers later in the season, at a time when pest numbers have increased (Agnew et al., 1981). This species also produces more eggs per female and is considered a more aggressive predator (Elkarmi et al., 1987).

Kuzentsova (1969) found the incubation period for *C. carnea* eggs to be 6.4 to 6.8 days at 20°C, dropping to 2.3 to 2.5 days at 35°C. Butler and Ritchie (1970) recorded incubation periods of 13 and 4 days at 15°C and 25°C, respectively, for *C. carnea*. Egg hatching could be rapidly induced by a sharp rise of temperature (Canard and Principi, 1984). Toschi (1965) found that the incubation period of eggs in the laboratory under varying conditions averaged five days.

Burke and Martin (1956) found that the larva completes two molts during the active or feeding period and undergoes the last molt within a cocoon. The newly hatched larva begins feeding shortly after emerging from the egg. The number of aphids consumed by a larva of *C. rufilabris* averaged 8.3 per day (Canard and Principi, 1984). Canard et al. (1984) showed that larval development generally might be completed within 1 to 2 weeks and several hundred eggs may be laid by an individual female.

The time of development from hatching to adult emergence ranged from 16 to 27 days with an average of 18 days (Toschi, 1965). The development threshold temperature for chrysopid

larvae is usually at least 8<sup>0</sup>C and larvae are not active early in the season (New, 1975). Average duration for immature stages at 24<sup>0</sup>C was 5.8, 3.5, 5.2, and 3.8 days for instars to 1 to 4 respectively; pupae needed 9.6 days at the same temperature (Toschi, 1965). Adult longevity of *C. rufilabris* ranged from 8 to 45 days with an average of 18 days. Females were in copulation with males soon after emergence and in most cases mating occurred within the first two days of adult activity (Burke and Martin, 1956). The oviposition period of *C. rufilabris* ranged from 2 to 30 days with an average of 11.3 days. Eggs are laid singly on stalks and rarely is more than one egg found per leaf under natural conditions (Toschi, 1965).

Malik (1981) stated that there are about five to six generations of *C. carnea* per year in Arizona. Toschi (1965) found three to five generations per year of *C. carnea* populations in California. Malik (1981) stated that the major factors that govern population fluctuations of *C. carnea* include climate, food, diseases, parasites and agricultural practices. He found that higher temperature and long photoperiod enhances development, whereas low temperatures and short photoperiods slow development of immature stages. Temperature plays a major role in the development of chrysopids, but as Canard et al. (1984) suggested, temperature alone cannot give meaningful conclusions if nutritional factors are not included.

Adults of chrysopids in the genus *Chrysoperla* feed on nectar, pollen and honeydew and this habit may attract them to the vicinity of aphids and other homopterans (New, 1975). Many chrysopid larvae invading agroecosystems are extremely general in feeding habits. For example, Barnes (1975) found preferences for a particular prey to be minimal for *C. zastrowi* (Esben-Petersen). Toschi (1965) found that larval development was completed with low number of aphids and normal sized adults were obtained. The diet of *C. rufilabris* apparently affects the development, survival, adult emergence, and body weight of newly emerged adults (Legaspi et

al., 1994). Larvae that were provided *Helicoverpa zea* (Boddie) and *Sitotroga cerealella* (Olivier) eggs developed faster, had an increased survival rate, and weighed more as newly emerged adults than larvae that were fed on a combined diet of lepidopteran eggs and sweetpotato whitefly or an artificial diet (Legaspi et al., 1994).

Major egg parasites of chrysopids are Hymenoptera within the families Trichogrammatidae and Scelionidae. *Trichogramma evanescens* (Westwood) has been reared from eggs of various chrysopids (Clancy, 1946) and scelionids are the most frequent parasites of chrysopid eggs. Species of this latter family have been reported in France, Italy, Britain, India and Egypt (Canard et al., 1984). Egg parasites are moderately important in causing chrysopid mortality (Canard et al., 1984).

Chrysopid larvae may be heavily parasitized. Clancy (1946) reported *Proctotrupoidea heloridae* (Townes) ( Heloridae) as a specific chrysopid larval parasite that kills the host before the pupal molt. Several species of *Tetrastichus* (Eulophidae) have been reared in North America and Europe (Canard et al., 1984). Of the Encyrtidae, Homalotylini and several species of *Isodromus* are among the most frequently recorded chrysopid parasites (Canard et al., 1984).

There is little information available about chrysopid adult parasites. The most important adult parasite (which is restricted to Europe) is an euphorine braconid, *Chrypophthorus chrysopimuginis* (Goidanich) (Canard et al., 1984). External mites have been observed associated with lacewings in Colorado (Cranshaw, personal communication).

Chrysopidae apparently do not have specific predators, however they are considered a part of the prey range of arthropods. A male empidid fly, *Empimorpha barbata* (Loew) has been recorded feeding on *C. carnea* (Toschi, 1965). Evans (1978) recorded that a sphecid wasp *Bembix stenebdoma* (Parker) nest contained ten chrysopid adults. Mehra and Dasgupta (1969)

recorded dragonflies capturing lacewings in flight. Adult chrysopids are also taken by robber flies (Asilidae) (Smith, 1922). Nabids have been seen to attack chrysopid larvae in Colorado (Cranshaw, personal communication).

**Chemical Control.** In 1911, prior to the identification of the causal agent, Johnson reported on the effectiveness of lime sulfur for control of psyllid yellows in tomatoes (Pletsch, 1947). Thorough coverage was essential to control. Control was effected by direct kill of adults during application time, repellence of ovipositing females, and a residual effect on the nymphs that settle on the sprayed surfaces.

Pack (1929) and Knowlton (1933c) reported that summer oil sprays, pyrethrum sprays, nicotine sulfate spray, and calcium cyanide dust also resulted in good nymphal control. Later, as synthetic organic insecticides were evaluated, Gerhardt and Turley (1961) reported effective insect control of tomato psyllid for approximately 100 days after planting following soil application of granules containing 10% phorate. Application to both sides of the seed piece of fertilizer-phorate mixture (0.2% phorate) also gave satisfactory control. Foliar application of systemic insecticides such as demeton, dimethoate and phorate similarly were effective to control tomato psyllid. Cranshaw (1989; 1994) reported that most pyrethroid and organophosphate insecticides applied as foliar sprays provide acceptable control when thorough coverage was performed.

#### **Other Potential Tomato Psyllid Control Products**

**Neem Insecticides.** Compounds extracted from the neem tree *Azadirachta indica* (A. Juss) look promising for control of many pest insects (Schmutterer, 1990). The neem tree has more than 100 common names that include also Limba and Indian lilac. It is a deciduous fast growing tree that may reach 25 m and has been introduced as shade tree to many countries because of its

tolerance to heat and poor soils (Koul et al., 1990). *Azadirachta indica* is widely distributed in arid tropical and subtropical zones of Asia, Africa, the Americas, Australia and the South Pacific islands (Schmutterer, 1995).

The insecticidal properties of *A. indica* were discovered more than 35 years ago (Ascher, 1993). The most important source of ingredients of neem affecting insects comes from the seeds (Schmutterer, 1990) which contain high levels of azadirachtin and other active compounds. Therefore, most insect control substances are prepared from seed extracts (Jacobson, 1988). Azadirachtin is also present in other *A. indica* tissues at lower levels such as bark and leaves (Ascher, 1993). Most of the active ingredients in the neem plant belong to the group of tetranortriterpenoids (Kraus, 1995). The tetracyclic triterpenoid tirucallol is suspected to be the parent compound to all biologically active neem compounds; all other products formed are considered successive rearrangement and oxidation products of tirucallol (Ascher, 1993). Although azadirachtin is responsible for the majority of biological effects observed in organisms exposed to neem compounds (Isman et al., 1990), 25 different biologically active compounds have been isolated from neem seeds (Lee et al., 1991).

Extracts of neem fruit, seeds, seed kernels, twigs, stem bark, and root bark are reported to possess insect antifeedant, insecticidal, and insect growth disrupting activity (Kraus, 1995). Other effects of neem derivatives on insects include prevention of insect settling, reduced fecundity and egg sterility, lessened insect fitness, and egg laying deterrence (Schmutterer, 1990).

The primary active ingredient of neem extracts is azadirachtin, which has strong antifeedant, insect growth regulatory and reproductive effects. Sensitivity between species to the antifeedant effects of azadirachtin is profound; members of the order Lepidoptera appear most

sensitive to azadirachtin's antifeedant effects, with Coleoptera, Hemiptera and Homoptera being generally less sensitive (Mordue and Blackwell, 1993). Neem-derived insecticides have been demonstrated effective against several Homoptera, including some psyllids. On systemically treated plants, high concentrations of azadirachtin (> 100 ppm) are required to produce primary antifeedant effects on green peach aphid, *Myzus persicae* (Sulzer). Cereal aphid *Rhopalosiphum padi* (L.) and *Sitobion avenae* (F.) were prevented from settling on barley seedlings when leaves treated with azadirachtin (Nisbet et al., 1993).

Saxena (1995) also reported the repellence of neem oil to the whitebacked planthopper *Sogatella furcifera* (Horvath). Asiatic citrus psyllid, *Diaphorina citri* was effectively repelled when treated with a spray application of 1.4% emulsified neem oil on citrus under laboratory and field trials. The sweetpotato whitefly *Bemisia tabaci* (Gennadius), was repelled on cotton when treated with neem seed extract.

**Imidacloprid.** Imidacloprid (1-[(6-Chloro-3pyridinyl) methyl]-N-nitro-2-imidazolidinone) is an insecticide developed worldwide by Bayer AG. Introduced to the United States in 1994 it is the first registered insecticide in the chloronicotinyl nitroguanidine class. Imidacloprid is a broad-spectrum systemic and contact insecticide exhibiting low mammalian toxicity, with primary activity on sucking insects such as Homoptera. It also has excellent potential in seed and soil applications (Schmeer et al., 1990). It is used as a crop and structural pest insecticide, a seed treatment, and a flea-control treatment. Commercially available products in North America that contain imidacloprid include Merit<sup>®</sup>, Admire<sup>®</sup>, Provado<sup>®</sup>, Imicide<sup>®</sup>, Gaucho<sup>®</sup>, Marathon<sup>®</sup>, Premise<sup>®</sup>, and Advantage<sup>®</sup>. These products come in different formulations including granules, liquids, wettable powders, seed treatments, flowables, and trunk injection concentrates.

The mode of action of imidacloprid involves disruption of the insect nervous system, acting as a competitive inhibitor at nicotinic acetylcholine receptors (Yie and Casida, 1993; Zwart et al., 1994). The signals that are induced by acetylcholine at the post-synaptic membrane are blocked effectively resulting in impairment of normal nerve function (Zwart et al., 1994). Insects may be killed by contact or ingestion (Lagadic et al., 1993).

Imidacloprid is primarily used to control sucking insects, primarily aphids and whiteflies, and is effective against adult or immature stages of various species (Elbert et al., 1990; Lagadic et al., 1993; Leicht, 1996). In greenhouse trials imidacloprid has been found to be effective against such insects as *B. tabaci*, *Amrasca biguttula biguttula* (Ishida) and *Aphis gossypii* Glover (Elbert et al., 1990). In agricultural settings, seed treatments of imidacloprid have been used to protect cereal crops from Russian wheat aphid, *Diuraphis noxia* (Mordvilko) (Pike et al., 1993) and cotton from cotton aphid, *A. gossypii* (Nauen and Elbert, 1994). On lettuce, Palumbo and Kerns (1994) reported effective control by imidacloprid for green peach aphid; sweetpotato whitefly was also well controlled on the crop (Palumbo et al., 1996). Natwick et al. (1996) found all imidacloprid treatments to be effective for control of green peach aphid, cabbage aphid (*Brevicoryne brassicae* (L.)), and whiteflies in cauliflower. The behavior of *M. persicae* is strongly affected by imidacloprid. Nauen (1995) found that low concentrations of imidacloprid depressed the honeydew excretion of apterous adults of *M. persicae* by almost 95% within 24 hours. Aphid nymphs were not found on plants systemically treated with low concentrations of imidacloprid 24 hours after treatment and when aphid adults were placed on treated leaves they stopped feeding and starved to death.

Formulations of imidacloprid (Admire, Provado) are currently registered on a variety of field-grown fruit and vegetable crops in the United States, including most vegetables in the

family solanaceae (e.g., potatoes, tomatoes, pepper, eggplant), crucifers (e.g., broccoli, Brussels sprouts, cabbage, cauliflower), leafy vegetables (e.g., lettuce, spinach, parsley) and pear (Anonymous, 1998a). Among insects listed on labels as being controlled are two psyllids, the pear psylla (*Cacopsylla pyri* (L.)) and the tomato psyllid. However, imidacloprid is not currently registered for use on any greenhouse-grown vegetable, which constitutes a separate site under EPA registration guidelines than does outdoor field grown vegetables.

