Investigations of the integrated pest management of Colorado potato beetle, *Leptinotarsa decemlineata* (Say): Host plant preference, development of semiochemical-based strategies, and evaluation of a novel insecticide

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Abstract

Exploiting the chemical ecology of an insect can unveil novel strategies for its pest management. Though much has been learned about the chemical ecology of Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae), a major pest of solanaceous crops in the U.S., there has been little use of this knowledge in pest management. To better understand host plant selection by CPB, field and laboratory-choice experiments were conducted in Virginia. In laboratory studies, CPB preferred potato over both tomato and eggplant foliage and eggplant over tomato foliage. However, field studies using counts of live beetles on untreated paired plants and counts of dead beetles on insecticide-treated plants revealed no significant preference for potato over eggplant. Additional studies showed that the presence of adult male CPB on foliage greatly impacted host plant selection, with significantly more adults being attracted to eggplant with male beetles than any other treatment combination.

Adult CPB have been shown to be attracted to (*S*)-3,7-dimethyl-2-oxo-oct-6-ene-1,3-diol [(*S*)-CPB I], a male-produced aggregation pheromone. Field studies were conducted to determine if the opposite enantiomer of the pheromone, (*R*)-CPB I had an effect on CPB in the field. Results revealed no differences in counts of all CPB life stages between untreated potato plots with and without rows inundated with (*R*)-CPB I lures. In addition, the relative attraction of CPB adults to various racemic forms of the (*S*)- and (*R*)-enantiomers was also investigated and showed that racemic blends that were less than 97%(*S)* were not attractive to CPB adults.

Combinations of the (*S*)-CPB I pheromone with synthetic plant volatiles consisting of (Z)-3-hexenyl acetate, (+)-linalool, and methyl salicylate were investigated in a trap crop strategy in potatoes, but failed to reduce CPB numbers in untreated middle rows of potatoes. Combinations of the (*S*)-CPB I pheromone with synthetic plant volatiles were also used in a novel CPB trap designed to catch colonizing adults in the field. Although the traps caught CPB adults, no differences were observed in traps baited with and without the attractant.

Metaflumizone, a novel semicarbazone insecticide, was recently shown to be highly efficacious on CPB. Laboratory studies found the combination of metaflumizone and a low concentration (0.39 ppm) of the pyrethroid esfenvalerate was slightly synergistic on CPB adults and early (1st-2nd) instar larvae. Field trials combining a low rate of esfenvalerate and metaflumizone at one tenth the field rate controlled beetles as well as the full rate of metaflumizone.

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Introduction

The Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae), is a major pest of solanaceous crops in the United States and Europe, where adults and larvae can cause complete defoliation of plants and subsequent crop loss. Although potato (*Solanum tuberosum* L.) has been reported as the preferred host plant of CPB, eggplant (*Solanum melongena* L.) grown in Virginia, attracts great numbers of CPB as well. Identification of the host preferences of CPB has the potential to play a role in the development of alternative control methods such as trap cropping.

Although most growers in the U.S. currently control CPB effectively with neonicotinoid insecticides such as imidacloprid and thiamethoxam, there have been several accounts of resistance development to these insecticides in isolated field populations of CPB throughout the northeastern states. Metaflumizone, an experimental insecticide with a novel mode of action, has high toxicity to CPB and could be used in insecticide resistance management.

Recently, a male-produced aggregation pheromone was identified for CPB, (*S*)-3, 7-dimethyl-2-oxo-oct-6-ene-1, 3-diol [(*S*)-CPB I]. Attractive synthetic kairomone blends based on potato volatiles were also derived for CPB, and the combination of the aggregation pheromone and kairomones is more attractive than either one alone. The potential exists for use of these semiochemicals into IPM approaches for CPB to reduce overall use of insecticides.

Research presented in this dissertation identifies the host preferences of CPB, investigates the use of pheromones and kairomones in an integrated pest management program, and evaluates the use of metaflumizone for control of CPB.

Chapter One

Literature review of *Leptinotarsa decemlineata*

History and significance of *Leptinotarsa decemlineata*

The Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae), is the most important insect pest of potato, *Solanum tuberosum* L., in both the United States and Europe. The beetle was first discovered by Thomas Nuttal in 1811, and later described by Thomas Say in 1824 (Jacques 2005). Say located the beetle in Missouri and Arkansas and placed it in the genus *Doryphora* (Say 1824). While the host species was not mentioned when it was first identified, subsequently, the original host species of CPB was documented as buffalo burr, *S. rostratum* Dunal, and the beetle was native to Mexico (Tower 1906, Lu and Lazell 1996). Potato, which is native to Peru, was not introduced into North America until 1621 when it was brought to Virginia (Harris 1968), and feeding on potato was first documented in the mid 1800's (Gauthier et al. 1981).

It is believed that the beetles' host species arrived in the U.S. when burs from the buffalo burr plant clung to the coats of cattle that were being driven from Mexico to Texas for sale (Lu and Lazell 1996). The cultivated potato was brought into the Mississippi Valley between 1845-1850 by settlers, and CPB quickly made the transition to potato as a food source (Towers 1906). This consequently resulted in the loss of a previously existing barrier to further eastward dispersion by the beetle (Tower 1906). By 1874, the beetle had spread to the East Coast of the U.S. (Gauthier et al. 1981). CPB was not identified as a pest of potatoes until 1865, when infestations were reported from Colorado and Nebraska into Iowa within five years (Walsh 1865). Farmers reported that the beetles were completely defoliating potato crops, and up to two bushels of the beetles could be collected from plants in a very short period of time (Walsh 1865). It was estimated that CPB was moving eastward at the rate of 97 km per year (Walsh 1865). In 1866, damage by CPB was estimated to cost consumers $1.75 million due to an increase in potato from $0.50/bu to $0.75/bu (Walsh 1866). Walsh (1866) further states that "We shall always have the Colorado bug in smaller or larger numbers among us, I have no more doubt, than that we shall always have more or less thunderstorms every year in the Valley of the Mississippi". One hundred and fifty years later, we are still in need of new methods to manage the beetle.

Early control methods for CPB consisted of hand picking beetles from plants, and using turkeys to feed on them (Walsh 1865). There was also an emphasis on the conservation of natural enemies such as predatory stink bugs and ladybird beetles (Walsh 1865). In 1872, Paris Green (lead arsenate) was recommended for control of CPB (Riley 1871), and arsenical insecticides continued to be used until the late 1940's (Gauthier et al. 1981). However, as early as 1912, CPB developed resistance to Paris Green (Gauthier et al. 1981). In the 1950's, growers switched to DDT for CPB control; however, by the mid-1950's, CPB showed resistance to this pesticide (Gauthier et al. 1981). Subsequently, CPB has developed resistance to many other insecticides (Whalon et al. 2007).

CPB is the major defoliator of potato throughout most of North America as both adults and larvae feed on foliage (Hare 1990). First instar larvae account for 3% of total leaf consumption, with second, third and fourth instars accounting for 5%, 15%, and 77% leaf consumption, respectively (Capinera 2001). For adults, leaf consumption occurs at a rate of 7-10 sq cm per day, while larval leaf consumption averages 35-45 sq cm per day (Capinera 2001). Uncontrolled populations can completely defoliate potato; when defoliation occurs early in the plant growth cycle, tuber production may be lost (Hare 1980). Potato plants just beginning to bloom and to form and fill tubers are the most susceptible to CPB damage (Shields and Wyman 1984, Zehnder and Evanylo 1989, Hare 1990, Zehnder et al. 1995). In tomatoes, *Lycopersicon esculentum* L., feeding by 1st generation CPB larvae substantially lowers yield (Schalk and Stoner 1979). Progeny of a simulated beetle density level of 0.002 females per plant were also found to cause yield losses in indeterminate cultivar tomatoes (Cantelo and Cantwell 1983). In eggplant, newly hatched 1st generation larvae temporarily disrupted flower production and temporarily reduced yield; however, following removal of 2nd generation larvae, the plants compensated for early losses (Cotty and Lashomb 1982). It is estimated that CPB causes hundreds of millions of dollars in crop losses and chemical control expense each year in North America (USDA 2000).

Life History and Biology

CPB is an oligophagous insect that feeds primarily on the Solanaceae (Order: Solanales). The beetle overwinters in the soil as an adult and emerges in early spring. In eastern Virginia, adults become active around the end of April into mid-May (Zehnder 1986). After emergence, beetles disperse by walking and by flight in search of solanaceous host plants (Voss and Ferro 1990). Since CPB do not usually fly until temperatures above 27˚C are reached, they will typically invade fields by walking (Weber and Ferro 1994). Females feed for 5-10 days before laying clusters of 20-60 eggs on the undersides of leaves (Capinera 2001). Laboratory studies show a mean fecundity of 3,348 eggs per female (Brown et al. 1980). Eggs hatch in approximately one week and the larvae then feed on plant foliage (Gauthier et al. 1981). Four instars occur over a three-week period. Larvae are reddish with two rows of black spots on either side of their abdomens. The four larval instars can be identified by the width of their head capsule: 0.65, 1.09, 1.67, and 2.5 mm for instars 1-4, respectively (Capinera 2001). Fourth instars burrow into the soil and pupate (Gauthier et al. 1981). Adults emerge from the soil after 1-2 weeks and the cycle begins again. CPB have two generations per year in Virginia, with the second-generation adults overwintering in the soil. If adults do not find food, they enter the soil in mid-summer to overwinter without reproducing (Nault et al. 1997).

A combination of photoperiod, temperature and host quality are responsible for inducing diapause in CPB (Capinera 2001). The beetle is a “long-day” insect and will enter diapause following exposure to short photoperiods (Hare 1990). Beetles vary in their responses to photoperiod with latitude, with southern beetle populations (Arizona and Texas) requiring a shorter photoperiod for diapause induction than northern beetles (Washington and Utah) (Hsiao 1981). The beetle will typically overwinter at a depth between 7.6 and 12.7 cm (Lashomb et al. 1984). CPB are capable of prolonged diapause where adults will remain dormant in the soil for more than one year (Biever and Chauvin 1990). A 10-yr field study that examined the prolonged dormancy found considerable variation in the incidence and duration of prolonged dormancy in natural and artificially selected CPB populations (Tauber and Tauber 2002).