**Spinosad.** Spinosad is an insecticide originally isolated from an organism found in soil samples taken in 1982. In 1988, the organism was identified as a new bacterium species, *Saccharopolyspora spinosa* and belongs to the order Actinomycetales (Anonymous, 1998b). The name "spinosad" comes from the active chemicals in the insecticide, known as spinosyns. The insecticide is not readily absorbed across the insect integument. Its main effect then is through ingestion, carrying with its effective use all the requirements of good spray coverage (Salgado, 1997).

The actual mode-of-action of spinosad has recently been determined. It acts on the insect nervous system at the nerve synapse. Salgado (1997) reported that spinosad depolarizes insect neurons by activating nicotinic receptors, causing widespread hyperactivity in the nervous system, which leads to involuntary muscle contractions and tremors. Eventually, insects become prostrate with tremors and, after prolonged exposure, become paralyzed from neuro-muscular fatigue. Success<sup>®</sup>, Tracer<sup>®</sup>, Spintor<sup>®</sup>, and Conserve<sup>®</sup> are among the commercial names for spinosad-containing insecticides.

### **Microbial Insecticides**

Fungi are an important natural control to insect populations. Successful use of pathogenic fungi for insect control depends mainly on proper environmental conditions such as temperature,

humidity and intensity of ultraviolet radiation. (Feng et al., 1994; Weinzierl and Henn, 1989). *Beauveria bassiana* (Balsamo) Vuillemin, *Metarhizium anisopliae* (Metsch.) and *Verticillium lecanii* (Zimm.) Viegas are among the most commonly used fungi for insect control.

***Beauveria bassiana.*** *Beauveria bassiana* is a hyphomycete insect-pathogenic fungus in the subdivision Deuteromycotina. It is found naturally on some plants and in soils worldwide. The very distinctive and noticeable white mummies of silkworms produced by infection led to its discovery by Agostino Bassi de Loid in 1835. The fungus was later named *B. bassiana* in honor of its discoverer (Tanada and Kaya, 1993). Over 200 species of insects in nine orders, mainly Lepidoptera and Coleoptera, have since been recorded as hosts (Li and Yang, 1988). The extensive list of hosts includes such important pests as whiteflies, aphids, grasshoppers, termites, Colorado potato beetle, Mexican bean beetle, Japanese beetle, boll weevil, cereal leaf beetle, bark beetles, lygus bugs, chinch bug, fire ants, European corn borer, and codling moth (Tanada and Kaya, 1993).

*Beauveria bassiana* can infect such a wide range of insects by producing spores that are resistant to environmental extremes. The spores (conidia) infect directly through the insect integument. Under favorable temperature and moisture conditions a conidium adhering to the host cuticle will germinate. The fungal hypha growing from the spore secretes enzymes, which attack and dissolve the cuticle, allowing it to penetrate the integument and grow into the insect body. Once inside the insect it produces a toxin (beauvericin) that weakens the host's immune system (Feng et al., 1994).

*Beauveria bassiana* has shown considerable potential for management of insect pests (Feng et al., 1994). However, the successful use of *B. bassiana* in IPM programs might be limited by many abiotic and biotic factors. Abiotic factors affecting *B. bassiana* include

temperature, humidity and light. Inglis et al. (1997) stated that the efficacy of *B. bassiana* was limited by solar radiation and temperature when used against acridid grasshoppers in field conditions. The survival of *B. bassiana* conidia was reduced by greater than 95% after 15 minute exposure to UV-B radiation in a controlled environment (Inglis et al., 1997). Free water or relative humidity of over 90% has been considered for many years as the most serious constraint for germination and growth. However, a number of studies indicate that infection occurs independently of relative humidity (Junianto and Sri, 1995; Marcandier and Khachatourians, 1987; Yasuda et al., 1997). High relative humidity is only required for fungal sporulation, which is necessary for horizontal transmission from cadavers (Ferron, 1978). Humidity also affects the survival of conidia, with long survival at low relative humidities (Lingg and Donaldson, 1981).

Temperature affects both germination and mycelial growth. For most strains, the optimal temperatures are thought to fall between 23°C and 25°C (Ferron, 1978). Temperatures above or below the optimum range prolong the lag phase and decrease the germination rate (Hywel-Jones and Gillespie, 1990). In general, *B. bassiana* can grow at a wide temperature range from 8 to 35°C, but there are isolate differences. Fargues et al. (1997) tested the maximum thermal threshold of 65 isolates of *B. bassiana*. The maximum thermal threshold occurred at temperatures of >35-37°C for 50 isolates; >32-35°C for 12; and 30-32°C for one isolate.

Biotic factors influencing *B. bassiana* include: microbial antagonists on the host integument, leaf surface or in soil; host susceptibility; and strain virulence (Lingg and Donaldson, 1981; Quintela et al., 1990). Insects also may escape infection during molts or because of some protective structures such as the chorion and elytra that prevent fungal penetration (Hunt et al., 1984). Therefore, thorough spray coverage is essential because fungal

spores must contact vulnerable areas of the insect for infection to occur (Tanada and Kaya, 1993).

The effect of fungicides on *B. bassiana* has been studied in the laboratory (Aguda, 1988; Gardner and Storey, 1985; Majchrowicz and Poprawski, 1993; Olmert and Kenneth, 1974; Poprawski and Majchrowicz, 1995) and followed by tests in the field (Clark et al., 1982; Hassan et al., 1991; Hassan et al., 1994; Loria et al., 1983). The entomopathogenic fungi are generally affected negatively by fungicides (Hassan et al., 1994; Majchrowicz and Poprawski, 1993). Todorova et al. (1998) found that all six tested fungicides (chlorothalonil, maneb, thiophanate-methyl, mancozeb, metalaxyl+mancozeb and zineb) tested in vitro inhibited *B. bassiana* mycelial growth and sporulation. The harmful effect of maneb, mancozeb, zineb was mentioned in many studies (Hassan et al., 1991; Loria et al., 1983; Majchrowicz and Poprawski, 1993; Olmert and Kenneth, 1974). In contrast Loria et al. (1983) showed that chlorothalonil had a neutral or stimulatory effect on *B. bassiana*.

*Beauveria bassiana* is available in commercial strains that better withstand ultraviolet light, temperature and humidity extremes commonly encountered in the field (Tanada and Kaya, 1993). There are several products that contain *B. bassiana*, including Naturalis<sup>®</sup>, BotaniGard<sup>®</sup>, and Mycotrol<sup>®</sup>.

***Verticillium lecanii***. The entomopathogenic fungus *V. lecanii* consists of a heterogeneous species aggregate (Evans and Samson, 1986). It has been reported to infect various insects including larvae of beetles, mites, aphids, scales and other homopterans particularly under greenhouse conditions (Hall, 1981). Meade and Byrne (1991) reported the successful use of *V. lecanii* worldwide to control greenhouse pests such as greenhouse whitefly and various species of aphids. Hall and Burges (1979) showed that a single spray of *V. lecanii* could control

populations of *M. persicae* in chrysanthemum crops. Sprays or dips of *V. lecanii* to chrysanthemum cuttings were effective against *A. gossypii* and ensured introduction of clean plants to the greenhouse (Sopp and Palmer, 1990).

The use of *V. lecanii* as a biological control agent is limited to particular environments. Infection of whiteflies and *M. persicae* required high relative humidity (Ekbom, 1981). Temperature of 15-25<sup>0</sup>C and relative humidity of 95% maintained at least for 10 to 12 hours daily is a strict environment for *V. lecanii* germination (Helyer et al., 1992).

Two *V. lecanii* strains have been developed for greenhouse pest biological control: *V. lecanii* strain 19.79 (Mycotal) to control whitefly, *T. vaporariorum* and *V. lecanii* strain 1.72 (Vertalec) to control aphids such as the chrysanthemum stem aphid, *Macrosiphoniella sanborni* (Gillette) (Chandler et al., 1993). In England both Vertalec and Mycotal are used in commercial greenhouses (Feng et al., 1990).

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## Objectives

- \* Develop techniques to improve the management of tomato psyllid on greenhouse grown tomatoes
  
- \* Identify improved methods for trapping tomato psyllid adults, as means of detecting incipient infestations
  
- \* Evaluate microbial, botanical and selective chemical insecticides for control of tomato psyllid, with emphasis on materials that are both efficacious and compatible with beneficial insects (e.g., parasitoids, pollinators) currently used in greenhouse tomato production.
  
- \* Investigate the potential of green lacewings as an applied biological control agent for tomato psyllid control.
  
- \* Determine the compatibility of soil-applied imidacloprid application on bumble bee pollinators of greenhouse grown tomatoes

## Chapter II

### Detecting and Trapping Tomato Psyllid *Paratrioza cockerelli* (Sulc) (Homoptera: Psyllidae) in Tomato Greenhouses

#### Introduction

Greenhouse tomatoes have, in a very short period, become a major crop in the US. In 1997 estimates of US greenhouse vegetable acreage was 220 to 260 hectares, with several operations indicating plans for expansion (Naegely, 1997). During the past decade Colorado has been the number one state in the rate of increase of the tomato greenhouse industry with cash receipts increasing about seven fold from 3.8 million in 1993 to 29 million in 1996; these currently exceed \$60 million in annual value (Hudson and Fretwell, 1998). More than 48 hectares were cultivated in Colorado in 1997 and presently almost half of the total US greenhouse tomato production occurs in the state (Naegely, 1997). The rapid growth of the greenhouse industry in Colorado can be attributed to many reasons including: high light intensity, relative low heating cost when produced in co-generation power facilities allowing use of waste heat, and central location of the production allowing ease of transportation.

The East Coast is the prime market for greenhouse tomatoes where prices can bring up to \$4 per pound in the winter. With these high prices and use of high yielding varieties, such as “Beefsteak”, which produces about 60 kg/square m (49 lb/plant), growers can realize a net profit of \$18.5 to \$28 per square m in a tomato greenhouse (Naegely, 1997).

The tomato (potato) psyllid, *Paratrioza cockerelli* (Sulc), has developed as a key insect pest of greenhouse tomatoes of western North America. Feeding by tomato psyllid causes a condition known as psyllid yellows disease. The injuries include removal of large quantities of plant nutrients

during feeding and mechanical injuries to the cells (Eyer, 1937). This may result in malformation and distortion of foliage, plant growth reduced to a standstill for weeks to months, widespread chlorosis, stimulated and/or aborted fruit set, and production of numerous and small poor quality fruit that lead to yield loss (Abernathy, 1991; Daniels, 1954).

Common methods for monitoring tomato psyllid on outdoor crops have long included use of a sweep-net to detect adults and careful inspection of plant parts to detect eggs and nymphs (Pletsch, 1947). However, a sweep-net can not be used in greenhouse tomatoes, tender plants that are easily damaged. In addition, sampling effectiveness is poor when there are small numbers of psyllid adults or the infestation is in the early stage and concentrated in small areas of the planting. Although examination of leaves and vines may be an appropriate sampling method for greenhouse tomatoes, it is tedious and time-consuming (Pletsch, 1947). However, certain color traps have been used to monitor psyllids (Adams and Los, 1989; Mensah and Madden, 1992).

Spatial patterns perceived by insects that might be used in traps include combinations of hue (dominant wavelength of reflected light), brightness (intensity of perceived reflected light) and saturation (spectral purity of reflected light) (Prokopy and Owens, 1983). In many herbivorous insects, plant spectral quality (particularly hue and intensity) appears to be the principal stimulus eliciting alightment on living plants. A considerable number of other insect species have been reported to be attracted to yellow. These include the pear psylla, *Cacopsylla pyricola* (Foerster), as well as many other homopterans (Adams and Los, 1989; Kennedy et al., 1961).

Kaloostian and Yeomans (1944) were first to report the effectiveness of yellow sticky-board traps to detect pear psylla. Subsequently, Adams et al. (1983) reported that several daylight fluorescent colored sticky traps also were effective for monitoring pear psylla adults on pear. Field

studies on color preferences of another psyllid, *Ctenarytaina thysanura* (Ferris & Klyver), similarly revealed attractiveness to yellow traps (Mensah and Madden, 1992).

Kaloostian and Wolf (1968) reported the attraction of pear psylla adults to black light. Black light may also be a possible detection method for tomato psyllid infestation. Pletsch (1947) found the excreted "psyllid sugar", resembling granulated sugar or salt, produced definite fluorescent properties when exposed to ultraviolet light in darkroom. When tested in a greenhouse at night even small numbers of psyllids could be detected. However, under field conditions no fluorescence was detected, likely due to disturbance by wind or rain removing the sugary excretions.