Host Preferences

Personal observations made by Walsh (1865) indicated that CPB had a preference to eggplant over potato and to potato over tomato. This preference was believed to occur because eggplant is botanically more closely related to CPB’s native host species buffalo burr, than potato or tomato, and potato is more closely related than tomato (Walsh 1865). Other early studies found that the preferences of CPB depended upon the plant species the insects were fed on prior to testing (McIndoo 1935), and that the beetle preferred some species over others (Brues 1940). More recently, CPB was found to feed on a variety of native and exotic solanaceous plants (Hsiao and

Fraenkel 1968, Bongers 1970). Although potato, *S. tuberosum,* was documented as the preferred host for CPB (Hsiao and Fraenkel 1968, De Wilde and Hsiao 1981, Matsuda 1988), other solanaceous crops such as eggplant, tomato, and pepper, as well as solanaceous weeds such as nightshades and nettles have been found to serve as suitable hosts (Hsiao and Fraenkel 1968, Bongers 1970, Hare and Kennedy 1986). The acceptability and suitability of 104 plant species for larvae of CPB were tested by placing larvae in Petri dishes with fresh leaves of the various plant species, and then recording the amount of biting and feeding on the leaves after 1, 2, and 24 h (Hsiao and Fraenkel 1968). Of the 104 plant species tested, only 15 supported growth of fourth-instars to pupation (Hsiao and Fraenkel 1968). Oviposition preference experiments were also conducted by placing newly emerged CPB adults in an oviposition cage with potato leaves and leaves of another plant, and then counting the number of egg masses laid on each plant species (Hsiao and Fraenkel 1968). Experiments examined 11 solanaceous and 6 nonsolanaceous plants, and in all but 2 cases (*S. rostratum* and *S. nigrum* L.) potato was the preferred plant for oviposition (Hsiao and Fraenkel 1968). Bongers (1970) studied the suitability of various species of *Solanum* and tomato by rearing beetles on the plants from the egg stage, and found that potato was the best food-plant for development followed by *S. dulcmara* L., *S. carolinense* L., and *S. rostratum*. In oviposition tests, the most eggs were laid on potato followed by *S. dulcamara*, tomato, and *S. lutem* (Bongers 1970). CPB has repeatedly displayed the ability to adjust its host range by accepting locally abundant *Solanum* species (Hare and Kennedy 1986, Horton et al. 1988, Mena-Covarrubias et al. 1996). The native host plants of CPB have been broadly characterized as colonized and weedy species that do well in sunny disturbed areas and produce large numbers of seeds that have been dispersed by humans and animals, and consequently have spread widely over North America (Hsiao 1981).

Host preferences of CPB vary geographically (Hsiao 1978, De Wilde and Hsiao 1981, Hare and Kennedy 1986). For instance, in Michigan, a host choice colonization study was conducted by placing seven potted solanaceous plants perpendicular from the edge of a horsenettle, *S. carolinense* stand (Mena-Covarrubias et al. 1996). Counts of CPB adults and egg masses on these solanaceous plants found that CPB populations restricted to feeding on horsenettle colonized potato and horesenettle over eggplant, nightshade, or tomato plants (Mena-Covarrubias et al. 1996). Although populations of CPB from North Carolina, Virginia, New Jersey and Connecticut differed in their ability to survive on the wild host *S. carolinense*, they did not differ in their ability to survive on potato (Hare and Kennedy 1986). Populations of CPB from Mexico and Arizona, which are associated with the host plants *S. elaeagnifolium* Cavanilles and *S. angustifolium* Mill, rarely feed or oviposit on potato (Hsiao 1978, Hare 1990). However, behavioral recordings and leaf consumptions studies indicate geographic populations of CPB adapted to different local plants have not lost their preference for feeding on their ancestral host species, *S. rostratum* (Harrison 1987). It is believed that host shifts evolve through selection for feeding in generalists in isolated populations, and do not require genetic or physiological changes in the host species (Harrison 1987, Horton et al. 1988). Moreover, survival rates are higher for populations that are reared on locally abundant and annually predictable host species present in their communities (Horton et al. 1988). While adult survivorship on potato, eggplant and tomato was not affected by the food plants, fecundity was greatest on potato followed by tomato then eggplant (Jansson et al. 1989). However, observations in Virginia revealed that significantly more CPB adults occur on eggplant than on potatoes or potatoes bordered by eggplant (T.P.K., unpublished data). It is feasible that CPB host preference could potentially be used in a trap crop pest management strategy.

Pest Management

Cultural Control Some of the earliest tactics developed to control CPB included releasing turkeys into potato fields and use of a horse drawn machine that knocked CPB into a box (Walsh 1866). Currently, crop rotation is the most important cultural practice for control of CPB. This practice is effective because a substantial number of diapausing CPB adults overwinter in fields where potatoes were grown the previous year (Voss and Ferro 1990). CPB populations are much lower in rotated potato fields than in non-rotated fields (Speese and Sterrett 1998). An important key to successful crop rotation is distance. Hough-Goldstein and Whalen (1996) demonstrated that fields that were > 0.4 km from previous potato fields had lower CPB densities than fields that were adjacent to fields planted with potatoes the previous year. Although there was a reduction in densities of CPB, chemical control was still necessary to keep populations below damaging levels.

Chemical control CPB has had a major impact on the development of insecticides and spray equipment. Powder guns such as the Leggett powder gun came into existence to specifically kill CPB in potato fields (Gauthier et al. 1981). Early field trials were also conducted at the Virginia Truck Experiment Station (now the Eastern Shore Agricultural Research and Extension Center) with traction sprayers in an attempt to control CPB populations (Sanderson 1912). The first arsenical insecticide, Paris Green, was initially used to control CPB (Gauthier et al. 1981, Casagrande 1987). DDT became an important control for the beetle, but by the 1950's, CPB showed resistance to it (Gauthier et al. 1981). Since the loss of effectiveness of DDT in the 1950's, potato growers have had to change insecticides every few years because of resistance problems (Forgash 1985). As of the early 1990's, CPB had developed resistance to over 25 insecticides (Roush et al. 1990) in five major insecticide classes (Forgash 1985, Harris and Turnball 1986, Tisler and Zehnder 1990, French et al. 1992). Since then, CPB has developed resistance to at least 49 different active ingredients and all major insecticide classes (Whalon et al. 2007).

Since its registration in 1995, imidacloprid (Admire 2F, Bayer Crop Science), a contact and systemic neonicotinoid insecticide, has been widely used throughout the United States to control CPB. Studies in Michigan indicate that control costs plus yield losses caused by the beetle dropped by more than half following the release of imidacloprid (Grafius 1997, Michigan Potato Industry Commision 1995, 1996). Since then, a few other neonicotinoids have been developed and registered for control of CPB (ie., thiamethoxam, acetamiprid, and dinotefuran). Neonicotinoids act by targeting the nicotinic acetylcholine receptors (nAChRs) in the insect central nervous system (Bai et al. 1991, MingYie and Casida 1993). Imidacloprid has been the most widely used neonicotinoid for control of CPB in potatoes. The insecticide is typically applied as an in-furrow treatment to the whole field at planting, which places heavy selection pressure on CPB populations. Albeit neonicotinoids are still highly effective in most areas, resistance to imidacloprid has been reported in New York, Delaware, Michigan, southern Maine and Canada (Grafius and Bishop 1996, Mota-Sanchez et al. 2000, Olson et al. 2000, Zhao et al. 2000, Tolman et al. 2005, Alyokhin et al. 2006, Mota-Sanchez et al. 2006). Although other registered neonicotinoids besides imidacloprid are also currently being used to control the beetle, imidacloprid-resistant beetles have shown cross-resistance to these neonicotinoids, suggesting that rotation of imidacloprid with other neonicotinoids is not an effective option for growers (Mota-Sanchez et al. 2006, Alyokhin et al. 2007). A key component of any resistance management plan is the rotation of insecticides with different modes of action. In 1999, spinosad, which is a mixture of the bacterial fermentation products spinosyns A and D, was introduced for use against CPB. Spinosad also works on nicotinic acetylcholine receptors in insects; however, it is believed that it acts on an unidentified allosteric site that differs from the neonicotinoid binding site (Salgado 1998, Thompson et al. 2000). Due to its different mode of action, spinosad has been a popular choice for potato growers as a "rescue" foliar application following neonicotionid in-furrow treatments at planting. However, low levels of resistance were recently found to spinosad in an imidacloprid-resistant strain of CPB (Mota-Sanchez et al. 2006).

Populations of CPB in Virginia remain highly susceptible to imidacloprid treatment (T. P. Kuhar and Galen Dively, personal communication). The environmental implications and potential for resistance with such intensive insecticide dependence demands exploration of alternative management tactics.

Chemical Ecology

The use of plant attractants and chemical signals may provide an alternative management strategy for CPB. One of the earliest studies investigating insect attraction to plant volatiles was conducted by McIndoo (1926) and showed that CPB are attracted to undamaged potato plants. In wind tunnel studies, adult male and female beetles walk upwind in response to potato plant volatiles (Visser 1976). Later studies demonstrated CPB attraction to both artificially damaged and insect-damaged potato plants (Bolter et al. 1997, Landolt et al. 1999). Bolter et al. (1997) showed that potato plants that were mechanically wounded or damaged by CPB larvae were more attractive than undamaged potato plants. In their study, the green leaf volatile derivatives (*Z*)-3-hexenol and (*Z*)-3-hexenyl butyrate, and the sesquiterpenes β-caryophyllene and β-selenine were some of the many compounds emitted by potato plants being fed upon by CPB larvae (Bolter et al. 1997). Landolt et al. (1999) demonstrated that unmated adult female CPB moved upwind to potato plants damaged by CPB larvae or potato plants treated with regurgitant from CPB larvae or cabbage looper larvae (*Trichoplusia ni* Hübner). Coupled gas chromatography/electroantennographic detector recordings showed CPB antennae detected 18 compounds from damaged potato plants (Schütz et al. 1997). Some of these compounds were strongly attractive to CPB at concentrations corresponding to damaged plants 12h after damaged (Schütz et al. 1997). In addition, adults were able to perceive some of these volatiles in the field at a distance of 10-50 m from an odor source (Schütz et al. 1997). In addition to potato plants, studies have also demonstrated the attraction of CPB to several other solanaceous species volatiles (DeWilde et al. 1969, Visser and Nielsen 1977).

Recently, Dickens (1999, 2000, 2002) identified *S. tuberosum* (‘Kennebec’) volatiles that are attractive to larval and adult CPB. The most attractive blend of volatiles were (*Z*)-3-hexenyl acetate, (±)-linalool, and methyl salicylate (Dickens 2000, 2002). Laboratory and greenhouse studies utilizing potato plants baited with the synthetic blend of host volatiles found that attractant-baited plants were more attractive then unbaited potato plants (Martel et al. 2005a). Field studies investigating use of these synthetic host volatiles in a trap crop found more postdiapause, colonizing adults, egg masses, and small larvae in attractant-baited trap crops than in unbaited trap crops (Martel et al. 2005b). Field studies have also indicated that small CPB larvae were controlled when synthetic host plant volatiles were combined with a pyrethroid insecticide (Martel et al. 2007).

The presence of a sex pheromone for CPB has been a controversial issue. DeWilde et al. (1969) initially reported that female odor in wind tunnels attracted male beetles. Levinson et al. (1979) showed that in a binary-choice arena, male CPB showed a preference to a wire screen arena that was situated 8 mm above a piece of filter paper treated with an n-pentane extract of female elytra, compared to a section above male elytral extract. In studies conducted by Jermy and Butt (1991), males did not perceive females kept in small wire cages; however, an ethanol extract of CPB female elytra did elicit normal mating behavior in male CPB when applied to glass rods in amounts as low as 0.3 female equivalents. It was later reported that female sex pheromones attracted males from a distance of at least 50 cm although the percentage of males responding was low (Edwards and Seabrook 1997). In contrast to these previous studies, a male produced aggregation pheromone for the CPB was recently identified as (*S*)-3, 7-dimethyl-2-oxo-oct-6-ene-1, 3-diol [(*S*)-CPB I] (Dickens et al. 2002). The male aggregation pheromone has been found to be attractive to both male and female adult CPB (Dickens et al. 2002). Field studies utilizing the synthetic aggregation pheromone in a trap crop management system demonstrated its potential for use in control of CPB (Kuhar et al. 2006). Furthermore, field and laboratory bioassays showed that adult CPB prefer the mixture of plant attractants and pheromone over either alone (Dickens 2006). Fourth instar larvae orient to the plant attractant alone (Dickens 2002), or the blend of pheromone and plant attractants in the lab (Hammock et al. 2007). The potential use of this pheromone for pest management purposes should be explored.