The objective of this study was to develop effective detection and monitoring systems for tomato psyllid in greenhouse tomato. This included evaluation of several visual traps, effects of their placement and shading.

## **Materials and Methods**

**1997 Trials.** Three trapping experiments were conducted in a tomato psyllid-infested greenhouse tomato planting at Colorado State University, Fort Collins, Colorado in 1997. In each experiment, trap configuration involved four replicates of 7.5 x 12.5-cm rectangles constructed of colored poster board, covered with clear plastic film and coated with a thin layer of Tangle-Trap<sup>®</sup>. Tomato plants used for these studies were of the same age, approximate height, and planted in 3.8 l size container. Tomato plants were placed on two benches with double lines on each bench. The tomato lines were placed in an east-west direction. There were about 40 plants per line, a total of 80 plants per bench.

All traps were hung at a 45-cm distance from the tomato canopy and at height of 150-cm. The color traps were placed 15 to 20 cm apart from each other. All the 1997 traps were hung as a single line parallel to the south side of the planting, facing north. Psyllids trapped were counted every other day for at least 10 days. Following each count, trapped psyllids were removed and new

coat of Tangle-Trap<sup>®</sup> was applied before being mounted again. Trials were established in a randomized complete block design with four replications.

In the first experiment colors were yellow, neon (fluorescent) orange, red, neon green, orange, and a "standard" yellow commercial trap (Stiky Strips<sup>®</sup>, Olson Products, Medina, OH) as a control. In addition, a series of color patterns were observed to determine if color patterns also affected psyllid capture. These involved comparison of solid yellow-green traps, to those with alternating yellow and green horizontal stripes, or alternating yellow and green vertical stripes.

The second experiment compared the response of adult psyllids to white, black, blue, light blue, silver, gold, beige, pink, magenta, and neon orange. In the third experiment, colors that had previously attracted most psyllids were re-tested: neon orange, neon green, beige, and the "standard" yellow commercial trap. Blue was also added to this trial as a previously non-attractive color for a control.

**1999 Trials.** Five additional experiments were conducted in 1999. Similar techniques were employed as in the 1997 trials, with some modifications, indicated below. Trapped psyllids were counted at 1, 3, and 6 days after treatment (DAT).

In the first experiment neon green and neon orange colored traps, previously indicated as being highly attractive, were compared to each other. This trial was conducted with only these two colors to confirm the results of 1997 trials.

In the second experiment treatments included shading or not shading neon green and neon orange traps. This was to determine if there was a possible shading effect on psyllid adult capture. Cardboard with dimensions of 25 x 35 cm were centered on top of the trap for the shaded traps. Traps were hung in a single line, on the north side of the planting and faced south.

In the third experiment neon green and neon orange were oriented at different directions to investigate the effect of direct sunlight on trap capture of psyllid adults. This included a comparison of captures on north facing traps along the south side of the planting and south facing traps on the north side.

Trap orientation was further examined in the fourth experiment. It included neon green traps vertically oriented wither east or west, and also included horizontal (up and down) orientations. All traps were placed along the north side of the planting.

All previous experiments involved traps hung at a standard distance and height from the tomato plants. In the fifth experiment neon orange traps were hung at two different levels. High level traps were hung at the top of the tomato plants (150 cm); low-level traps at the base (30 cm).

Because of interest in there being possible temperature differences in traps, based on orientation, in the latter trials surface temperatures of traps were measured. Four temperature loggers (HOBO<sup>®</sup> Temp, Environmental Sensors, Inc., San Diego, CA) were placed on the north and south sides of the planting. Temperature readings were recorded at two hour intervals, June 9 to June 16, 1999.

All trap catch data, except experiments one and five of 1999, were submitted to analysis of variance and the Student-Newman-Keuls (SNK) test for mean separation (SAS Institute, 1990). The remaining two trials were analyzed by a Two-tailed t-test.

## Results and Discussion

**Experiment One 1997.** Of the nine colors tested, adult psyllids were captured in significantly lower numbers on red traps compared to neon green, neon orange, and standard yellow (Table 2.1). There were no differences among the other tested colors, which involved various shades of green, yellow, and orange. Changes in color patterning (e.g., vertical or horizontal stripes) also did not significantly affect trap captures.

**Table 2. 1. Capture of adult *Paratrioza cockerelli* on different colored and patterned traps in a tomato greenhouse, Ft. Collins, CO, 1997**

Trap color	Psyllid adults/trap
Neon-Green	7.0a
Neon-Orange	6.3a
Standard Yellow	6.2a
Yellow	4.8ab
Yellow-Green	4.0ab
Yellow and Green/Vertical stripes	3.9ab
Orange	3.9ab
Yellow and Green/Horizontal stripes	2.2ab
Red	0.6b

Means followed by the same letter are not significantly different ( $P < 0.05$ , SNK). N= 4.

**Experiment Two 1997.** Neon-orange was significantly more attractive to *P. cockerelli* ( $P < 0.05$ ) than the other 9 colors. Neon-orange captured four-fold more than the second most attractive color (beige) (Table 2.2).

**Table 2. 2. Capture of *Paratrioza cockerelli* on different colored traps in a tomato greenhouse, Ft. Collins, CO, 1997**

Trap color	Psyllid adults/trap
Neon-Orange	88.0a
Beige	22.9b
Gold	20.9b
Pink	9.3b
Silver	7.3b
White	4.9b
Light-Blue	4.8b
Black	4.8b
Blue	2.8b
Magenta	0.1b

Means followed by the same letter are not significantly different ( $P < 0.05$ , SNK). N= 4.

**Experiment Three 1997.** Among the 5 colors tested, significantly more tomato psyllids ( $P < 0.05$ ) were captured on neon-orange traps. Captures on beige, neon green, and the standard yellow were not significantly different from each other. They all, however, captured significantly more psyllids than blue (Table 2.3).

**Table 2. 3. Capture of *Paratrioza cockerelli* on different colored traps in a tomato greenhouse, Ft. Collins, CO, 1997**

Trap color	Psyllid adults/trap
Neon-Orange	14.7a
Neon-Green	6.7b
Yellow	5.1b
Beige	4.1b
Blue	0.6c

Means followed by the same letter are not significantly different ( $P < 0.05$ , SNK). N= 6.

**Experiment One 1999.** There was no significant difference between neon green and neon orange in capture of *P. cockerelli* adults (Table 2.4).

**Table 2. 4. Capture of *Paratrioza cockerelli* on neon green and neon orange sticky traps in a tomato greenhouse, Ft. Collins, CO 1999**

Trap color	Psyllid adults/trap
Neon-Green	95.5a
Neon-Orange	102.3a

N= 4.

**Experiment Two 1999.** Among the four tested colors treatments, significantly more tomato psyllids ( $P < 0.05$ ) were captured on un-shaded neon green traps (Table 2.5).

**Table 2.5. Effects of shading on capture of *Paratrioza cockerelli* on colored traps in a tomato greenhouse, Ft. Collins, CO, 1999**

Trap color and shading	Psyllid adults/trap
Un-shaded Neon-Green	43.8a
Un-shaded Neon-Orange	23b
Shaded Neon-Green	12.5b
Shaded Neon-Orange	10.3b

Means followed by the same letter are not significantly different ( $P < 0.05$ , SNK). N= 4.

As Table 2.5 indicated there was significant difference between the un-shaded green and orange unlike the results presented in Table 2.4.

**Experiment Three 1999.** Color traps facing north were significantly more attractive to *P. cockerelli* ( $P < 0.05$ ). South orientated traps captured from two to four-fold more than north oriented traps (Table 2.6).

**Table 2.6. Effect of different orientation of green and orange traps on capture of *Paratrioza cockerelli* in a tomato greenhouse, Ft. Collins, CO, 1999**

Trap color and orientation	Psyllid adults/trap
Neon-Green/North orientation	95.5a
Neon-Orange/North orientation	102.3a
Neon-Green/South orientation	43.8b
Neon-Orange/South orientation	23.0b

Means followed by the same letter are not significantly different ( $P < 0.05$ , SNK). N= 4.

**Experiment Four 1999.** There was no difference in psyllid capture on traps oriented east or west (Table 2.7). Lowest captures were on traps oriented down.

**Table 2.7. Effect of different orientations of neon green traps on capture of *Paratrioza cockerelli* in a tomato greenhouse, Ft. Collins, CO, 1999**

Trap orientation	Psyllid adults/trap
East	61.5a
West	65.5a
Down	42.0ab
Up	19.3b

Means followed by the same letter are not significantly different ( $P < 0.05$ , SNK). N= 4.

**Experiment Five 1999.** Traps placed near the top of the canopy caught significantly more tomato psyllids than traps placed near the base of the plant (Table 2.8). Approximately twice as many psyllid were caught at the upper level.

**Table 2.8. Capture of *Paratrioza cockerelli* on neon orange traps placed at two different heights adjacent to greenhouse tomatoes, Ft. Collins, CO, 1999**

Trap location	Psyllid adults/trap
High level (150 cm)	176.5a
Low level (30 cm)	96.0b

Means followed by the same letter are not significantly different ( $P < 0.05$ , Two-tailed Student *t*-test).  $N= 4$ .

The results demonstrate that *P. cockerelli* adults are highly attracted to some daylight fluorescent colors, notably neon-orange, neon-green and standard yellow (Table 2.3). However, there were no significant differences among the most attractive colors (neon-green, neon-orange and standard yellow) (Tables 2.1, 2.4). Thus results are similar to that of Adams et al. (1983) who found no significant differences among signal green, lightning yellow, Saturn yellow, arc yellow and blaze orange when used in color traps for monitoring the pear psylla. The range of these colors in the electromagnetic radiation spectrum (green, 500-560 nm; yellow, 560-590 nm; and orange, 590-630 nm) fall in the homopteran visual sensitivity range (350 to 600 nm) identified by Prokopy and Owens (1983).

Attractiveness of neon-green, neon-orange, and yellow colors might be due, in part, to their similarity to stimuli eliciting alightment on plants. For example, a considerable number of other homopterans, including *C. pyricola*, have been reported to be attracted to yellow (Adams and Los, 1989; Kennedy et al., 1961). This is may be due to a higher than average available nitrogen content in plant tissues is characteristic of newly developing leaves, which look more yellow. Similarly,

tomato psyllids may have developed a preference toward alighting on more yellow colored leaves. Also, high levels of chlorophyll fluorescence in tomato leaves are an indication of high photosynthesis of the plant (Lorenzini et al., 1997).

Hue and intensity of reflection appears to play an important role in attracting tomato psyllid adults to color traps as traps that did not receive direct sunlight captured significantly fewer psyllid adults than those exposed to direct sunlight (Table 2.5). However, under field conditions, the majority of eggs and nymphs were found on the shaded or the lower side of the leaves (Knowlton and Janes, 1931; Pletsch, 1947). The micro-climate of the tomato plants may have some effect on color trap capture. Personal observations in the tomato greenhouses indicated that psyllids typically concentrated among plants growing on the south bench. When temperatures of plants were recorded, a difference of about  $5^{\circ}\text{C}$  was found in the daily maximum temperatures between plants grown on the two benches. This may, in part, explain why color traps hung along the south captured significantly more psyllids than traps hung along the north (Table 2.6). The tomato psyllid is very sensitive to extreme temperatures. According to List (1939a; 1939b) on the effects of temperature on tomato psyllid in the greenhouse a temperature of  $26.6^{\circ}\text{C}$  seemed most favorable to psyllid development and survival. Oviposition is reduced under  $32.2^{\circ}\text{C}$ , while  $37.7^{\circ}\text{C}$  is lethal to eggs and nymphs and female stop egg laying at this temperature. Areas of excessively high temperatures thus could be avoided by psyllids.

In addition, the height of the trap above the tomato crop canopy was found to affect the number of adult psyllids captured. When color traps were hung near the top of the tomato plants more psyllids were captured than when hung near the bottom of the plant (Table 2.8). Adams and Los (1989) similarly found that more pear psylla were captured on color traps placed at higher levels relative to the plant.

Based on these results it is suggested that an optimal color-based trap for tomato psyllid would have a reflected spectrum between 500 and 630 nm (green, yellow, orange). Intensity of reflection would assist in capture, as indicated by the increased attraction of neon (daylight fluorescent) colors. Traps should be oriented to receive direct light and placed at or above the crop canopy.