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Chapter Two
Host plant preference in Colorado potato beetle

The Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae), is considered one of the most destructive pests of potatoes (*Solanum tuberosum* L.) in North America, Europe and Asia, and may feed on other solanaceous plants including eggplant (*S. melongena* L.) and tomato (*Lycopersicon esculentum* L.) (Hare 1990, Jacques 2005). CPB has developed resistance to numerous conventional insecticides (Forgash 1985, Hare 1990, Kennedy and French 1994, Stewart et al. 1997, Olson et al. 2004, Mota-Sanchez et al. 2006). While the development and registration of new insecticides has been the traditional strategy to combat this important pest (Casagrande 1987), more sustainable approaches that utilize reduced amounts of insecticides, biorational control treatments (e.g. attractants and/or repellents), or non-chemical tactics for controlling CPB are needed.

Understanding host plant preferences of CPB may provide information for the development of alternative control strategies, such as trap cropping (Hunt and Whitfield 1996). CPB probably originated in South America, where it fed primarily on the wild host, buffalo burr, *S. rostratum* Dunal (Walsh 1865). While CPB feeds on a wide variety of native and exotic solanaceous plants (Brues 1940, Hsiao and Fraenkel 1968, Jacques 1988, Hare 1990), feeding and oviposition preferences for one solanaceous species over another have been noted. Walsh (1865) suspected that CPB preferred eggplant over potato and potato over tomato based on the botanical similarity of these host plants to their wild host buffalo burr. In Massachusetts, CPB thrived on buffalo burr, eggplant, and potato, but were unable to survive for more than two seasons on other potential host plants (*S. subinerme* Jacquin., *S. marginatum* L., *S. dulcamara* L., *S. torvum* Swartz, *S. barbisetum* Nees, and *L. esculentum*). Several studies have identified potato as the preferred host for CPB (Hsiao and Fraenkel 1968, De Wilde and Hsiao 1981, Matsuda 1988, Hare 1990); however, other solanaceous crops such as eggplant, tomato and pepper, *Capsicum annum* L., as well as solanaceous weeds such as nightshades and nettles can also serve as suitable hosts (Hsiao and Fraenkel 1968, Hare 1990). CPB has repeatedly displayed the ability to expand its host range by accepting locally abundant *Solanum* species (Hare and Kennedy 1986, Horton et al. 1988, Mena-Covarrubias et al. 1996). Host preferences among geographically separated allopatric populations of CPB varies (Hsiao 1978, De Wilde and Hsiao 1981, Hare and Kennedy 1986). While populations of CPB from

North Carolina, Virginia, New Jersey and Connecticut differed in their ability to survive on the wild host *Solanum carolinense* L., they did not differ in their ability to survive on potato (Hare and Kennedy 1986). Populations of CPB from Mexico and Arizona which are associated with the host plants *S. elaeagnifolium* Cavanilles and *S. angustifolium* Mill rarely feed or oviposit on potato (Hsiao 1978, Hare 1990).

In order to better understand host preferences of native CPB and factors which may contribute to these preferences, I conducted field- and laboratory-choice experiments utilizing solanaceous crops available to local CPB populations in the field (potato, eggplant, tomato, and pepper). I also sought to determine if the presence of colonizing male CPB feeding on eggplant or potato affected the number of new CPB adults coming to those plants in the field.

Materials and Methods

Insects and Host plant. Laboratory host-plant choice experiments were conducted at the Virginia Tech Eastern Shore Agricultural Research and Extension Center (ESAREC) in Painter, VA (USA) using a field population of CPB collected from potato plants.

The study initially included potato (‘Superior’), eggplant (‘Black Beauty’), tomato (‘Florida 47’), and bell pepper (‘Paladin’). However, bell pepper was removed from the study as local populations of CPB rarely fed upon or developed on this host plant.

CPB adults collected within 1-2 days of emergence from the soil were used for the study. Beetles were collected from untreated potato plants in the field, separated by sex and then placed individually in Petri dishes with a moistened cotton ball. Eggplant foliage and tomato foliage were obtained from 10-12 wk old plants potted in Pro-mix potting soil/vermiculite mix (~4 liter pots), whereas potato foliage was obtained from field-grown potato plants. Plants ranged in height from 25 – 35 cm. Single-stems weighing approximately 4.8 g, were removed from the plants and mechanically injured by making approximately 1-cm slits around the perimeter of the leaf (Dickens 1999).

Olfactory laboratory comparison. An open Y-track olfactometer (“Flying T”) apparatus modified after Visser and Piron (1998) and described in detail by Dickens (1999) was used for laboratory choice experiments. Hydrocarbon-free air, supplied at the rate of 1 L/min, was humidified by passing it through distilled water prior to delivery of the plant volatiles to the apparatus. Beetles were considered to have made a choice after traveling 1 cm up either arm of the "Flying T". Foliage was replenished after 30 min.

Experiments were conducted in a darkened room at 24°C where the only light source was from the bioassay apparatus. For each assay, 20 adult males and 20 adult females were tested. Beetles were starved for 24 h then dark-adapted for 1 h prior to testing. The following tests were conducted: potato vs. tomato; potato vs. eggplant; and eggplant vs. tomato. This experiment was conducted three times, once in 2006 and twice in 2007. Data were analyzed by testing the hypothesis that the binomial proportion was significantly different from a probability of 50% using the standard normal approximation (Ott and Longnecker 2001).

Field Comparisons: tomato versus eggplant. Field comparisons were conducted during the summer of 2005 in Painter, VA. The multiple-crop choice study utilized eggplant ('Black Beauty'), tomato ('Florida 47'), and pepper ('Paladin') as potential host plants. However, since bell pepper plants tested in the study had significantly fewer CPB adults, larvae, and egg masses than eggplants or tomatoes, data collected for bell pepper were omitted.

The three crops were transplanted in a single row and each crop was replicated four times. Each row consisted of a 6-m section of eggplant, 6-m section of tomato, and a 6-m section of pepper for a total row length of 18 m. The order was randomized for each replicate. There were three treatments: 1) all plants were treated prior to transplanting with a drench of imidacloprid (255.5 g[AI]/ha); 2) eggplants only were treated with the imidacloprid drench prior to transplanting; and 3) all plants were untreated. Numbers of CPB dead adults within 30 cm of the row, live adults, larvae, and egg masses on each crop in each block were counted twice a week for approximately one month (total of 7 sampling events). All data were transformed using a square root (x + 0.05) transformation prior to analysis. Data were analyzed using ANOVA, and mean comparisons were separated using Fisher's LSD at the $P \leq 0.05$ significance level (Analytical Software 1998). Untransformed data were used in all figures.

Field Comparisons: potato versus eggplant. Field comparisons were conducted during the summer of 2006 and 2007 in Painter, VA. In 2006, the field comparisons were conducted once, while in 2007 the field comparisons were conducted twice using two separate areas of the research farm. Individual plots consisted of two 1.5-m long rows separated by 0.91-m. One row contained 5 containerized eggplants, while the other row contained 5 potato plants. Fifteen-cm containerized potted eggplants were buried in the soil at the start of the study with the rim of the container even with the soil surface. Potatoes were planted in the field in March following standard cultivation and production practices for Virginia (Kuhar et al. 2006). In the first experiment, I counted the number of live CPB adults on paired rows of untreated potato and eggplant. In the second experiment, I counted the number of dead CPB adults found on or at the base of plants in paired rows of potato and eggplant treated with imidacloprid (255.5 g[AI]/ha). Both treatments were replicated four times in each experiment.

In 2006, potato plants were carefully pruned to approximately match the leaf area in the eggplants 24 h prior to the start of the study. Plants were pruned by removing one to two stems from each potato plant. I understand that injury to potato plants as a result of pruning might change the volatile profile emitted by the plant; however, since pruning was done 24 h prior to testing, and observations were made over a period of one month, any prolonged effects on the attractiveness of the plants due to pruning are likely to have been minimal (personnel communication, J.C. Dickens). However, to the extent this is true I cannot be certain. In 2006, CPB numbers (including dead adults and live adults) in each block were counted every 2-3 days for approximately two weeks (total of 4 sampling events). Both beetles and egg masses were removed from the plants at the end of every sampling event to avoid recounting.

Eggplants used for the 2007 study matched the leaf area of the potato plants, and were therefore not pruned. Since CPB populations were low in 2007, beetles collected from nearby potato fields were released around each block on 13 and 20 June. For each release, 40 beetles were placed around the perimeter of each block (10 beetles to each side of the block). CPB numbers (including dead adults and live adults) in each block were counted every 2-3 days for approximately two weeks (total of 4 sampling events). For each sample date, beetles and egg masses were removed from the plants to avoid recounting.

For all field comparisons, beetle numbers were compared using a paired *t*-test at the $P \leq 0.05$ significance level. All data were transformed using a square root (x + 0.05) transformation prior to analysis. Untransformed data are presented in the results.

Effect of colonizing male CPB adults feeding on eggplant and potato. 'Superior' potatoes were planted in March of 2006 and 2007 in Painter, VA. Potted eggplants at the 6-leaf stage were obtained (Hampton Roads Agricultural Research and Extension Center, Virginia Beach, VA) in April and May for use in the experiment. The study was initiated on 28 April 2006 and 7 May 2007 immediately after CPB emerged from overwintering. Six treatments were arranged in a randomized complete block design with 6 replicates. Replicates were separated from one another by placing them within alleyways of 6-row blocks of potatoes. There were 3.7 m of bare ground and a 6-row block of potatoes separating the replicates while individual test plants within a replicate were separated by 9.1 m from one another.

Test plants were either eggplant or potato treated with imidacloprid drench (imidacloprid 255.5 g[AI]/ha) and were approximately 25-30 cm tall at initiation of the experiment. Test plants were either paired with an imidacloprid treated eggplant or potato plant that was covered by a mesh bag that contained two newly-emerged CPB males that had been collected from untreated potatoes at Painter, VA. There were six treatments which consisted of the following pairs of plants: potato (treated) + potato (CPB); potato (treated) + eggplant (CPB); potato (treated) + potato (no CPB); eggplant (treated) + potato (CPB); eggplant (treated) + eggplant (CPB); and eggplant (treated) + eggplant (no CPB). Numbers of live and dead CPB adults on or within a 30 cm radius of each test plant were recorded at 24, 48, 72, and 96 h. For each evaluation, all beetles found on a test plant were removed to avoid recounting for the following evaluation. Data were analyzed using analysis of variance procedures, and mean comparisons were separated using Fisher's LSD at the $P \leq 0.05$ level of significance (Analytical Software 1998).

Results

Olfactometer laboratory comparison. Results were similar for all three experiments conducted in 2006 and 2007, and were therefore combined for analysis. CPB adults oriented to the odorous blend emitted from mechanically-damaged potato foliage over volatiles of mechanically-damaged eggplant foliage ($P \leq 0.01$). Adults also oriented to the odorous blend emitted from mechanically-damaged potato foliage over volatiles of mechanically-damaged tomato foliage ($P \leq 0.01$). In addition, volatiles arising from mechanically-damaged eggplant foliage were preferred over volatiles from mechanically-damaged tomato foliage ($P \leq 0.01$) (Table 2.1).

Field comparison test 1: Tomato versus eggplant. On untreated plots (no insecticides), more CPB adults ($F = 18.93$; df = 2, 134; $P < 0.0001$), larvae ($F = 13.01$; df = 2, 134; $P < 0.0001$), and egg masses ($F = 12.24$; df = 2, 134; $P < 0.0001$) were noted on eggplants than on tomatoes (Fig. 2.1). In imidacloprid treated plots, more dead CPB adults ($F = 8.56$; df = 2, 136; $P = 0.0004$) were found in eggplant plots than tomato plots. Across all sampling dates, there were 297 dead CPB adults found in eggplant plots compared to 20 dead adults found in tomato plots.

Field comparison test 2: Potato versus eggplant. The mean number of live adults in the untreated experiments for 2006 and 2007 are shown over time in Fig. 2.2. In 2006, more live CPB adults were found on untreated eggplant than on untreated potato for first sample date, 16 May (t = 5.09; df = 3; P = 0.0147) (Table 2.2). There were no significant treatment effects for the number of adults on untreated eggplant and untreated potato for the remainder of the sampling dates for both 2006 and 2007 (Table 2.2).

The mean number of dead adults in the imidacloprid-treated experiments for all sample dates in 2006 and 2007 are shown in Fig. 2.3. For the imidacloprid-treated plants, there were no significant differences in the numbers of dead CPB adults found on treated eggplant and treated potato in 2006 and 2007 for all sample dates (Table 2.2).

Effect of colonizing male CPB adults feeding on eggplant and potato. There was a significant difference in the total number of CPB adults found coming to the test plants over time in 2006 (F = 2.76; df = 5, 25; P = 0.0406) and 2007 (F = 3.12; df = 5,25; P = 0.0252), with the eggplant (treated) + eggplant (CPB) treatment having significantly more beetles than all other treatments (Figs. 2.4A and 2.4B).

Discussion

My findings indicate that CPB adults collected from potato plants in the field prefer mechanically-damaged potato foliage over eggplant foliage in the laboratory. In 2006, it was observed that CPB adults also preferred mechanically-damaged potato foliage over tomato foliage. Although there was no significant difference in the number of CPB adults choosing mechanically-damaged potato foliage over tomato foliage in both 2007 trials, the data follow the same trends observed in 2006 with more beetles choosing damaged potato foliage over tomato foliage. When a choice of mechanically-damaged eggplant and tomato foliage was offered, CPB in 2006 preferred eggplant. In the 2007 trials conducted, adult CPB followed this same trend, despite no significant difference in the data.

Dickens (2000) found that although CPB preferred mechanically-damaged potato foliage to mechanically-damaged soybean foliage, there was no preference when offered mechanically-damaged potato vs tomato foliage. One possibility for the difference in preference between potato and tomato foliage observed in 2006 and the earlier study (Dickens 2000) could be differences in cultivars of potato (*S. tuberosum* 'Superior' in my current study vs. *S. tuberosum* 'Kennebeck' in

Dickens 2000) and tomato (*L. esculentum* 'Florida 47' in my current study vs. *L. esculentum* 'Pik Red' in Dickens 2000) used in the two studies.

Field studies reported here show that counts of live beetles on untreated plants and counts of dead beetles on imidacloprid-treated plants on the Eastern Shore showed a significant preference for eggplant over tomato. When testing the number of live CPB on untreated eggplant and potato, only one sample date showed a significant difference in the number of beetles, with more beetles coming to eggplant. However, in ten out of the twelve sample dates for all three experiments, numerically more live beetles were found on untreated eggplant than on untreated potato. By comparison, for the twelve sample dates on the imidacloprid-treated eggplant and potato, there were four sample dates where more dead CPB were found on potato, five sample dates where more dead CPB were found on eggplant, and three sample dates where there were no differences observed in the numbers of dead beetles found on eggplant or potato. This leads me to believe that CPB do have a preference for untreated eggplant over untreated potato plants. This finding differed from my laboratory olfactometer studies in which mechanically-damaged potato foliage was preferred over both eggplant and tomato foliage. Since plants utilized in both the laboratory and field studies were damaged (either mechanically or by feeding CPB), colonization appears to be affected by another factor.

Geographic variation in host preferences for phytophagous insects may occur for a variety of reasons. These variations may be due to ecological differences such as availability of acceptable food sources (Fox and Morrow 1981, Thompson 1988, Bowers et al. 1992, Funk and Bernays 2001). In some cases preferences may change on a seasonal basis (Rausher 1980). It has been clearly demonstrated that there is considerable variability in host species for geographically separated populations of CPB (Hsiao 1978, De Wilde and Hsiao 1981, Hare and Kennedy 1986). For example, research conducted in Michigan on CPB populations restricted to feeding on horsenettle, *Solanum carolinense*, found that there were significantly more CPB adults colonizing potato and horsenettle than eggplant, nightshade, or tomato plants (Mena-Covarrubias et al. 1996). These geographically distinct populations of beetles that have adapted to different local plants still maintain their preference for feeding on their ancestral host species, *S. rostratum* (Harrison 1987). It appears that these evolutionary changes in host preference for geographic host-adapted populations are unrelated to adaptations of alkaloids in host plants (Harrison and Mitchell 1987). Survival rates are higher for CPB populations that are reared on locally abundant and annually predictable host species present in their communities than on unfamiliar hosts (Horton et al. 1988). Research investigating adult survivorship and fecundity on food plants potato, eggplant, and tomato found that, although adult survivorship was unaffected by the food plants, fecundity was greatest on potato followed in decreasing order by tomato and eggplant (Jansson et al. 1989). More recent research indicates that greater survival and growth of CPB on one host versus another host may be genetically based (Lu et al. 1997). Furthermore, this host plant adaptation may involve unusual genetic mechanisms (Lu et al. 2001). There is evidence that adaptation to plants by CPB may require evolution of both behavioral and post-ingestive physiological characteristics (Forister et al. 2007).

CPB is estimated to have reached the Eastern Shore of Virginia around 1874, where potato was abundantly grown (Gauthier et al., 1981). Although *S. rostratum*'s host range extends to the Eastern Shore of Virginia, it has not been seen in the area of these experiments (E. Hitchner and T. Kuhar, personal observations). Horsenettle is a common weed species on the Eastern Shore; CPB are often found on it late in the season after potato plants have senesced. Potato has always been an important commodity for the Eastern Shore, and CPB has been the major pest of potatoes in this area for the last 130 years. Tomatoes are another important commodity in Virginia, and in some areas, CPB has been identified as causing serious economic loss (Schalk and Stoner 1979, Kennedy et al. 1983). However, CPB are not well adapted to tomato on the Eastern Shore and generally are not an important pest of this crop (VA Crop Profile 2001). These host preference studies support that observation.

One of the earliest studies investigating insect attraction to plant volatiles was conducted by McIndoo (1926) with CPB. That study and various others since have verified that adult CPB are attracted to undamaged potato plants (McIndoo 1926, Schanz, 1953, DeWilde et al. 1969, Visser 1976). Later studies showed CPB attraction to both artificially damaged and insect-damaged potato plants (Bolter et al. 1997, Landolt et al. 1999). CPB is more attracted to the odor from damaged potato plants than the odor from undamaged plants (Bolter et al. 1997, Landolt et al. 1999, Schütz 1997). Recently, Dickens (1999, 2000, 2002) identified a kairomone of *S. tuberosum* ('Kennebec') that is attractive to both larval and adult CPB. This kairomone is composed of (Z)-3-hexenyl acetate, (±)-linalool, and methyl salicylate (Dickens 2000). This three-component plant blend has been tested for its use in managing CPB movement in the field (Martel et al. 2005a, b) and was recently found to effectively reduce small CPB larvae when combined with a pyrethroid (Martel et al. 2007).

Recently a male-produced aggregation pheromone was identified for CPB, and may potentially play a role in host preference selection (Dickens et al. 2002). The pheromone is comprised of a single enantiomer (*S*)-3,7-dimethyl-2-oxo-oct-6-ene-1,3-diol [(*S*)-CPB I] and is attractive to both male and female beetles (Dickens et al. 2002). My studies, in which two newly-emerged male beetles were placed in a mesh bag on either an eggplant or a potato plant and paired with another plant indicate that the aggregation pheromone, may in fact play a role in host preference selection in CPB. The combination of eggplant (imidacloprid-treated) + eggplant (CPB) was significantly more attractive than any other plant combination.

Host plant volatiles affect the response of insects to aggregation pheromones. The combination of host plant volatiles and an aggregation pheromone may evoke a greater response than the individual components alone. For example, there was an increase in catches of the boll weevil, *Anthonomus grandis* Boheman, when traps were baited with both the aggregation pheromone and green leaf volatiles (Dickens 1989, Dickens et al. 1990). In the southern pine beetle, *Dendroctonus frontalis* Zimmerman, the response to the aggregation pheromone, -frontalin, is enhanced by the host monoterpene α-pinene (Renwick and Vite 1969). Dickens (2000, 2006) has investigated attraction of CPB to the combination of the (*S*)-CPB I pheromone and a three-component plant blend based on potato volatiles. Both field and laboratory studies demonstrated that CPB prefer the combination of the pheromone + plant blend over either attractant alone (Dickens 2006). Although the attraction of CPB to potato volatiles has been clearly demonstrated, data on eggplant volatiles and CPB attraction have not been reported. My field test results indicate that CPB prefer eggplant to potato plants; however, when the plants were treated with a systemic insecticide, there was no difference in numbers of dead beetles observed in the area of either host plant. This explains why CPB preferred potato to eggplant foliage in the lab, but not in the field. It appears the beetles were able to feed on untreated eggplants and potatoes, and likely released their aggregation pheromone while feeding. Insect mortality on imidacloprid-treated plants made pheromone release unlikely. The effect of newly-emerged male CPB feeding on host plants further supports this observation with a clear preference for the eggplant (treated) paired with eggplant (CPB) combination, indicating that host plant preference in the Colorado potato beetle involves both plant volatiles and a male-produced aggregation pheromone.

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Table 2.1. Behavioral response of CPB adults to volatiles emitted by mechanically-damaged foliage from solanaceous plants in a laboratory olfactometer for three combined experiments: 2006 and 2007, Painter, VA. Asterisks indicate that responses differ for paired treatments (*P* < 0.01) by testing the hypothesis that the binomial proportion is significantly different from P = 50% using the standard normal approximation (Ott and Longnecker 2001).

	Mechanically damaged plants		
Orientation of *L. decemlineata*	**A. Potato vs. B. Tomato**	**A. Potato vs. B. Eggplant**	**A. Eggplant vs. B. Tomato**
Combined experiments 2006 & 2007	76:44	80:40	76:44
% response to A	63.3*	66.7*	63.3*

Table 2.2. Paired t-test results for live and dead CPB adults for all sample dates on untreated eggplant and potato and imidacloprid treated eggplant and potato; Painter,VA 2006 and 2007.