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## Chapter III

# Evaluations of Conventional and Non-conventional Insecticides and Repellents for Control of Tomato Psyllid, *Paratrioza cockerelli* (Sulc) (Homoptera: Psyllidae), on Greenhouse Tomato

## Introduction

The tomato (potato) psyllid, *Paratrioza cockerelli* (Sulc), has emerged as a serious problem in production of greenhouse tomatoes in the Rocky Mountain West, an industry that has expanded rapidly to currently have greater than \$60 million annual value in Colorado alone. *Paratrioza cockerelli* is a native insect first described by Sulc, from a sample collected on peppers in Boulder, Colorado (Pletsch, 1947). It has a wide host range of cultivated and non-cultivated plants. Although most commonly found on members of Solanaceae, *P. cockerelli* has been reported on many other plant families (Wallis, 1946).

Distribution of tomato psyllid extends from northern Mexico to southern Canada and from Texas to parts of southern California. Utah, Colorado, Wyoming and Nebraska, historically have sustained the most severe and frequent damage by this insect (Pletsch, 1947). Tomato psyllids migrate every summer to northerly locations from the desert breeding grounds in southern Arizona. They overwinter in these sites from January to May with populations peaking in April or May. They then disappear until late in October or early in November when a returning influx is observed (Romney, 1939).

The importance of tomato psyllid to members of the Solanaceae is due to its ability to produce “psyllid yellows”, a disease condition first described by Richards (1928). Symptoms of psyllid yellows are caused by a chlorosis-inducing toxemia that is produced mainly by nymphal

feeding (Carter, 1950). In tomato severe symptoms of this disease lead to production of small, poor quality fruit, disrupted fruit set and yield loss (Abernathy, 1991; Daniels, 1954).

Under field conditions, many insecticides have been reported to be effective for tomato psyllid control. Lime sulfur was the first effective tomato psyllid control method and was widely used during the 1930's and 1940's (List, 1935). Summer oil sprays, pyrethrum sprays, nicotine sulfate sprays, and calcium cyanide dusts also provided nymphal control during the first half of the century (Knowlton, 1933; Pack, 1929). Among the modern synthetic organic insecticides Gerhardt and Turley (1961) reported effective tomato psyllid control with the systemic organophosphate insecticides phorate, demeton and dimethoate. More recently it was reported that most of the tested pyrethroid and organophosphate insecticides, and the chlorinated hydrocarbon endosulfan, applied as foliar sprays provide acceptable control of tomato psyllid, although thorough coverage was critical (Cranshaw, 1994).

Greenhouse tomato, and other greenhouse vegetables, are considered a "minor crop" and registered insecticidal control options are very limited (e.g., some microbial insecticides and formulations of soaps, horticultural oils, pyrethrins-based and neem-derived insecticides). Furthermore, pesticide use is restricted by the extensive use of beneficial insects in crop production, notably bumblebees for pollination and parasitoids [e.g., *Encarsia formosa* (Gahan), *Eretmocerus californicus* (Howard)] for management of greenhouse whitefly *Trialeurodes vaporariorum* (Westwood). Therefore, the use of insecticides with selective effects are most suitable for management of tomato psyllids in such environment.

Horticultural oils and soaps have broad applications against insect and mite pests (Baxendale and Johnson, 1990; Nielsen, 1990a; Nielsen, 1990b). There has been numerous research done with oils on greenhouse pests that can affect greenhouse tomatoes, particularly

various whiteflies. Sieburth et al. (1998) evaluated the horticultural oil SunSpray for effects on nymphs of the silverleaf whitefly *Bemisa argentifolii* Bellows & Perring and showed that among nymphs, pupae, and crawlers emerging from treated eggs approximately 50 to 75 percent were killed outright. When SunSpray was compared with a broad spectrum pyrethroid for residual toxicity, SunSpray as a dip proved to be at least as effective as a pyrethroid for control of *B. argentifolii* on tomatoes under greenhouse and laboratory conditions (Liu and Stansly, 1995).

Although specific trials on tomato psyllid have not been reported, Baxendale and Johnson (1990) found the horticultural oil SunSpray 6E Plus to be highly effective against many homopterous insects, such as boxwood psyllid *Psylla buxi* (L.) and calico scale *Eulecanium cerasorum* (Cockerell). They further noted that the spray oil has very good ovicidal action against *P. buxi* with decreased egg viability by 95% when applied in the spring

Repellent effects of horticultural oils have been reported in many studies (Larew and Locke, 1990; Liu and Stansly, 1995; Weissling et al., 1997; Xie and Isman, 1995). Weissling et al. (1997) found oils significantly reduced winter form oviposition rates of pear psylla, *Cacopsylla pyricola* (L.).

Compounds extracted from the neem tree, *Azadirachta indica*, look promising to provide various control means to many pest insects (Schmutterer, 1990). Greenhouse pests controlled by neem extracts include of chrysanthemum leafminer *Liriomyza trifolii* (Burgess) (Parkman and Pienkowski, 1990). Margosan-O<sup>®</sup> also was found to prevent greenhouse whitefly emergence while emergence among untreated controls ranged 75 to 85% (Knauss and Walter, 1995). When three sprays of Margosan-O<sup>®</sup> were used at 7-day intervals numbers of western flower thrips, *Frankliniella occidentalis* Pergande, were significantly reduced. The beet armyworm *Spodoptera exigua* (Hubner), was effectively controlled by Margosan-O<sup>®</sup> and treatments produced high

mortality of young instars. Neem also is reported to work as a repellent for some insects. For example, greenhouse studies found neem oil spray at concentrations of 12% to be an oviposition deterrent to *Nilaparvata lugens* (Stal) (Krishnaiah and Kalode, 1991).

Microbial insecticides with some potential for use in greenhouse tomatoes include various entomopathogenic fungi. *Beauveria bassiana* (Bals.) Vuill. is found naturally on some plants and in soils worldwide and has shown considerable potential for management of insect pests (Feng et al., 1994). First described by Agostino Bassi De Loid in 1835 (Tanada and Kaya, 1993) in the association with the silkworm *Bombyx mori* (L.), over 200 species of insects in nine orders, mainly Lepidoptera and Coleoptera, have been recorded hosts of *B. bassiana* (Li and Yang, 1988). The extensive host list includes such important pests as whiteflies, aphids, grasshoppers, termites, Colorado potato beetle, Mexican bean beetle, Japanese beetle, boll weevil, cereal leaf beetle, bark beetles, lygus bugs, chinch bug, fire ants, European corn borer, and codling moth (Tanada and Kaya, 1993). The successful use of *B. bassiana* in IPM programs can be limited by ultraviolet and other environmental factors (Ingliš et al., 1997). However, *Beauveria bassiana* strains are commercially available that better withstand ultraviolet light and the temperature and humidity extremes commonly encountered in the field. Currently, there are several products that contain *B. bassiana*, including Naturalis<sup>®</sup>, BotaniGard<sup>®</sup> and Mycotrol<sup>®</sup> (Tanada and Kaya, 1993; Vandenberg et al., 1998).

The entomopathogenic fungus *Verticillium lecanii* (Zimm.) Viegas also has been reported to infect various insects including larvae of beetles, mites, aphids, scale insect and other homopterans particularly under greenhouse conditions (Hall, 1981). Several thrips species, such as *F. occidentalis*, *Thrips tabaci* Lindeman and *F. tenuicornis* (Uzel), have been found to be infected by *V. lecanii* (Schreiter et al., 1994). Meade and Byrne (1991) reported the successful

use of *V. lecanii* worldwide to control greenhouse pest such as the greenhouse whitefly and various species of aphids. However, the use of *V. lecanii* as a biological control agents is limited to particular environments, particularly high relative humidity required for infection (Ekbohm, 1981). Temperatures of 15-25<sup>0</sup>C and relative humidity of 95% is a strict environment for *V. lecanii* germination (Helyer et al., 1992). Two *V. lecanii* strains have been developed for greenhouse pest biological control. In England both Vertalec<sup>®</sup> and Mycotal<sup>®</sup> are used in commercial greenhouses (Feng et al., 1990).

Spinosad is a newly developed microbial-derived insecticide with active ingredients isolated from the bacterium *Saccharopolyspora spinosa* (Actinomycetales) (Anonymous, 1998). The common name spinosad refers to the insecticidal components known as spinosyns. Commercial formulations of spinosad (Success<sup>®</sup>, Conserve<sup>®</sup>, Tracer<sup>®</sup> and Spintor<sup>®</sup>) are a mixture of spinosyns A and D (Sparks et al., 1995). Spinosad has a novel mode of action initially causing involuntary muscle contractions and tremors by exciting neurons in the central nervous system. Furthermore, it is shown that spinosyns can excite the nervous system directly, when applied to isolated ganglia at submicromolar concentrations. After prolonged periods of spinosyn-induced hyperexcitation, insects become paralyzed, apparently due to neuromuscular fatigue caused (Salgado, 1998). Although spinosad currently is labeled only for control of Lepidoptera and certain Thysanoptera, spinosyns and spinosoids are have some broad spectrum activity and control has been reported against some insects in the orders Coleoptera, Diptera, Homoptera, Hymenoptera, Isoptera, Orthoptera and Siphonaptera, as well as mites (Salgado et al., 1997).

Although, spinosad has broad spectrum activity against many pests, it also has showed great selectivity to beneficial arthropod populations (Boyd and Boethel, 1998). Spinosad also appears to have little negative impact on most beneficial insects (Anonymous, 1998; Eger Jr, 1998).

Pymetrozine (GCA215944) is a newly developed synthetic organic insecticide with selectivity of action. Pymetrozine controls sucking pests in rice and vegetables (Lutz, 1998) and is a selective compound active against Homoptera. It is highly active against susceptible and resistant aphids and whiteflies in vegetables, ornamentals, cotton, field crops, and deciduous fruits and citrus. Effects on the behavior of treated homopterous insects include cessation of feeding and ultimately death from starvation. Pymetrozine has minimum effects on beneficial insects, therefore it is useful in IPM programs (Fluckiger et al., 1992). In 1997, pymetrozine was registered in Turkey (Chess<sup>®</sup>), Germany (Plenum<sup>®</sup>), the Czech Republic, Panama and Costa Rica. It is also registered in Taiwan, Malaysia and expected to be registered in the US this year.

Another new insecticide in development is acetamiprid. Acetamiprid has a mode of action is similar to imidacloprid and similarly acts at the nicotinic receptor. Acetamiprid has appeared highly effective against whitefly adults (Kerns and Tellez, 1997). And was as effective as imidacloprid for controlling cotton whitefly *Bemisia tabaci* (Gennadius) with longer field residual activity (Horowitz et al., 1998). Very high levels of control also have been reported against the hop aphid, *Phorodon humuli* Schrank, (Vostrel, 1996) and western flower thrips (Szabo and Vasiliu, 1998). Acetamiprid has been shown to have potential for control of insect pests belonging to Hemiptera, Lepidoptera, Coleoptera and Isoptera (Matsuda and Takahashi, 1996).

The objective of this study was to evaluate insecticides that have potential application in management of tomato psyllid in greenhouse tomatoes, with particular attention to those

compatible with biological control and pollinators. These include microbial insecticides (e.g., *B. bassiana*, *V. lecanii*), microbial derived insecticides (spinosad), selective botanical products (e.g., neem) and selective synthetic insecticides (e.g., acetamiprid, pymetrozine). Also, the use of various insect repellents as a means of deterring tomato psyllid oviposition were evaluated.

## **Materials and Methods**

***Beauveria bassiana* Spray Chamber Trial 1997.** A trial was conducted with two different *B. bassiana* formulations to determine their ability to produce infection in *P. cockerelli*. In addition, treatments were expanded to evaluate if an organosilicant wetting agent (Kinetic<sup>®</sup>) could affect activity of the treatments. Tested *B. bassiana* formulations included BotaniGard WP<sup>®</sup> (Mycotech, Bozeman, MT) or Naturalis-L<sup>®</sup> (Troy Biosciences, Inc. Phoenix, AZ) alone or in combination with Kinetic (0.1% concentration) and included a water check. Treatments were applied by passing the leaves through a spray chamber, located in the Weed Science Building of the Colorado State University campus, to ensure uniform distribution. Each replicate consisted of a psyllid infested tomato leaf, with at least 50 psyllid nymphs, attached to a piece of Oasis Block<sup>®</sup> and placed in a petri dish. A completely randomized design with four replications was used. Following application, the petri dish was sealed using Parafilm<sup>®</sup> and kept at room temperature.

Pretreatment counts of psyllid nymphs were made immediately prior to application. Evaluations of psyllid mortality were made five days after treatment. Data were submitted to ANOVA and Student-Newman-Keuls (SNK) test (SAS Institute, 1990).

***P. cockerelli* Insecticide Trial 1997.** Trials of several insecticides were conducted 25 June on greenhouse grown tomatoes ('Blitz F1') infested with tomato psyllid. Plots consisted of individual leaves known to be infested with at least 20 psyllids based on pretreatment counts.