Sample Date	**Experiment 1 Live Adults**			**Experiment 2 Dead Adults**		
	Untreated eggplant and potato			Imidacloprid treated eggplant and potato		
	t	df	*P*	*t*	df	*P*
16 May 2006	5.09	3	0.0147*	1.23	3	0.3071
19 May 2006	1.93	3	0.1487	0.54	3	0.6264
22 May 2006	2.76	3	0.0703	1.59	3	0.2109
26 May 2006	0.21	3	0.8454	1.12	3	0.3455
15 June 2007a	1.96	3	0.1449	1.33	3	0.2579
15 June 2007b	0.49	3	0.6578	0.11	3	0.9196
19 June 2007a	1.06	3	0.3660	0.21	3	0.8480
19 June 2007b	0.58	3	0.6013	0.72	3	0.5223
22 June 2007a	1.71	3	0.1862	0.96	3	0.4058
22 June 2007b	1.88	3	0.1566	0.13	3	0.9023
26 June 2007a	0.32	3	0.7704	0.33	3	0.7654
26 June 2007b	1.96	3	0.1449	0.43	3	0.6971

* Statistically significant at α = 0.05

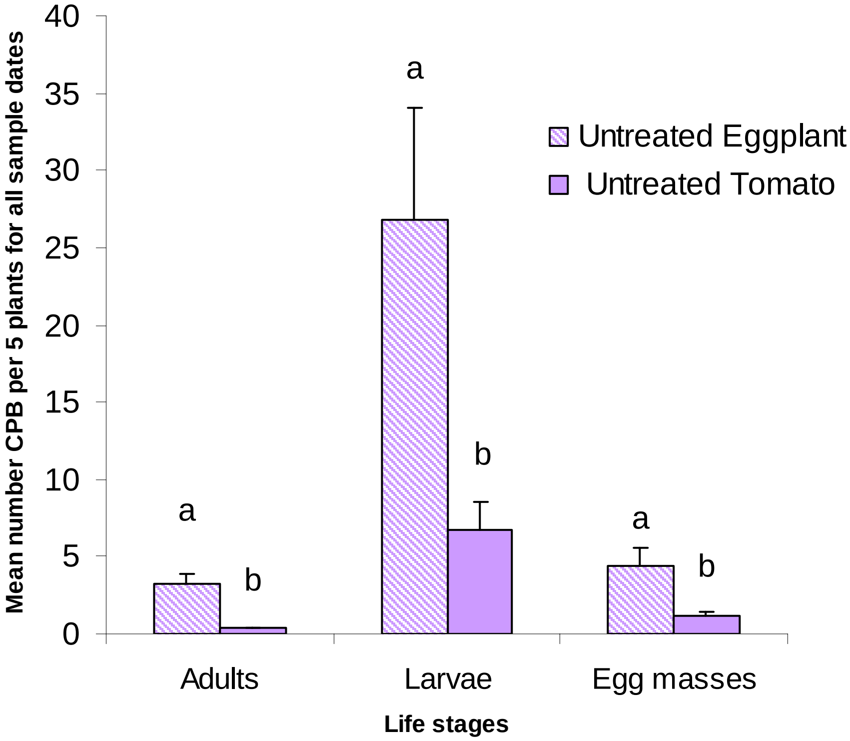
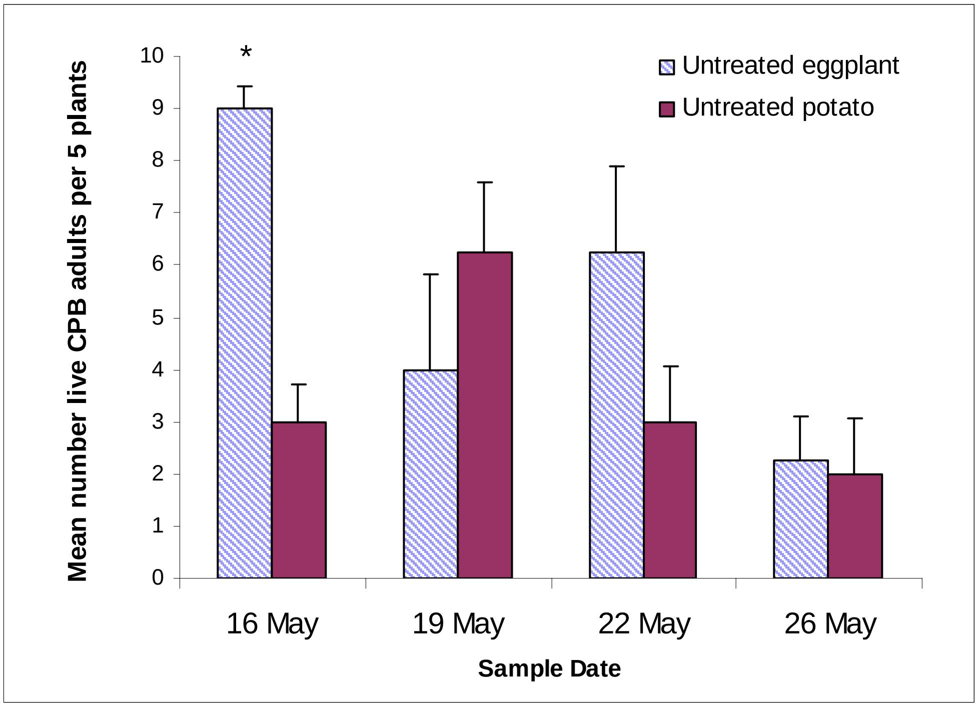
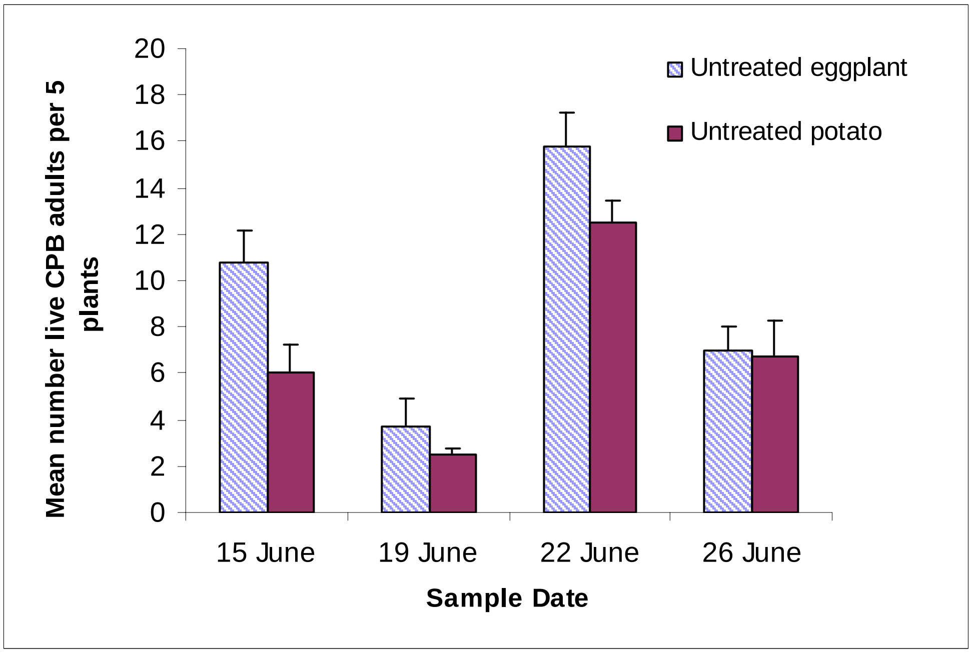


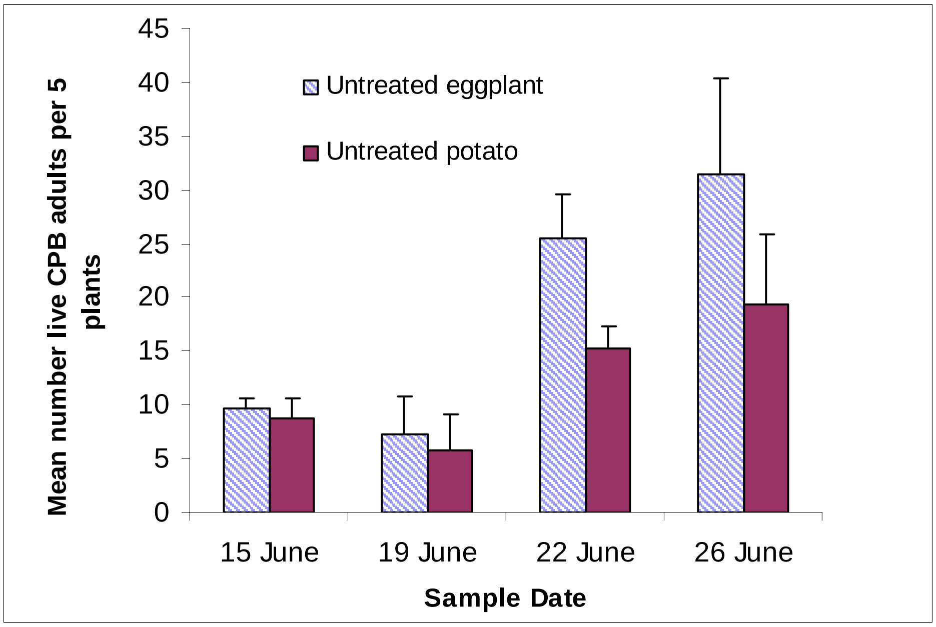
Fig. 2.1. Number of live Colorado potato beetle adults, larvae and egg masses (Mean ± SE per five plants) on untreated eggplant and tomato across seven sample dates; Painter, VA, 2005. Data were analyzed by ANOVA and differences between means were determined with Fisher's LSD ($\alpha = 0.05$). Bars surmounted by different letters are significantly different.



(A) 2006

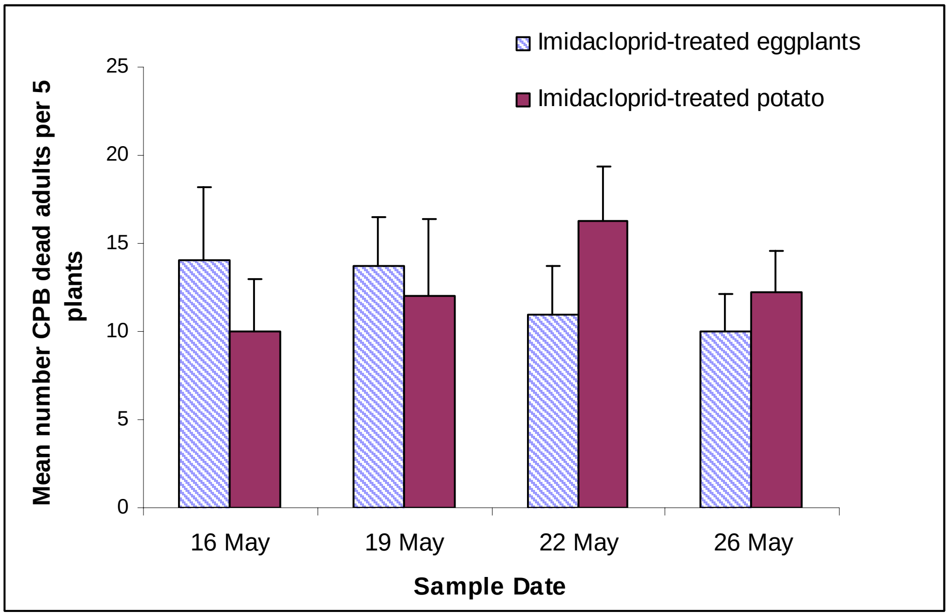


(B) 2007a

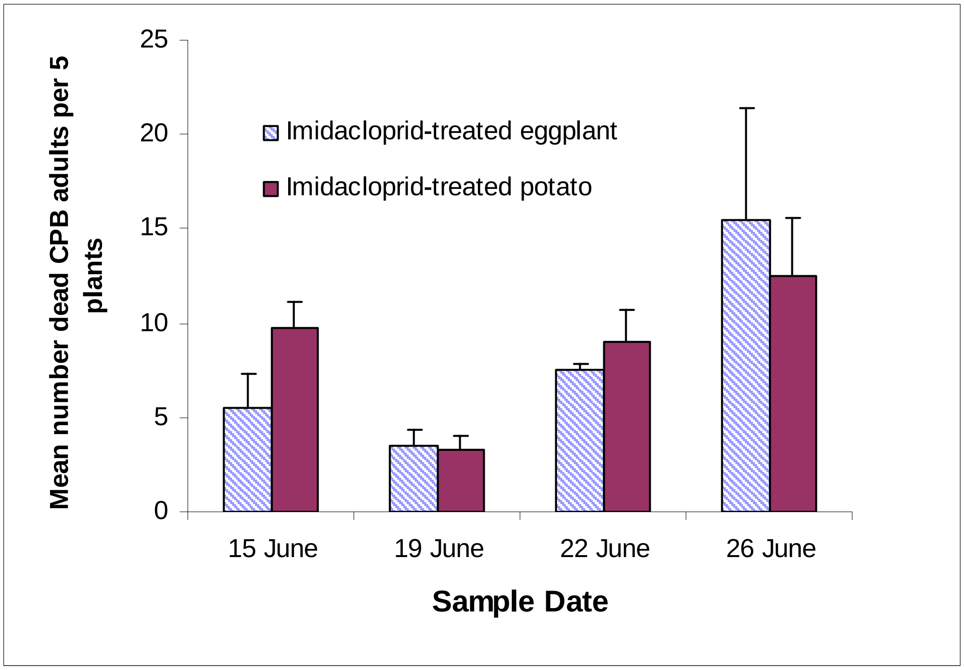


(C) 2007b

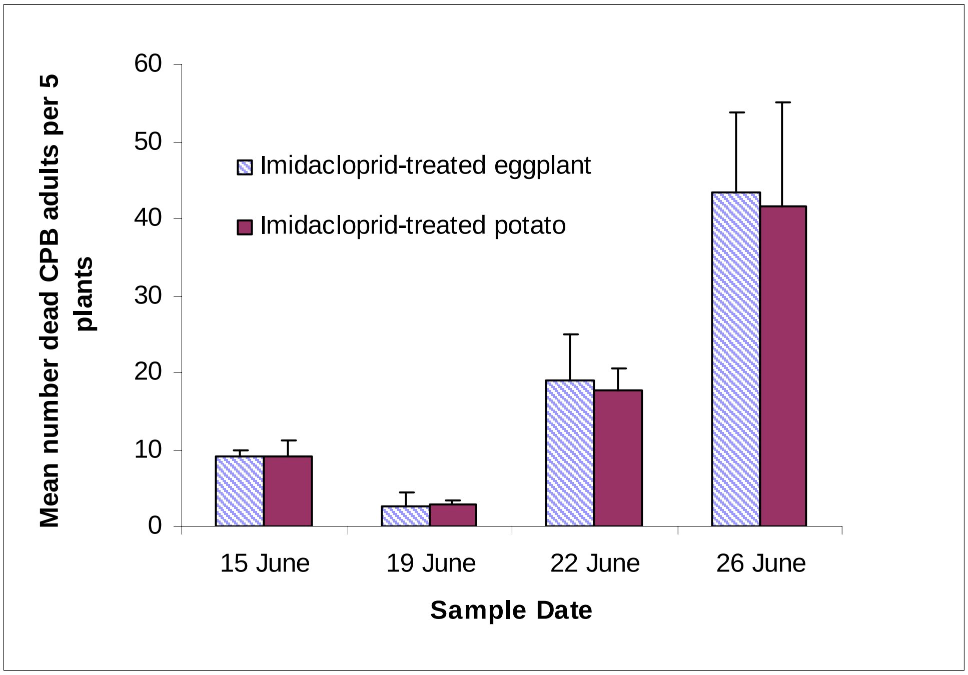
Fig. 2.2. Number of live CPB adults (Mean ± SE per five plants) found on untreated eggplant and potato paired plots for four sample dates in 2006 (A), 2007a (B) and 2007b (C) in Painter, VA. Live beetle numbers were compared using a paired *t*-test at the $P \leq 0.05$ significance level. Asterisks indicate a significant difference in the number of adults coming to a host plant ($P \leq 0.05$).



(A) 2006

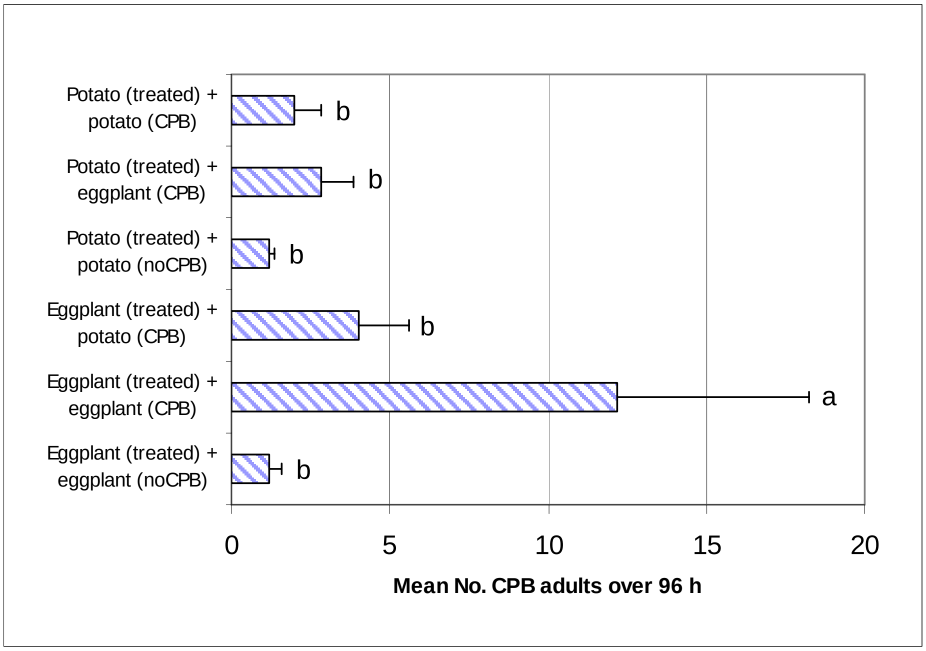


(B) 2007a

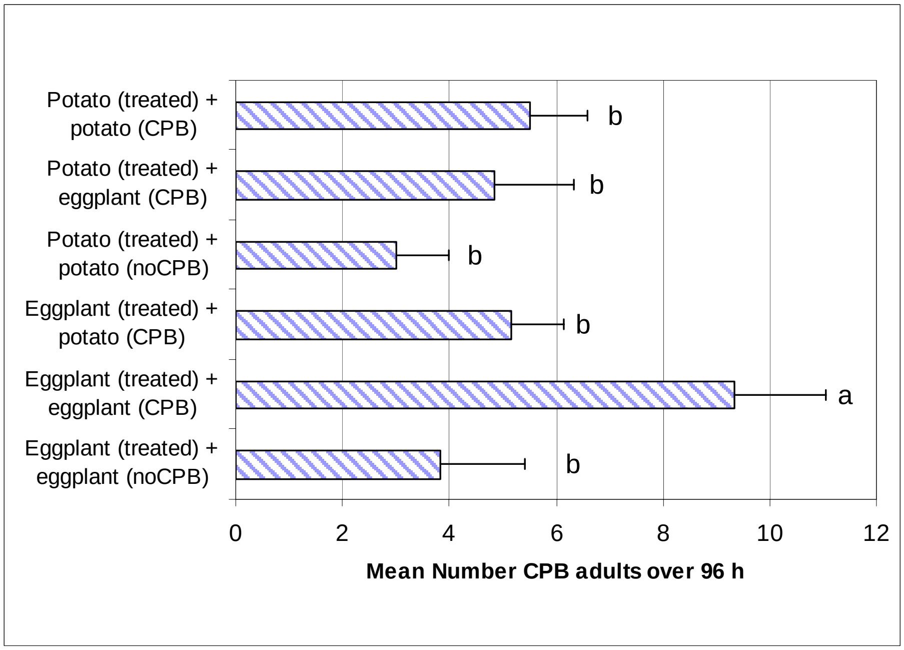


(C) 2007b

Fig. 2.3. Number of dead CPB adults (Mean ± SE per five plants) on imidacloprid-treated eggplant and paired plots for four sample dates in 2006 (A), 2007a (B) and 2007b (C) in Painter, VA. Dead beetle numbers were compared using a paired *t*-test at the $P \leq 0.05$ significance level.



(A) 2006



(B) 2007

Fig. 2.4. Mean ± SE cumulative numbers of CPB adults coming to combinations of paired eggplant and potato plants (CPB = 2 males per plant) over 96 h in 2006 (A) and 2007 (B) in Painter, VA. Data were analyzed by ANOVA and differences between means were determined with Fisher's LSD ($\alpha = 0.05$). Bars surmounted by different letters are significantly different.

Chapter Three

Field response of Colorado potato beetle to the (*R*)-enantiomer of the male-produced aggregation pheromone CPB I and determination of optimal blends of the (*S*)- and (*R*)-enantiomers of the pheromone

Recently, a male aggregation pheromone for the Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae), was identified as (*S*)-3, 7-dimethyl-2-oxo-oct-6-ene-1, 3-diol [(*S*)-CPB I] (Dickens et al. 2002). (*S*)-CPB I pheromone is only produced by male beetles (Dickens et al. 2002). It is attractive to both CPB larvae (Hammock et al. 2007) and adults (Dickens et al. 2002). Field studies utilizing the synthetic (*S*)-CPB I aggregation pheromone in a trap crop demonstrated its attractiveness in the field as well as its potential for integrated pest management of CPB (Kuhar et al. 2006).

Although electroantennograms from Colorado potato beetles were significantly greater for the (*S*)-enantiomer compared to the (*R*)-enantiomer, an intermediate response was observed for equal amounts of the racemate (Dickens et al. 2002). Behavioral responses in an open Y-track olfactometer, however, found that while both male and female beetles oriented to the (*S*)-enantiomer of the CPB I pheromone, they had no preference for the (*R*)-enantiomer or racemate blend of the enantiomer (Dickens et al. 2002). It is possible that a mixture containing predominately the (*S*)-enantiomer with trace amounts of the inactive or inhibitory (*R*)-enantiomer might be active in the field. Such a blend of enantiomers might decrease the cost of synthesis while still providing a useful attractant for large scale field use. Therefore, the goals of this study were to determine effects of (*R*)-CPB I on field populations of CPB and to identify the activity of various blends of the optimal isomer of the aggregation pheromone.

Materials & Methods

Effect of (R)-CPB I under field conditions. A randomized complete block experiment was conducted in potatoes to assess the effect of (*R*)-CPB I on CPB behavior. Spatially-isolated blocks of ‘Superior’ potatoes were planted in March of 2006 at the Virginia Tech Eastern Shore Agricultural Research & Extension Center in Painter, VA. Each plot comprised eight 7.6 m rows planted on 91 cm row centers, with potatoes spaced 30 cm apart within the row. Treatments included the following: 1) a ‘conventional insecticide border’ in which the middle four rows were untreated and the outer two rows were treated at-planting with an in-furrow application of imidacloprid at the rate of 255.5 g[AI]/ha, 2) a ‘conventional insecticide full plot’ in which all eight rows were treated with imidacloprid as previously described, 3) a ‘conventional insecticide border + (*R*)-CPB I whole plot’ in which the outer two rows were treated with imidacloprid as previously described while lures containing the (*R*)-CPB I were placed throughout the entire plot, 4) a ‘(*R*)-CPB I whole plot’ in which all rows were untreated and (*R*)-CPB I lures were placed throughout the entire plot, and 5) an ‘untreated control’ in which all eight rows were left untreated. The experiment had four replicates for a total of twenty plots of potatoes.