Experimental design was completely randomized with 8 replications. Treatments included acetamiprid (Rhone Poulenc Ag., Triangle Park, NC), the neem-derived insecticide/repellent Azatin XL (Olympic Horticultural Products Co., Mainland, PA), the *Beauveria bassiana* formulation BotaniGard ES (Mycotech Co, Butte, MT), the pymetrozine formulation Relay/CGA-215944 50W (Novartis Crop Protection, Greensboro, NC), the spinosad formulation Spintor SC (Dow AgroSciences, Indianapolis, IN), a petroleum-derived horticultural oil SunSpray (Sun Refining and Marketing Co., Memphis, TN), the neem oil formulation Trilogy (W.R. Grace and Co., Columbia, MD) gal) and included an untreated check. These insecticides were applied using a hand sprayer to point of run-off. Evaluations were made 1, 2 and 5 days after treatment (DAT). Data were submitted to two-way ANOVA and Student-Newman-Keuls test (SNK) in the manner described above.

***P. cockerelli* Insecticide Trial 1998.** A subsequent experiment was conducted to further examine the efficacy of the selective insecticides Spintor SC (spinosad), Relay (pymetrozine), and BotaniGard (*B. bassiana*) under greenhouse conditions. In addition, a second entomopathogenic fungus was evaluated, Mycotrol® (*Verticillium lecanii*). Plots consisted of whole plants with at least four infested leaves. Leaves were designated as replicates. Treatments were applied to single infested tomato leaves (Super Sweet 100) and experimental design was completely randomized with four replications. Pretreatment counts of psyllid nymphs on both sides of the leaves were made. Treatments were applied 25 November and included serial dilutions of four insecticides, in addition to the water check. Insecticide applications were made to point of run-off. Evaluations were made one, three, and five days after treatment by counting number of nymphs found on leaves. Data were submitted to two-way ANOVA and Student-Newman-Keuls (SNK) test in the manner described above.

***P. cockerelli* Insecticide Trial 1999.** The final insecticide trial was conducted to again reevaluate the efficacy of *B. bassiana* (Mycotrol) (Mycotech Co., Bozeman, MT) and spinosad (Spintor SC) and to further examine an additional entomopathogenic fungus, *Metarhizium*

*anisopliae* (TAE-001-0L2 Strain F52) (Taensa, Inc., Fairfield, CT). Trials were again conducted on individual leaves of greenhouse grown tomatoes (Super Sweet 100) infested with tomato psyllid. Insecticide applications were made 6 July to point of run-off. Evaluations were made one and five days after treatment by counting number of live nymphs found on leaves. Data were submitted to two-way ANOVA and Student-Newman-Keuls test (SNK) in the manner described above.

**Oviposition Deterrent Trial 1997.** A trial was conducted to determine if tomato psyllid oviposition could be deterred by the use of several materials recognized to have some activity as insect repellents. Whole tomato (Blitz F1) plants were used as individual plots and first treated 17 May to point of run-off. Treatments included neem oil (Trilogy), an azadirachtin-enriched neem insecticide (Azatin XL), a horticultural oil (SunSpray) and a garlic-derived insect repellent (Garlic Barrier). Experimental design was a complete randomized design with 4 replications (leaves). Treatment application was repeated on 20 and 24 May. Data were collected by counting the number of eggs on a leaf 19, 21, 23, 26 and 28 May. Data were submitted to two-way ANOVA and a Student-Newman-Keuls test (SNK) in the manner described above.

## Results and Discussion

***Beauveria bassiana* Spray Chamber Trial 1997.** Both *B. bassiana* formulations produced significant reduction of *P. cockerelli*. The addition of the wetting agent Kinetic did not promote additional control (Table 3.1). This was the first demonstration that *B. bassiana* was capable of infecting tomato psyllid, and high levels of infection, producing mortality of up to 96 percent, were achieved. Furthermore, as *B. bassiana* is considered to be a "reduced risk" microbial insecticide, with several formulations that have extremely broad labeling, including greenhouse grown vegetables, such products may have application for control of tomato psyllid in Colorado greenhouses.

There are some other reports of other psyllids being susceptible to this fungus. Chazeau (1987) reported the infection of psyllid *Heteropsylla cubana* Crawford in the field by entomogenous fungi such as *B. bassiana*. Subsequent field applications of *B. bassiana* conidial suspension to a heavily-infested *Leucaena* plantation reduced *H. cubana* infestation by 81.5% (Liu et al., 1990). Within three days, *B. bassiana* caused mortality to *C. pyricola* (Puterka et al., 1994).

**Table 3. 1. Mortality of tomato psyllid nymphs following exposure to various *Beauveria bassiana* formulations in spray tower applications, Fort Collins, CO 1998**

Treatment	Rate	Percent mortality*
BotaniGard WP	23 gr/380 l	96.4a
BotaniGard WP and Kinetic	23 gr/380 l	82.1a
Naturalis-L	23 ml/380 l	95.4a
Naturalis-L and Kinetic	23 ml/380 l	95.0a
Water Check	-	23.6b

\*Average percent mortality per 4 leaves. Kinetic included in spray mixture at a rate of 0.1%.

Numbers followed by the same letter are not significantly different ( $P > 0.05$ , SNK).

Evaluations made 5 days after treatment. N= 4.

***P. cockerelli* Insecticide Trial 1997.** Evaluation at 1 and 2 DAT indicated that acetamiprid and spinosad (Spintor SC) provided significant control of tomato psyllid (Table 3.2). Final evaluation (5 DAT) indicated that all treatments, except Trilogy, were significantly different from the check.

Acetamiprid is known to have a fast acting effect due to a mode of action that kills insects both by contact and ingestion (Lagadic et al., 1993). However, its chemical similarity to imidacloprid may indicate potential problems with non-target organisms, notably bumble bee pollinators (Chapter Five).

On the other hand the high level of activity of spinosad is of particular interest and somewhat surprising as few Homoptera have been identified as susceptible species in the development of the product (Salgado et al., 1997). This insecticide is currently receiving very broad registration on greenhouse crops and outdoor vegetables under trade names such as Spintor, Conserve, and Success.

The microbial insecticide (BotaniGard ES) and the neem-derived insecticide (Azatin XL) were not effective until the fifth day of treatment. In the case of entomopathogenic fungi, a sequence of germination and growth of the fungus is required to produce mortality of infected insects. The virulence (degree of pathogenicity within a group or species) of *B. bassiana* and pathogenicity of isolates towards any insect varies (Feng et al., 1994). In a bioassay of a collection of over 50 *B. bassiana* isolates from around the world on the Colorado potato beetle, *Leptinotarsa decemlineata* (Say), produced LT50 (Lethal Time) values ranging from 2 to 10 days. According to Inglis et al. (1997) mycosis of *B. bassiana* treated grasshoppers in the greenhouse took 3 to 4 days after treatment. It took the same time for *Sitophilus zeamais* Motschulsky (Adane et al., 1996) and for *Coptotermes formosanus* Shiraki (Jones et al., 1996). Within three days, *B. bassiana* caused mortality to *C. pyricola* (Puterka et al., 1994).

Relatively slow activity of the neem-derived insecticide Azatin XL might be due to its anti-feedant activity and insect growth disrupting mode of action. These effects are more subtle

than produced by typical nerve poison insecticides (e.g., acetamiprid) and thus require some time before causing mortality (Kraus, 1995).

**Table 3. 2. Mortality of *Paratrioza cockerelli* nymphs following application of different insecticides in a tomato greenhouse, Fort Collins, CO, 1997**

Treatment	Rate	Percent mortality*		
		26 Jul (1 DAT)	27 Jul (2 DAT)	30 Jul (5 DAT)
Acetamiprid	0.06gr/380 l	79c	83c	93b
Azatin XL	480 ml/380 l	56bc	60abc	80b
BotaniGard WP	23 gr/380 l	39ab	60abc	86b
Relay 25W	150 ml/380 l	46abc	52abc	79b
Spintor SC	240 ml/380 l	62bc	67bc	87b
SunSpray	7.6 l/380 l	42ab	45ab	63b
Trilogy	3.8 l/380 l	46abc	60abc	63ab
Untreated check	-	17a	30a	45a

\*Average percent mortality per eight leaves. Numbers followed by the same letter are not significantly different ( $P > 0.05$ , SNK). N= 4.

***P. cockerelli* Insecticide Trial 1998.** Spinosad (Spintor SC) again showed very good activity producing over 95 mortality at 3 DAT (Table 3.3). There was no difference in effectiveness between the higher (300 ml/380 l) and lower (150 ml/380 l) rates.

Pymetrozine (Relay 25W) had fair-good efficacy, first demonstrated at 3 DAT. Control at the high rate (15 gr/380 l) was similar to that of spinosad at the end of the trial. Among the entomopathogenic fungi, *B. bassiana* (BotaniGard) did again produce significant mortality at 5 DAT. Significant effects were not observed prior to this, reflecting the required period to produce mycosis. *V. lecanii* (Mycotal) was not effective. This could be because of the strict

environmental requirements for germination of this fungus which include temperatures of 15-25°C and a relative humidity in excess of 95% (Helyer et al., 1992).

**Table 3.3. Mortality of *Paratrioza cockerelli* nymphs following application of different insecticides in a tomato greenhouse, Fort Collins, CO, 1998**

Treatment	Rate	Percent mortality*		
		25 Nov (1 DAT)	27 Nov (3 DAT)	29 Nov (5 DAT)
BotaniGard ES	0.95 l/380 l	4ab	42a	57bc
BotaniGard ES	1.9 l/380 l	25a	50a	62bc
BotaniGard ES	3.8 l/380 l	0a	62a	87c
Mycotal	23gr/380 l	0ab	0a	0ab
Mycotal	45gr/380 l	0ab	0a	2abc
Mycotal	90gr/380 l	0ab	0a	0a
Relay	75 ml/380 l	0ab	31a	42bc
Relay	150 ml/380 l	49ab	47a	89c
Spintor SC	150 ml/380 l	62ab	97a	97c
Spintor SC	300 ml/380 l	60ab	97a	100c
Water check	-	0ab	19a	19bc

\*Average percent mortality per 4 leaves. Numbers followed by the same letter are not significantly different ( $P > 0.05$ , SNK).  $N = 4$ .

***P. cockerelli* Insecticide Trial 1999.** Preliminary evaluation 1 DAT of all treatments showed that only Spintor SC provided significant control of psyllid nymphs producing a mortality of 52% (Table 3.4). At the 5 DAT evaluation both Spintor SC and Mycotrol 22 WP were significantly different from other treatments with mortality of 97% and 89% respectively. The *M. anisopliae* formulation demonstrated significant mortality compared to the untreated check, suggesting that further studies of this organism may be warranted.

**Table 3.4. Effect of different insecticides on *Paratrioza cockerelli* nymphs in a tomato greenhouse, Fort Collins, CO, 1999**

Treatment	Rate	Percent mortality*	
		7 Jul (1 DAT)	12 Jul (5 DAT)
Mycotrol 22 WP	0.12 gr/100ml	0a	89c
Spintor SC	1.28 ml/l	52b	97c
TAE-001-OL2	5 ml/l	17a	67b
Untreated check	-	0a	10a

\* Average percent mortality per 4 leaves. Numbers followed by the same letter are not significantly different ( $P > 0.05$ , SNK). N= 4.

**Oviposition Deterrent Trial 1997.** Oviposition was deterred during several evaluations by use of the horticultural (petroleum) oil SunSpray and the neem oil Trilogy (Table 3.5). There were no effects from application of an oil-free azadirachtin-enriched neem insecticide (Azatin) nor from a garlic derived product (Garlic Barrier).

**Table 3. 5. Effect of different deterrents on oviposition of *Paratrioza cockerelli* on greenhouse grown tomatoes, Fort Collins, CO, 1997**

Treatment	Rate	Number of eggs/4 leaves*				
		19 May (2DAT)	21 May (1DAT)	23 May (3DAT)	26 May (2DAT)	28 May (4DAT)
Azatin XL	480 ml/380 l	126.3ab	44.3a	76.3a	138.8a	270.0ab
Guardian Garlic	38 l/380 l	182.5a	117.0a	65.3a	121.5a	172.3ab
SunSpray	7.6 l/380 l	21.5b	16.0a	9.5a	25.5a	48.0b
Trilogy	3.8 l/380 l	9.0b	42.0a	13.8a	23.3a	74.5b
Water check	-	113.8ab	80.5a	69.5a	183.0a	429.3a

\* Average of four leaves. Numbers followed by the same letter are not significantly different ( $P > 0.05$ , SNK). N= 4.