(*R*)-CPB I obtained from J.C. Dickens (USDS-ARS, BARC) was 96% optical purity [4% (*S*)-CPB I] (Mori & Tashiro, 2004). (*R*)-CPB I was released from red rubber septa obtained from Fisher Scientific Co., Cat. No. 03-215-5. Prior to treatment, septa were washed several times in hexane. Individual septa were then treated with 20-µl of a 25-µg/µl solution in hexane or 50% hexane:50% ethyl acetate to give 500 µg of (*R*)-CPB I per septum. After the solvent evaporated, the septa were individually wrapped in aluminum foil and placed in plastic freezer bags. Septa were prepared on 27 April and held on ice until installation in the appropriate trap crop rows on 28 April, soon after overwintering adult CPB had emerged. A metal flag was used to pierce the edge of each rubber septum and situate them 15-30 cm above the soil surface. For the (*R*)-CPB I treatments, each plot received a total of 100 lures spaced evenly throughout the plot. The lures were placed between all eight rows, and along the outer rows such that lures were approximately 0.61 m apart. Old lures were replaced on 23 May with fresh lures that were prepared as described above.

On 2, 5, 10, 15, 22, 26 and 30 May the number of CPB egg masses, larvae and adults were counted on 10 plants in the inner four rows of each plot. By 9 June, newly eclosed adults had emerged and began feeding, subsequently, percent defoliation ratings were visually taken on each plot. On 10 July, the middle two rows of each plot were harvested with a single-row mechanical potato harvester. Potatoes were evaluated according to U.S. standards (Grade B, small A, large A and Chef) and then weighed to evaluate tuber yield.

The numbers of CPB egg masses, larvae and adults over time were analyzed using analysis of variance procedures (Analytical Software 1998). Data were square root ($x + 0.05$) transformed prior to analysis. Proportion defoliation data were arc-sine, square root transformed prior to analysis. All means were separated using Fisher's protected least significance difference at the $P \leq 0.05$ level of significance. Untransformed data were used in all figures.

Optimal ratios of the (S)- and (R)-enantiomers. Field experiments were conducted in June and July of 2007 at the Virginia Tech Eastern Shore Agricultural Research & Extension Center in Painter, Virginia. Four 0.008 hectare plots were cultivated to remove any weeds and create a level ground surface. In each of these four 0.008 hectare spatially-isolated locations, a 9-m diameter circle was laid out with a total of twelve pitfall trap stations situated equally around the circumference of the circle such that all stations were 4.5 m from the center of the circle (release point for insects), and 2.4 m from each other. Pitfall trap stations consisted of a 25-cm long sheet of aluminum flashing placed vertically in the ground as a barrier and on both ends, an 11-cm diameter cup was buried flush to the ground. Pitfall cups were filled with water and 30 ml of liquid soap detergent to kill insects falling into the trap. A metal flag was placed between the two cups to mark the station location and to attach the lure 40 cm above the soil surface. Blends of enantiomers were prepared from (*S*)- and (*R*)-3,7-dimethyl-2-oxo-oct-6-ene-1,3-diol [(*S*)-CPB I and (*R*)-CPB I, respectively] by mixing the synthetic enantiomers previously dissolved in a 1:1 mixture of hexane and ethyl acetate. (*S*)-CPB I and (*R*)-CPB I were 96.5% and 96% optically pure, respectively (Mori and Tashiro, 2004). Blends were released from red rubber septa obtained from Fisher Scientific Co., Cat. No. 03-215-5. Prior to treatment, septa were washed several times in hexane. Individual septa were then treated with 50 µl total of 10-µg/µl solutions of (*S*)- and (*R*)-CPB I in 50% hexane:50% ethyl acetate to give 500 µg of CPB I enantiomers per septum. The solvent was allowed to evaporate prior to wrapping the septa individually in aluminum foil and placing them in plastic freezer bags in an ice chest for transport to the field. The following blends of enantiomers were prepared and tested: 97%(*S*):3%(*R*), 87% (*S*):13%(*R*), 73%(*S*):27%(*R*), and 50%(*S*):50%(*R*) (racemic blend). The solvent control consisted of 50 µl of a 50% hexane:50% ethyl acetate solution applied to a septum as described above for the experimental treatments.

For each of the four circles, every other pitfall trap station (stations 2, 4, 6, 8, 10, and 12) received a rubber septum with the designated ratio of the enantiomer, while the other six stations (odd stations) were controls and received a rubber septum treated with the solvent. Experiments were conducted on 12 and 20 June and 18 July. Immediately following each lure placement in the field, 400 CPB adults were collected from a nearby potato field and released into the center of each plot. After 24 h traps were assessed and beetles were taken to the laboratory where they were sexed. Catches of CPB were evaluated for differences using a Student's *t*-test (df = 5) at the $P \leq 0.05$ level of significance.

Results

Effect of (R)-CPB I under field conditions. Adult CPB were active throughout the month of May, with peak numbers in plots occurring in the middle of the month (Fig. 3.1). However, because of defoliation of untreated plants and adult CPB movement to more lush foliage, sample counts of adults after 15 May were misleading, and consequently were not analyzed. There was a significant effect of treatment on CPB adults on 5 and 10 May (Table 3.1). On both sample dates, the imidacloprid full plot had the fewest number of adults, which was expected. No differences were observed between the untreated control and all other treatments. The imidacloprid border treatment significantly reduced the numbers of CPB adults in the (*R*)-CPB I plots, but not in the control plots.

CPB egg masses (Fig. 3.2) also peaked in mid-May which coincided with adult activity (Fig. 3.1). Since CPB egg masses followed the same pattern as observed with CPB adults (movement into the foliage of treated plots after 15 May, following defoliation of untreated plots), counts after 15 May were not analyzed (Table 3.1). There were significant differences between treatments for all sample dates analyzed. The imidacloprid full treatment had fewer egg masses than all other treatments for all sampling dates. Mean comparisons showed no significant differences in CPB egg masses between the untreated control and (*R*)-CPB I + no imidacloprid treatments. This was also observed with the no (*R*)-CPB I + imidacloprid border and (*R*)-CPB I + imidacloprid border treatments. However, in both cases, the imidacloprid border treatment appeared to reduce the number of egg masses in the middle potato plots.

Larval activity did not begin until mid-May and peaked on the last sample date (Fig. 3.3). Significant differences in treatments were observed for all sampling dates analyzed (Table 3.1). The imidacloprid full plot had significantly fewer larvae than all other treatments. Mean comparisons showed that larvae in the untreated control and (*R*)-CPB I + no imidacloprid treatments were not significantly different across all sampling dates. Larvae found in the no (*R*)-CPB I + imidacloprid border and (*R*)-CPB I + imidacloprid border treatments were also not significantly different for all sampling dates.

There was a significant effect of treatment on percentage defoliation (*F* = 68.76; df = 4, 12; *P* <0.0001), but not on marketable tuber yield (*F* = 1.49; df = 4, 12; *P* = 0.2269). Defoliation averaged less than 3% in the imidacloprid full plot, which was significantly less than all other treatments, which ranged from 82-84% (Table 3.2).

Optimal ratios of the (S)- and (R)-enantiomers. The sex ratio of male to female beetles caught in pitfall traps was approximately 50%, thus catches of both were combined for data analysis and reporting. In trial 1 (12 June), mean number of CPB adults in pitfall traps unbaited and baited with the racemic blend were the same, therefore a *t*-test was not performed. No significant differences were found for CPB catch over 24 h for the unbaited traps and 73(*S*):27(*R*) and 87(*S*):13(*R*) enantiomer ratio traps (Fig. 3.4A, Table 3.3). Significantly more CPB were caught in the 97(*S*):3(*R*) baited traps than unbaited traps.

In trial 2 (20 June), there were no significant differences in mean number of CPB observed between the unbaited traps and all of the (*S*)- and (*R*)-enantiomer ratios and racemate (Table 3.3, Fig 3.4B). However, the 97(*S*):3(*R*) ratio appeared to have a noticeable numeric effect on CPB adult catch (Fig. 3.4B).

In trial 3 (18 July), there were also no significant differences in numbers for CPB between the unbaited and each of the (*S*)- and (*R*)-enantiomer ratios and racemate (Table 3.3, Fig. 3.4C). Again, however, there appeared to be a numeric difference between numbers of CPB adults in the 97(*S*):3(*R*) ratio baited traps.

Discussion

The proper enantiomeric ratio can be critical to the effectiveness of an insect pheromone. For example, the majority of European corn borer, *Ostrinia nubilalis* Hübner, populations respond to a 96:4 ratio of (*Z*)-11-tetradecenyl acetate and (*E*)-11-tetradecenyl acetate respectively (Klun et al. 1973, Bartels et al. 1997). However, there is a strain (first discovered in New York) that is attracted to the converse formulation of the isomers, *Z*:*E* = 4:96 (Roelofs et al. 1972, DuRant et al. 1995). In general, the two strains do not respond to each other's pheromone (Glover et al. 1987). Numbers of grape root borer moths, *Vitacea polistiformis* Harris, collected increased from three to seven times with the addition of 1% (3*Z*,13*Z*)-3,13-octadecadienyl acetate to (2*E*,13*Z*)-2,13-octadecadienyl acetate (Snow et al. 1987). Both the redbanded leafroller, *Argyrotaenia velutinana* Walker, and the smartweed borer, *Ostrinia obumbratalis* Lederer, also utilize the 11-tetracedenyl acetate isomers as their sex pheromones, however, the redbanded leaf roller is most attracted to a 92:8 ratio of the *Z*:*E* isomers, while the smartweed borer responds to a 50:50 ratio blend (Klun et al. 1973). The pure synthetic sex pheromone of the Japanese beetle, *Popillia japonica* Newman, (*R*,*Z*)-5-1-decenyl)-dihydro-2(3*H*)-furanone, is highly attractive to male beetles, whereas the racemic *Z* isomer and the *S*,*Z* enantiomer were strong inhibitors of male response (Doolittle et al. 1979). *Phyllotreta cruciferae* Goeze are attracted to four synthetic racemic himachalene derivatives, but are not attracted to a blend of the opposite enantiomers (Tóth et al. 2005).

Dickens et al. (2002) was able to identify the male-produced aggregation pheromone for the CPB by performing an antennectomy and topically applying juvenile hormone III, which resulted in a 200-fold increase in the production of the pheromone, enabling its identification. Electroantennogram responses indicated that antennal receptors for CPB I responded selectively to the (*S*)-enantiomer, but did not respond to the (*R*)-enantiomer (Dickens et al. 2002). Equal amounts of the racemate were found to evoke an intermediate response in CPB antennal receptors (Dickens et al. 2002). Behavioral tests utilizing an open Y-track olfactometer found that both male and female CPB adults were attracted to the (*S*)-enantiomer, but showed no preference for the (*R*)-enantiomer or the racemate (50:50 blend) (Dickens et al. 2002).

Field studies conducted here found no differences for all CPB life stages between untreated potato plots with and without plots inundated with (*R*)-CPB I lures. There were also no differences observed in numbers of CPB adults, larvae and egg masses between the insecticide border treatments with and without the (*R*)-CPB I lures. In general, plots that received a whole field treatment of imidacloprid had the fewest number of CPB adults, egg masses and larvae. The amount of (*R*)-CPB I lures placed in the treated plots was high, with each plot receiving 100 lures.