Perhaps the most promising result of the insecticide trials was the identification of spinosad as an effective material for tomato psyllid. In addition to the very low mammalian toxicity of spinosad, it also appears to have little negative impact on most beneficial insects (Anonymous, 1998; Eger Jr, 1998). For example, when *Orius insidiosus* (Say), *Geocoris punctipes* (Say), *Hippodamia convergens* Guerin-Meneville and *Chrysoperla carnea* Stephens were exposed to ten insecticides, spinosad was less toxic than all other insecticides tested on all species (Elzen et al., 1998). Murray and Lloyd (1997) reported that spinosad was not disruptive to predator populations in Australian cotton and suggested that the product has an important role in integrated management programs. Duffle et al. (1997) stated that in field trials in cotton and soya bean, of 17 insecticides or insecticide mixtures applied, following a single application, spinosad had the least adverse effect on populations of *Geocoris* spp. on cotton. Also, following two applications, numbers of coccinellids were similar between the spinosad and untreated areas.

(Pietrantoino and Benedict, 1997) rated spinosad as harmless (causing <25% mortality) to *Cotesia plutellae* (Kurdjumov). Toxicity of spinosad (24 hour LC50s in ppm) for some beneficial arthropods are: *Apis mellifera* L. 27, *E. formosa* 29, *O. insidiosus* 200, *H. convergens* >1500, *C. carnea* 520 and *Phytoseiulus persimilis* >200 (Anonymous, 1998).

Acetamiprid also appears to have good selectivity of action against predatory and parasitic insects. When tested in the laboratory on adults of *Rodalia cardinalis* (Mulsant) and *Leptomastix dactylopii* Howard, acetamiprid was found to be not toxic to both beneficial insects. (Viggiani et al., 1998). A laboratory test of acetamiprid on mature larvae of *E. formosa* within pupal cases of *T. vaporariorum* indicated no significant effect on the development and adult emergence. Furthermore the same insecticides did not prevent the emergence of Braconidae, Chalcidoidea and Cynipoidea from mummies of *Aphis spiraecola* Patch (Viggiani et al., 1998). Toda and Kashio (1997) in evaluation of a direct dip test and a residual contact test of acetamiprid on first instar of *C. carnea* reported low toxicity in the dip test, but high toxicity in the residual contact test.

The novel mode of action and limited spectrum of activity shows pymetrozine to be safe to beneficial insects such as *C. carnea*, *G. punctipes*, *Coccinella* spp. and hymenopterous parasitoids (Ferguson et al., 1997). Pymetrozine also has low acute toxicity to mammals, terrestrial and aquatic wildlife and has favorable ecochemical properties that can make it useful in IPM programs (Fluckiger et al., 1992). This suggests that its use may be compatible with greenhouse tomato production practices, although other products (e. g., spinosad) were identified as being more efficacious for tomato psyllid control in this study.

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## Chapter IV

### Evaluation of Green Lacewings (Neuroptera: Chrysopidae) as Biological Control Agents for *Paratrioza cockerelli* (Homoptera: Psyllidae) in Greenhouse Tomatoes

#### Introduction

Colorado is the number one state in the production of greenhouse tomatoes with more than 48 hectares in 1997 and presently almost half of the total US greenhouse tomato production (Naegely, 1997). However, the tomato psyllid, *Paratrioza cockerelli* (Sulc), is often a limiting factor in greenhouse tomato production in western North America. The feeding of the psyllid on the vegetation induces a condition known as “psyllid yellows” that affects plant growth and fruit production (Daniels, 1954; Pletsch, 1947; Richards, 1928).

Several predators have been reported to attack tomato psyllids including several species of lady beetles, green lacewings, nabids [*Nabis ferus* (L.)], a syrphid fly, and big-eyed bugs (*Geocoris decoratus* Uhler) (Knowlton, 1933a; 1933b; 1933c; 1934; Knowlton and Allen, 1936). Among the predators, Pletsch (1947) felt that only the chrysopids, lady beetles and big-eyed bugs warranted further research attention in regards to being potentially important in psyllid management.

Unidentified chrysopids have been observed to destroy psyllid nymphs under field conditions (Knowlton, 1933a). Romney (1939) stated that numbers of psyllid eggs and nymphs on matrimony vine in Arizona desert areas were reduced by predators such as coccinellids and chrysopids. Newly hatched chrysopid larva showed no hesitation in attacking psyllid eggs when offered and an individual larva was observed to consume 20 to 30 psyllid eggs at a rate exceeding one egg per minute. When chrysopid larvae were reared only on tomato psyllids, the first instar consumed 110 eggs; the second instar 318 eggs; and the third instar 125 eggs and 67 small nymphs,

a total during the larval life span of 553 eggs and 67 nymphs (Pletsch, 1947). With this magnitude of prey consumption and the several hundred eggs typically laid by an individual female, chrysopids appear to have high potential to exercise a considerable check on *P. cockerelli* populations.

Chrysopids are a widely available species for biological control use (Hunter, 1997) and available at low cost (Cranshaw et al., 1996). The potential use of *Chrysoperla carnea* Stephens and other lacewing species to control greenhouse species is substantial (New, 1975). Greenhouse experiments demonstrated that the green peach aphid, *Myzus persicae* (Sulzer), was successfully controlled by second instar of *C. carnea* when released at 1:40 predator prey ratios (Hassan et al., 1985). Wildermuth (1916) determined the number of aphids eaten by *C. carnea* larvae. Based on the results of laboratory rearing, he estimated that a single individual, developing through three larval instars, consumed a total of 300 to 400 aphids in the field. Larvae of *C. carnea* also were very effective against *Helicoverpa zea* (Boddie) and *H. virescens* (F.). Van den Bosch and Hagen (1966) mentioned that the green lacewings are among the most important beneficial insects in California cotton fields and chrysopids are abundant in a wide variety of crops, corn, alfalfa, sorghum, tobacco, citrus and grapes (Canard et al., 1984).

The potential use of green lacewings in IPM programs is enhanced by their relatively high tolerance to most insecticides (New, 1975). Pyrethroids have little effect on all life stages except the eggs (Hurej and Dutcher, 1994). In addition, the ease of mass production and development of semi artificial diets for rearing allows use in enclosed environments such as greenhouses (New, 1975).

The overall objective of this study was to investigate the potential use of green lacewing as an applied biological control for tomato psyllid. This was pursued by observations on tomato psyllid consumption by two commercially available chrysopids [*C. carnea*, *C. rufilabris* (Burmeister)] in laboratory and field settings.

## Materials and Methods

**Growth Chamber Trial 1998.** A preliminary trial was conducted to determine if green lacewings could be reared solely on a diet of tomato psyllid nymphs. Two species of lacewings were tested, *C. carnea* and *C. rufilabris*, obtained from Rincon-Vitova Insectaries, Inc., Ventura, CA.

Newly hatched lacewing larvae were individually reared in petri dishes and placed with excised tomato psyllid infested tomato leaves placed in a small block of Oasis Block<sup>®</sup>. Eight larvae were reared of each species, starting on 9/30/98 and lasting until 10/20/98. The petri dishes were sealed with Parafilm<sup>®</sup> and placed in a growth chamber with a temperature of 27<sup>0</sup>C and a 16 hour photoperiod.

**Comparison of Lacewing Prey on Development 1999.** Development and prey consumption by *C. carnea* and *C. rufilabris* were compared between diets of tomato psyllid or green peach aphid. Newly hatched green lacewing larvae were placed in individual petri dishes in a growth chamber at a constant temperature of 27<sup>0</sup>C and a photoperiod of 16 hours. Trials consisted of twenty newly hatched *C. carnea* or *C. rufilabris* provided either tomato psyllid or green peach aphids. Green peach aphids were maintained on cabbage and tomato psyllids on tomato. The number of prey consumed by lacewing larvae was recorded and new prey provided every two days. Data on survival, length of the larval and pupal development periods were collected.

**Prey Choice Test Trial 1999.** The relative preference of *C. carnea* and *C. rufilabris* on tomato psyllid versus green peach aphid was assessed. Individually reared two day old lacewing larvae were provided a choice of 100 green peach aphids and 100 psyllids in petri dish. Numbers of prey consumed by the lacewing larva were counted after 24 hours. Four replications of this trial were conducted.

**Field Trial 1999.** A field trial was conducted using applications of *C. carnea* eggs to control tomato psyllids and green peach aphid on potato at the Horticulture Field Research Center in Ft. Collins, CO. Individual plots consisted of two rows divided into eight plots, about 11 m (35 ft) in length arranged in a randomized complete block design. On July 3, eight plots were each treated with fifty eggs of *C. carnea* applied using a backpack mister (Backpack Mister Model MD 150DX, Maruyama MFG. Co., Inc., Tokyo, Japan). The other eight plots were left untreated. Data were collected seven and 14 days following treatment by counting number of aphids and psyllids on 50 leaves from the center of each plot. Data were submitted to two-tailed Student t-test (SAS Institute, 1990).

## Results and Discussion

**Growth Chamber Trial 1998.** In this preliminary trial six of eight *C. rufilabris* and one of eight *C. carnea* successfully developed to pupation. Larval development averaged approximately 19 days. The results indicated that both species are capable of developing on a diet of tomato psyllids; better adaptation of *C. rufilabris* to tomato psyllid was also indicated in this preliminary experiment.

**Comparison of Lacewing Prey on Development 1999.** Survival of both chrysopid species to the pupal stage was better on tomato psyllid, 45 percent for both species, than on green peach aphid (Table 4.1). Survival of green lacewings reared on a diet solely of green peach aphid was 25 percent and 0 percent for *C. carnea* and *C. rufilabris* respectively. The average period to complete larval development was eight, 12 and 14 days for *C. rufilabris* on tomato psyllid, *C. carnea* on tomato psyllid, and *C. carnea* on green aphid, respectively.

**Table 4. 1. Prey consumption and survival of *Chrysoperla carnea* and *C. rufilabris* reared on tomato psyllid or green peach aphid, Fort Collins, CO, 1999**

Chrysopid species	Prey	Survival of larvae (%)*	Daily prey consumed	Total prey consumed
<i>C. carnea</i>	<i>P. cockerelli</i>	45	24.4	245
<i>C. carnea</i>	<i>M. persicae</i>	25	24.5	246
<i>C. rufilabris</i>	<i>P. cockerelli</i>	45	17.5	139
<i>C. rufilabris</i>	<i>M. persicae</i>	0	11.5	91

\* N = 20.

During development *C. carnea* consumed more prey than did *C. rufilabris*. Total consumption of psyllids was by *C. carnea* 245, averaging 24.4/day during its larval development. Total consumption by *C. rufilabris* averaged slightly more than half this amount, a total of 139, psyllids that were consumed at an average of approximately 17.5 day.

*Chrysoperla carnea* could successfully complete its development on both prey with larval development faster on psyllids (12 days). Where development was 18.3 days at 20°C (Toschi, 1965). *Chrysoperla rufilabris* was unable to complete development on the aphid diet but developed more rapidly (8 days) than *C. carnea* on tomato psyllid. Effects of different prey previously demonstrated food quality effects on the success of rearing and survival of *C. rufilabris* (Legaspi et al., 1994). For example, development of *C. rufilabris* larvae was longer when larvae were provided sweetpotato whitefly [*Bemisia tabaci* (Gennadius)], aphids or an artificial diet alone compared with combination diets of the various food sources. Hydorn and Whitcomb (1979) reported an increase in fecundity and survival for *C. rufilabris* when *Drosophila melanogaster* Meig. and *Tribolium castaneum* (Herbst) were replaced with aphids and *Phthorimaea operculella* (Zeller) eggs as food.

**Prey Choice Test Trial 1999.** *Chrysoperla carnea* and *C. rufilabris* showed no prey preference when provided choices of either green peach aphid or tomato psyllid as prey (Table 4.2).

**Table 4. 2. Consumption of green peach aphid and tomato psyllid *Chrysoperla carnea* and *C. rufilabris* in a choice test, Fort Collins, CO, 1999**

Lacewing species	No. of prey consumed	
	<i>M. persicae</i>	<i>P. cockerelli</i>
<i>C. carnea</i>	70.0a	59.3a
<i>C. rufilabris</i>	63.8a	39.8a

Two tailed Student t-test was used for analysis and no significant differences was noticed. N= 20.

Legaspi et al. (1994) reported a preference by *C. rufilabris* for eggs of *Sitotroga cerealella* (Olivier) to those of *H. zea*, *Manduca sexta* (L.) and to *B. tabaci*. *C. plorabunda* (Fitch) captured *R. padi* more often than *Diuraphis noxia* (Mordvilko) (Bergeson and Messina, 1998). Legaspi et al. (1994) stated that preference among lacewing larvae for different prey is probably a function of food quality.

**Table 4. 3. Effect of a *Chrysoperla carnea* application on numbers of tomato psyllids and green peach aphids in a potato planting, Fort Collins, CO, 1999**

Treatment	Aphid		Psyllid	
	July 10 (7 DAT)	July 17 (14 DAT)	July 10 (7 DAT)	July 17 (14 DAT)
<i>C. carnea</i> eggs applied	0.3	0.2	0.4	0.4
No application of eggs	0.3	0.2	0.6	0.3

Two tailed Student t-test was used for analysis and no significant differences was noticed.

**Field Trial One 1999.** There was no significant difference on number of psyllids or green peach aphids between lacewing egg-treated and untreated plots (Table 4.3). Similar poor results have been reported, such as Raupp et al. (1994) who reported that release of *C. carnea* and *C. rufilabris* on hawthorn (*Crataegus phaenopyrum*) infested with *A. fabae* in planter boxes in landscape settings provided no evidence of reductions in aphid populations. Absence of differences could have been obscured due to presence of high numbers of wild lacewings in the plot.

However, there have been successful uses of green lacewing applications for biological control of plant pests. Kaitazov and Kharizanov (1977) reported excellent control of *M. persicae* on tobacco using *C. carnea*. In field tests *C. carnea* successfully control *Aphis gossypii* Glover (Beglyarov and Ushchekov, 1974). Radzivilovskaya (1980) stated that use of *C. carnea* in early

spring is promising for the control of aphids on cotton. Also, applications of *C. carnea* eggs at 1, 3 and 10 eggs/aphid reduced populations of *Rhopalosiphum padi* (L.) by 21, 51 and 91%, respectively (Rautapaa, 1977).

Both *C. carnea* and *C. rufilabris* may well fit into an integrated pest management program for tomato psyllid in greenhouse grown tomatoes. As *C. rufilabris* is reportedly better adapted to humid, irrigated field or greenhouse conditions than *C. carnea* (Legaspi et al., 1994) it may prove the better species for Colorado greenhouses, although both are capable of developing on a tomato psyllid diet.

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## Chapter V

### Effect of Imidacloprid on *Bombus terricola occidentalis* (Hymenoptera: Apidae) Colonies

#### Introduction

In a very short period greenhouse-grown tomatoes have become a major crop in Colorado, with current value in excess of \$60 million annually (Basham, personal communication). It is a very intensively managed crop and with one requirement being insect pollination. Although the honey bee, *Apis mellifera* L., is generally the most common commercial pollinator, bumble bees (*Bombus* spp.) are the superior pollinators of greenhouse tomatoes. This is because the tomato flower under greenhouse conditions needs vibration for proper pollination and fruit set. The bumble bee does this in an optimal way using the buzz pollination mechanism; honey bees are incapable of buzz pollination (Sowig and Paulus, 1988).

The anthers of about 20,000 species of plants, including tomatoes, are periodically dehiscent and only certain bees and syrphid flies are able to collect pollen from these flowers by vibrating (buzzing) the anthers (Harder and Barclay, 1994). Among these buzz-pollinated plant species the anthers generally function as dispensing mechanisms and reduce pollen loss. In order to release pollen from anthers, a bee vibrates its indirect flight muscles, producing a loud intermittent buzzing sound. This is achieved by bumble bees by producing buzz frequencies up to 400 Hz (Harder and Barclay, 1994). In the resulting pollen cloud the ventral parts of the bees thorax and abdomen became covered with pollen (Buchmann, 1974). Most buzz pollinated flowers are nectarless (Dukas and Dafni, 1990).

In tomatoes, it is possible to achieve nearly 100% pollinated flowers with bumble bees (Asada and Ono, 1996; Heinrich, 1979). Furthermore, in commercial pepper greenhouses using

bumble bees for pollination, the average early and total yields increased 29.6% and 22.4% compared with control. Fruit weight, diameter, volume and flesh thickness was also positively influenced by the activity of bumble bee pollinators (Abak et al., 1997).

Bumble bees also are better adapted to perform under confined greenhouse conditions. Colonies are relatively small and easily transferred within the greenhouse and bumble bees are less inclined to look for alternative sources of pollen and nectar outside the greenhouse. Furthermore, they have branched hairs allowing easy pick up and transfer of pollen.

There are about 30 species of native bumble bees in North America (Heinrich, 1979). At least two have been utilized in commercial agriculture. New technological developments have allowed colonies to be available on a year-round basis. In western North America, *Bombus occidentalis* Greene (= *B. terricola occidentalis* Greene) is the bumble bee commercially available as a pollinator; in the east, *B. impatiens* Cresson. *Bombus terricola occidentalis* is a common and widely distributed species native to Colorado.

Colonies used in greenhouses production typically are short lived. High temperatures tend to shorten the active life of bumble bee colonies (Palumbo et al., 1996). Chen and Hsieh (1996) reported that temperature increases to 40<sup>o</sup>C inside the greenhouse dramatically reduced the pollination activity and colony longevity of bumble bees. Effective pollination by a colony lasted only 30 to 40 days in April, while during the cool season (December to February) it lasted for two months with better effect.

A further limitation on the use of bumble bees as pollinators is the need to concurrently manage insect pests in the greenhouse. In Colorado these include greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood), tomato psyllid, *Paratrioza cockerelli* (Sulc), and flower

thrips, *Frankliniella* spp. As insecticides are widely used in greenhouses, an important consideration with insect pollinated crops is their compatibility with the pollinator.

Currently one of the most widely used insecticides in North American greenhouses is imidacloprid (Marathon<sup>®</sup>, Merit<sup>®</sup>, Admire<sup>®</sup>, Provado<sup>®</sup>, Imicide<sup>®</sup>, Gaucho<sup>®</sup>). The mode of action of imidacloprid is disruption of the nervous system acting as a competitive inhibitor at nicotinic acetylcholine receptors (Yie and Casida, 1993; Zwart et al., 1994). Insects are killed by contact and/or ingestion (Lagadic et al., 1993). It is a broad spectrum contact and systemic insecticide, primarily used for control of whiteflies, various aphids and other homopterans (Schmeer et al., 1990). It is also highly efficacious for control of tomato psyllid (Cranshaw, 1994). A member of the chloronicotiny insecticide class it is currently labeled for production of field grown tomatoes and has tolerance in the harvested crop ranging from 3 parts per million in tomato puree to 6 parts per million in tomato paste. However, it is not currently labeled for greenhouse vegetables in the United States.

Concerns of side effects of imidacloprid on beneficial non-target insects have been raised. Delbeke et al. (1997) found imidacloprid to be harmful to *Orius leavigatus* (Fieber) and resurgence of spider mites on imidacloprid treated plants also was documented as being related to incidental mortality of *O. insidiosus* (Say) (Sclar et al., 1998). Elzen et al. (1998) found imidacloprid to be highly toxic to *Chrysoperla carnea* Stephens, intermediate in toxicity to *Hippodamia convergens* Guerin-Meneville, and low in toxicity to *O. insidiosus* and *Geocoris punctipes* (Say). Imidacloprid caused 78% mortality to adult *H. convergens* after exposure to residues at 127.4 ppm for 48-72 h (Kaakeh et al., 1996; Mizell and Sconyers, 1992). Predatory insects such as lady beetles, hover flies, and lacewings populations decreased by at least 50% following imidacloprid spray application (Pfluger and Schmuck, 1991). Imidacloprid was toxic to *Deraeocoris nebulosus* (Uhler), larvae and

adults of *Olla abdominalis* (*O. v-nigrum*) (Mulsant), adults of *H. convergens*, eggs and adults of *C. rufilabris* (Burmeister) and adults of *G. punctipes* at concentrations near the recommended field rate (Mizell and Sconyers, 1992). Toda and Kashio (1997) found that imidacloprid showed low toxicity to instar I *C. carnea* when dipped in an aqueous solution but high toxicity in the residual contact test.

Among parasitoids, Bethke and Redak (1997) reported effects on the development of *Encarsia formosa* Gahan on whiteflies infesting imidacloprid treated plants. When applied to on mature larvae of *E. formosa* within pupal cases of *T. vaporariorum*, the development of the parasitoid was not significantly affected and adult emergence of the parasitoid took place normally. Gonzalez et al. (1997) classified imidacloprid as moderately harmful to pupae of *Eretmocerus mundus* Mercet, an important and widespread parasitoid of *Bemisia tabaci* (Gennadius). Hayashi (1996) reported harmful effects on both nymphs and adults of *E. formosa*. However, the same insecticides did not prevent the emergence of braconid, Chalcidoidea and Cynipoidea parasitoids (Viggiani et al., 1998). Schuld and Schmuck (1997) also observed no adverse effects when the pre-adult stages of *Trichogramma cacoeciae* Marchal, an egg parasitoid of *Cydia pomonella* (L.), was exposed to imidacloprid. However, when imidacloprid-treated *C. pomonella* eggs were stored under greenhouse conditions, emergence of parasitoids was significantly adversely affected. Adults of *T. japonicum* Ashmead treated with imidacloprid could oviposit normally, and the fecundity, net reproduction rate, innate capacity for increase of the treated and the next generation showed no significant differences (Zhang and Hirai, 1997).

Effects on pollinators have also been investigated. Mayer and Lunden (1997) monitored *A. mellifera*, *Megachile rotundata* (F.) and *Nomia melanderi* Cockerell caged with *Medicago sativa* (L.) foliage collected from plots that had been sprayed with different concentrations of midacloprid.

They found mortality increased with concentration, but older residues (greater than eight hours) were generally less toxic than two hour old residues. In tests at an artificial feeder, the addition of 2-500 ppm imidacloprid to sugar syrup reduced honey bee visits by 7-85%. In field tests, spraying flowers with imidacloprid reduced honey bee visits 0.5 and 1 hour after spraying but not at four hours. Mayer et al. (1994), in reporting on the acute toxicity of 16 insecticides, including imidacloprid, to caged bees using a standard residue bioassay test reported that imidacloprid can be applied in late evening with minimal hazard to bumble bees, honey bees, alkali bees and leafcutting bees. Furthermore, mortality of honey bees following exposure to 14 insecticides, including imidacloprid, was reduced by adding the adjuvant Sylgard 309 silicone surfactant (Mayer and Lunden, 1994). When applied as a spray in flowering crops during the foraging time, imidacloprid is to be classified as hazardous to honey bees. There is a noticeable repellent effect which, depending on the application rate and crop, can last more than a week (Mayer and Lunden, 1994).

An application of imidacloprid as a seed dressing or soil application has been stated to largely preclude a direct effect on honey bees (Pfluger and Schmuck, 1991). Also, seed treatment or application as a systemic soil drench are methods that may reduce the harmful effects to some biological control agents (Mizell and Sconyers, 1992).

It should be pointed out that all published reports of effects on honey bees, or other pollinator species, involved evaluation of acute effects on adult bee over a short duration of time. However, there have been reported effects of imidacloprid on other social insects, notably termites. Low concentrations (0.01%) imidacloprid significantly reduced *Heterotermes tenuis* (Hagen) termite populations when impregnated in bait traps (Almeida et al., 1998). At very low concentrations imidacloprid is able to modify the behavior of termites *Reticulitermes flavipes* (Kollar) (Leicht, 1993). Imidacloprid is currently sold as a termiticide in the United States.

Non-refereed reports from Israel have indicated that imidacloprid is taken up by the plant and can be transported into the flowers, including the pollen, and adversely affect bumble bees (Anonymous, 1996). Once in the plant, it may take a number of weeks before that active ingredient has reduced to a level that is not harmful to bumble bees. Reported residual effects from treatment range from one week when applied in soil to more than 7 weeks when applied in Rockwool®. Characteristics of a bumble bee colony poisoned by imidacloprid include a cessation of foraging shortly after the hive has been introduced and the presence of dead (black) larvae around the edges inside the colony was reported.

Imidacloprid has substantial potential use in controlling key insect pests of greenhouse-grown tomatoes, including whiteflies, aphids, and the tomato psyllid. However, if its use precludes effective pollination by bumble bees or other insect pollinators then its use on greenhouse tomato production is incompatible with current production practice. Therefore the objective of this study was to determine if the use of soil-applied imidacloprid affects the viability of bumble bees foraging on greenhouse tomatoes.

## Materials and Methods

A series of four trials were conducted during 1997 and 1998. In all trials plots consisted of individual greenhouse bays, each housing 200-500 greenhouse tomatoes. Treatments consisted of either treating all tomatoes in the greenhouse bay with imidacloprid or leaving them untreated.

**Preliminary Trial 1997.** In the preliminary, 1997, trial run ca 500 plants of the variety 'Trust' were grown hydroponically on Grodan<sup>®</sup> blocks. In one house imidacloprid (Admire<sup>®</sup>) was introduced at the base of each plant at a rate equivalent to 6.5 fl oz/1000 row-ft; the other house was maintained in a similar manner but plants did not receive any imidacloprid. Immediately after treatment bumble bee colonies were introduced into each house and maintained for ca two months. In this trial, and all subsequent trials, bumble bee colonies used were BioBest<sup>®</sup> "mini-hives" purchased through International Technology Services, Inc. of Boulder, CO. At the end of the experiment both colonies were placed in freezer. Colony evaluations included number of bumble bees found within hives at the end of a trial run, production of honey pots in colonies and number of pupae.

**1998 Trials.** In the 1998 trials, two greenhouse bays were used for each treatment in each trial run. Because the available greenhouse area was smaller, a cherry tomato variety ('Super Sweet 100') was used to increase flower density. Plants were grown in 1-gal pots in an artificial soil mix (Metro-Mix<sup>®</sup> 350, Horticultural Products Company, Marysville, OH) and were individually drip irrigated. The imidacloprid formulation Merit 75W was applied on July 15, at a rate of 0.13 grams/pot applied as a dilute drench. *Bombus t. occidentalis* "mini-hives" were introduced into each of the four bays after treatment and maintained for 25 days. At the end of this period a second set of four colonies were subsequently placed in the greenhouses and maintained for the following 20 days. A third set of bumble bee colonies were added immediately following the previous trial and were maintained for another 20 days.

General observations on overall colony activity and appearance were periodically conducted through opening the hive cover and counting the number of bees active on the surface of the colony. In the end of the experiment the bumble bee colonies were removed and placed in a cooler for one hour to calm down bumble bee adults. And the number of live bees within hives were counted. Also, counts were made of the number of pupae.

**1999 Trials.** In the 1999 trials, most of the same methods used in 1998 trials were repeated but involved only two greenhouse bays. Trials were repeated three times at seven day intervals. Additionally, 200 flowers in each greenhouse were examined for bumble bee visitation. Signs of flower visitation by bumble bees were bite marks made by the mandibles when the bumble bee grasps the flower during pollen collection and pollination. Flowers that had such markings were considered to have been visited and flowers with no bites were not. Also, the number of foraging bees in each greenhouse was recorded for one hour beginning at 7:00 am on two dates to give an additional estimation of hive activity. Where appropriate, data were submitted to a two-tailed Student t-test (SAS Institute, 1990).

## Results and Discussion

Substantial adverse effects on bumble bee activity and colony survival were observed among bumble bees foraging on greenhouse tomatoes that had been treated with soil applications of imidacloprid.

**Preliminary Trial 1997.** Number of adults in the colony foraging on untreated tomatoes was more than triple the number of adults in the colony foraging tomatoes treated with imidacloprid (Table 5.1). Furthermore there was dramatic differences appearance of colonies at the end. The colony foraging in the untreated greenhouse remained active and vigorous; only a few bees moving erratically were observed in the colony exposed to imidacloprid.

**Table 5. 1. Number of adult bees, honey pots, and pupae in bumble bee colonies placed in imidacloprid treated and untreated tomato greenhouses, Fort Collins, CO, 1997**

Imidacloprid treatment exposure	Bees	Honey pots	Pupae
Imidacloprid treated	7.0	3.0	7.0
Untreated check	28.0	14.0	11.0

N= 2.

**Trial One 1998.** At the end of this trial there were few differences between the number of bumble bee adults and pupae in colonies located within treated and untreated greenhouses (Table 5.2). This may be due to having frozen the colony before determining whether bees in the hive were alive or dead. As a result this evaluation technique was subsequently abandoned with later trials. However, observations of colony activity prior to freezing indicated dramatic differences in colony health; the colony in the untreated greenhouse was very vigorous while the colony in the imidacloprid treated greenhouse was in poor condition.

**Table 5. 2. Number of adult bees, honey pots, and pupae in bumble bee colonies placed in imidacloprid treated and untreated tomato greenhouses for 25 days after imidacloprid application, Fort Collins, CO. Total of two replications (colonies). Trial One, 1998**

Imidacloprid treatment exposure	Bees	Honey pots	Pupae
Imidacloprid treated	128.0	26.0	139.0
Untreated check	83.0	57.0	78.0

N= 4.

**Trial Two 1998.** The number of live adults was significantly lower in colonies exposed to imidacloprid treated tomatoes (treated 2.5/colony, untreated 37.5/colony) (Table 5.3). Also the number of honey pots were significantly lower in treated greenhouses; treated colonies lacked a single honey pot at the end of the experiment while untreated had an average of 30/colony. Activity of colonies also was very different, with essentially no bumble bee adults observed moving on the top of the colonies that had been in imidacloprid-treated tomato greenhouses for 25 days (Table 5.4). Untreated colonies were significantly much more active than treated ones (Table 5.4).

**Table 5.3. Number of adult bees, honey pots, pupae and adult mortality percent in bumble bee colonies placed in imidacloprid treated and untreated tomato greenhouses for 20 days after imidacloprid application, Fort Collins, CO. Total of two replications (colonies). Trial Two, 1998**

Imidacloprid treatment exposure	Bees	Mortality (percent) of adults	Honey pots	Pupae
Imidacloprid treated	5.0b	60.8	0b	108.0
Untreated check	75.0a	28.0	59.0a	116.0

\*Mortality was determined by dividing number of dead bees within the colony by total number of bumble bee adults found in the colony in the end of the experiment. Means followed by the same letter are not significantly different ( $P > 0.05$ , Two-tailed Student t-test). N= 4.

**Trial Three 1998.** The most noticeable difference between treated and untreated colonies was the colony activity (Table 5.4). Untreated colonies were much more active than untreated ones (treated, 1.3 adults observed/colony; untreated, 19.8 adults observed/colony). There was no significant difference between numbers of pupae in treated and untreated colonies (Table 5.5). Numbers of honey pots in untreated colonies were significantly higher than those in treated ones.

**Table 5. 4. Number of adult bees observed moving on top of bumble bee colonies (index of activity) placed in imidacloprid treated and untreated tomato greenhouses, Fort Collins, CO. Trial Two, and Three 1998**

Imidacloprid treatment exposure	No. of bumble bee adults	
	Trial two	Trial three
Imidacloprid treated	0b	1.3b
Untreated check	30.0a	19.8a

Means followed by the same letter are not significantly different ( $P > 0.05$ , Two-tailed Student t-test). N= 4.

**Table 5.5. Mean of honey pots and pupae per colony in imidacloprid treated and untreated tomato greenhouses, Fort Collins, CO. Combined data from trials two and three, 1998**

Imidacloprid treatment exposure	No. of honey pots per colony	No. of pupae per colony
Imidacloprid treated	7.8b	57a
Untreated check	39.8a	57.5a

Means followed by the same letter are not significantly different ( $P > 0.05$ , Two-tailed Student t-test). N= 4.

**Table 5.6. Mean no. of adults, adult mortality, mean no. of pupae and mean no. of honey pots per colony in bumble bee colonies placed in imidacloprid treated and untreated tomato greenhouses, Fort Collins, CO. Total of three replications (colonies)\*, 1999**

Imidacloprid treatment exposure	No. of adults	Mortality (percent) **	No. of pupae per colony	No. of honey pot per colony
Imidacloprid treated	55.0	74.3	51.3	20.0
Untreated check	201.0	28.0	46.7	88.3

\*Replications are made in three different dates (replication in time).

\*\*Mortality was determined by dividing number of dead on total number of bumble bee adults found in the colony in the end of the experiment. N= 3.

There was no significant difference in adult mortality between imidacloprid treated and untreated greenhouses (Table 5.6). There was no significant difference in number of pupae between treated and untreated greenhouses, or in the number of honey pots within colonies that had foraged between treated or untreated greenhouses. However, flower visitation, as evidenced by bite marks on flowers, was greatly reduced (Table 5.7).

**Table 5. 7. Numbers of visited flowers, foraging bumble bees and adults moving on top of the colony (index of activity) in imidacloprid treated and untreated tomato greenhouses, Fort Collins, CO. 1999 Means of three trial runs**

Imidacloprid treatment exposure	No. of visited flowers*	No. of foraging bees**	No. of bumble bee adults moving on top of the colony***
Imidacloprid treated	9.3b	0.5b	5.4b
Untreated check	128.7a	4.0a	23.0a

\*Mean number of flowers visited by bumble bee adults in treated and untreated tomato greenhouses. Means followed by the same letter are not significantly different ( $P > 0.05$ , Two-tailed Student t-test).

\*\*Mean number of foraging bumble bee adults during one hour in treated and untreated tomato greenhouses.

\*\*\*Mean number of bumble bee adults observed moving on top of colonies placed in treated and untreated tomato greenhouses.

Effects of imidacloprid have been studied with other pollinators. Ambolet et al. (1997) reported no effect on the foraging activity and mortality of honey bees when hives (6,000-1,0000 bees) were enclosed in plastic tunnels containing flowering sunflowers grown with seed treatment of imidacloprid (0.7 mg a.i./seed). Colombo (1997) stated that imidacloprid did not affect negatively the pollination activity of the flowers by *B. terrestris* nor adversely affect its survival. Application of imidacloprid to flowering apple trees did not reduce honey bee foraging (Mayer and Lunden, 1997).

However, the results of these studies do indicate harmful effects of imidacloprid on bumble bees. Numbers of live adults in the colonies placed in treated tomato greenhouses were

half those placed in untreated greenhouses (Table 5.3). The foraging of the bees in treated greenhouses was much lower at treated greenhouses. Using number of honey pots as indicator of foraging, number of honey pots was significantly less than those in untreated ones (Table 5.5). Colonies in treated greenhouses were consistently less vigorous of those in untreated ones and when count were made of the number of adults moving on top of the colony, there were significantly more bees found in untreated colonies (Table 5.4). Characteristics of bumble bee poisoning by imidacloprid, such as a cessation of foraging and the presence of dead (black) larvae around the edges inside the colony (Anonymous, 1996), were repeatedly observed in colonies confined with imidacloprid treated tomatoes. Also the number of flowers visited in the untreated greenhouse was significantly higher than in the imidacloprid-treated greenhouse (Table 5.7).

The bumble bee has a high tolerance to many insecticides. Stevenson and Racey (1967) reported an unusually high tolerance to four insecticides after contact application on *B. lucorum* (L.) and *B. pascuorum* (Scopoli). Laboratory dose response studies conducted with *A. mellifera*, *Andrena erythronii*, *M. rotundata* and *B. t. occidentalis*, using 6 insecticides (permethrin, mexacarbate, aminocarb, fenitrothion, carbaryl, and trichlorfon), found *B. t. occidentalis* the most tolerant species (Helson et al., 1994). The activity of the pollinators was not affected by insecticide pymetrozine and no repellent effect was observed for treated tomato flowers in tomato greenhouses (Freuler et al., 1996; Senn et al., 1994).

Although bumble bees showed high tolerance to insecticides, sublethal effects of insecticides on pollinators have been reported. For example, sublethal effects on honey bees include behavioral and physiological effects on adults and larvae such as disorganization of waggle dances and pollen gathering, decrease of life span and fecundity, and induction of

abnormalities in larval growth and pupation (Tasei et al., 1994). The lifespan, fecundity and larval growth duration in the solitary bee *M. rotundata* was adversely affected by low doses of deltamethrin (Tasei et al., 1994). In this study, the cessation of bumble bee foraging, the low number of visited flowers and the general weakness of the colony are signs of sublethal effects of imidacloprid. Among the social insects, Leicht (1993) found that a very low concentration of imidacloprid modifies the behavior of termites *Reticulitermes flavipes* (Kollar). Low concentrations (0.01%) of imidacloprid significantly reduced *H. tenuis* termite populations when impregnated in a baited trap (Almeida et al., 1998).

Although seed treatment with the insecticide imidacloprid had no immediate effect on the foraging activity of honey bees (Ambolet et al., 1997), effects of a soil application do not seem to be the same for *B. t. occidentalis*. This may be due to the way that both bee species pollinate flowers. In case of bumble bees, the foragers bite on the flower during buzz pollination, honey bees do not bite during pollination. During this process the bumble bee may be exposed to plant sap that can contain imidacloprid residues. Navalon et al. (1997) have recovered imidacloprid from vegetable sap samples including tomatoes.

In conclusion, this study indicates that soil applications of imidacloprid adversely affect foraging bumble bees in tomato greenhouses. This would suggest that imidacloprid use is thus incompatible with current greenhouse tomato production, where the bumble bee is depended upon for pollination.

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