Although pure (*R*)-CPB I does not appear to affect CPB behavior in the field, certain enantiomer ratios of (*S*)- and (*R*) might be as attractive as pure (*S*)-CPB I. Pitfall studies conducted across three test dates with various blends of the (*S*)- and (*R*)-enantiomers found only the 97%(*S*):3%(*R*) to be attractive to CPB adults. It should be noted that the physiological state of CPB is critical to responsiveness to attractants (Dickens 2007). In Dickens et al. (2002) studies, adult beetles were obtained from a colony where they were isolated upon emergence. These beetles were therefore unmated at the time of testing. The beetles used for both the 12 and 20 June tests reported here were newly emerged adults collected from untreated potato foliage; however, the age of these beetles and their mating status were unknown. For the 18 July test date, CPB used for the study were older adults who were more than likely mated at this point and preparing for overwintering. At this stage in the beetle's life, production and response to the aggregation pheromone may have been limited, which could explain the lack of response to the enantiomer blends.

Pitfall studies indicate that a mixture containing economically optimal amounts of the (*S*)- and (*R*)-enantiomers is not attractive to CPB adults in the field, which coincides with lab observations previously reported by Dickens et al. (2002). This research demonstrates the unattractiveness of the (*R*)-enantiomer or the racemate blends to CPB in the field. Although 97(*S*):3(*R*) was attractive to CPB adults in the field, the numbers of beetles drawn to these baited traps was relatively minimal. Following the release of 400 beetles, an average of 1 beetle was caught in the 97(*S*):3(*R*) baited traps. This indicates that additional research is needed to determine if the use of the aggregation pheromone to manipulate CPB populations is feasible.

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Table 3.1. ANOVA results for densities of CPB adults, egg masses and larvae for various (*R*)-CPB I treatments across all sampling dates, Painter, VA, 2006.

Sampling Date	Adults[b]			Egg masses[b]			Larvae[c]		
	F	df	*P*[a]	*F*	df	*P*[a]	*F*	df	*P*[a]
2 May 2006	3.20	4, 12	0.0528*	7.60	4, 12	0.0027*		-	
5 May 2006	4.57	4, 12	0.0180*	5.30	4, 12	0.0108*		-	
10 May 2006	3.87	4, 12	0.0303*	10.44	4, 12	0.0007*		-	
15 May 2006	2.24	4, 12	0.1251	6.45	4, 12	0.0052*	6.86	4, 12	0.0041*
22 May 2006		-			-		11.98	4, 12	0.0004*
26 May 2006		-			-		9.43	4, 12	0.0011*
30 May 2006		-			-		18.69	4, 12	0.0000*

[a] Source of variation significant at $\alpha = 0.05$

[b] Due to defoliation of untreated plots, and adult movement into plots with more foliage, sample counts of CPB adult and egg masses after 15 May were misleading, and therefore not analyzed.

[c] Larval activity did not begin until 15 May.

Table 3.2. Percentage defoliation and marketable yield of the inner four rows of potatoes with various (*R*)-CPB I treatments, Painter, VA 2006.

Treatment	Percentage Defoliation	Marketable Yield (cwt/acre)
Imidacloprid Full	2.75 ± 0.02 b	265.39 ± 40.26
No (*R*)-CPB I + imidacloprid border	86.25 ± 0.06 a	203.25 ± 44.69
(*R*)-CPB I + imidacloprid border	82.5 ± 0.08 a	217.36 ± 24.99
(*R*)-CPB I + No imidacloprid	93.75 ± 0.02 a	163.55 ± 29.16
Untreated control	90.00 ± 0.05 a	178.34 ± 24.40

Four replicates of the control and each treatment.

Means followed by the same letter within columns are not significantly different (ANOVA, LSD test; $P \leq 0.05$).

Table 3.3. Student's *t*-test results for CPB adults caught in pitfall traps unbaited and baited with various ratios of (*S*)- and (*R*)-enantiomers of the CPB I aggregation pheromone after 24 h, Painter, VA 2007.

(*S*):(*R*) racemic blend	Trial 1 (12 June)			Trial 2 (20 June)			Trial 3 (18 July)		
	t	df	*P*	*t*	df	*P*	*t*	df	*P*
50:50		-		0.79	5	0.4650	0.36	5	0.7316
73:27	0.60	5	0.5761		-			-	
87:13	2.39	5	0.0624	0.54	5	0.6109	1.39	5	0.2242
97:3	2.71	5	0.0422*	1.22	5	0.2752	0.98	5	0.3735

*****Source of variation significant at $P < 0.05$

- Mean number of CPB for unbaited and racemic blend baited traps were the same, so a *t*-test was not performed.

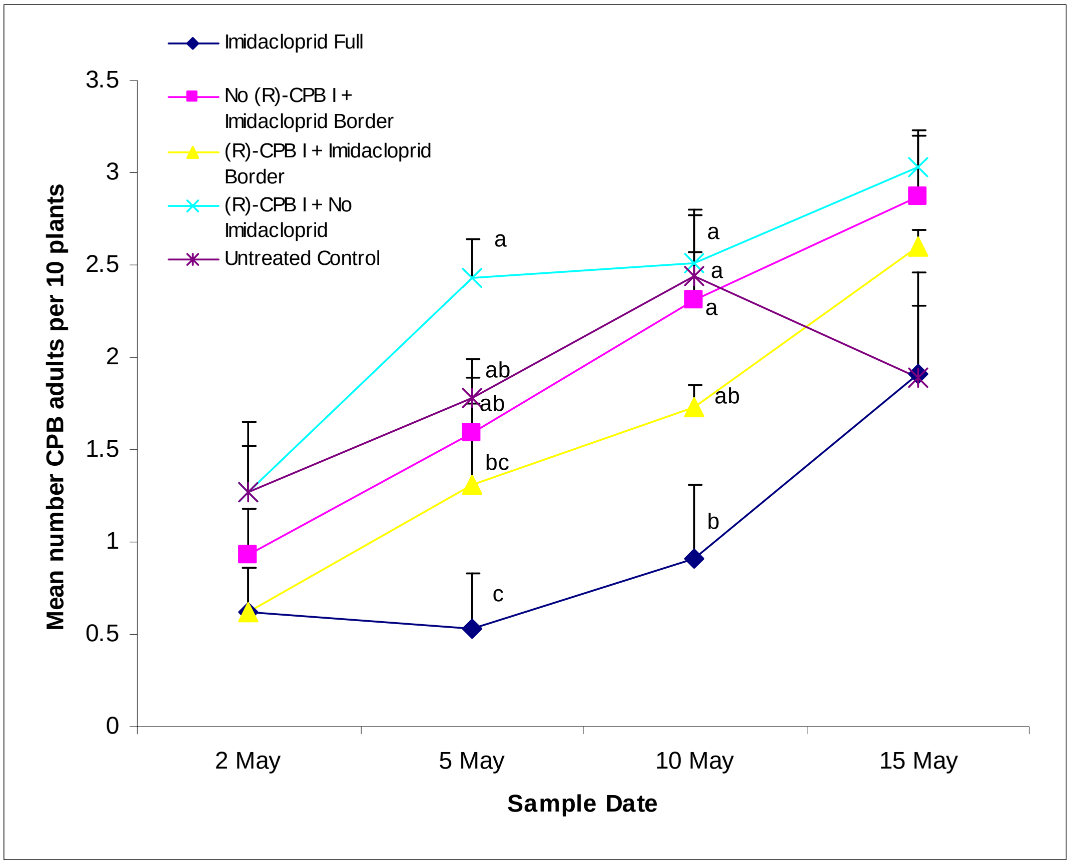


Fig. 3.1. Densities of CPB adults per ten plants (mean ± SE) in (*R*)-CPB I pheromone study for selected sample dates in Painter, VA 2006. Following a significant date x treatment interaction, data for each date were analyzed separately by ANOVA and means were separated using Fisher's protected least significance difference at the $P \leq 0.05$ level of significance. Bars with a letter in common are not significantly different.

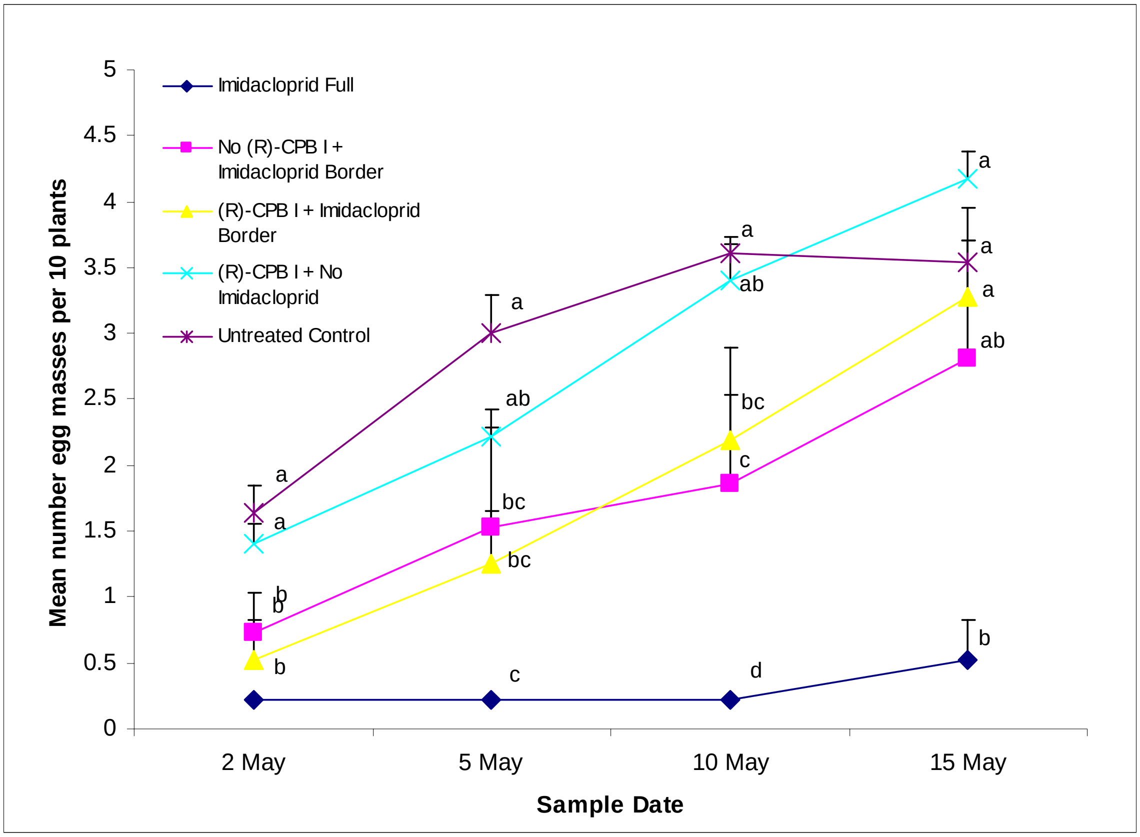


Fig. 3.2. Densities of CPB egg masses per ten plants (mean ± SE) in (*R*)-CPB I pheromone study for select sample dates in Painter, VA 2006. Following a significant date x treatment interaction, data for each date were analyzed separately by ANOVA and means were separated using Fisher's protected least significance difference at the $P \leq 0.05$ level of significance. Bars with a letter in common are not significantly different.

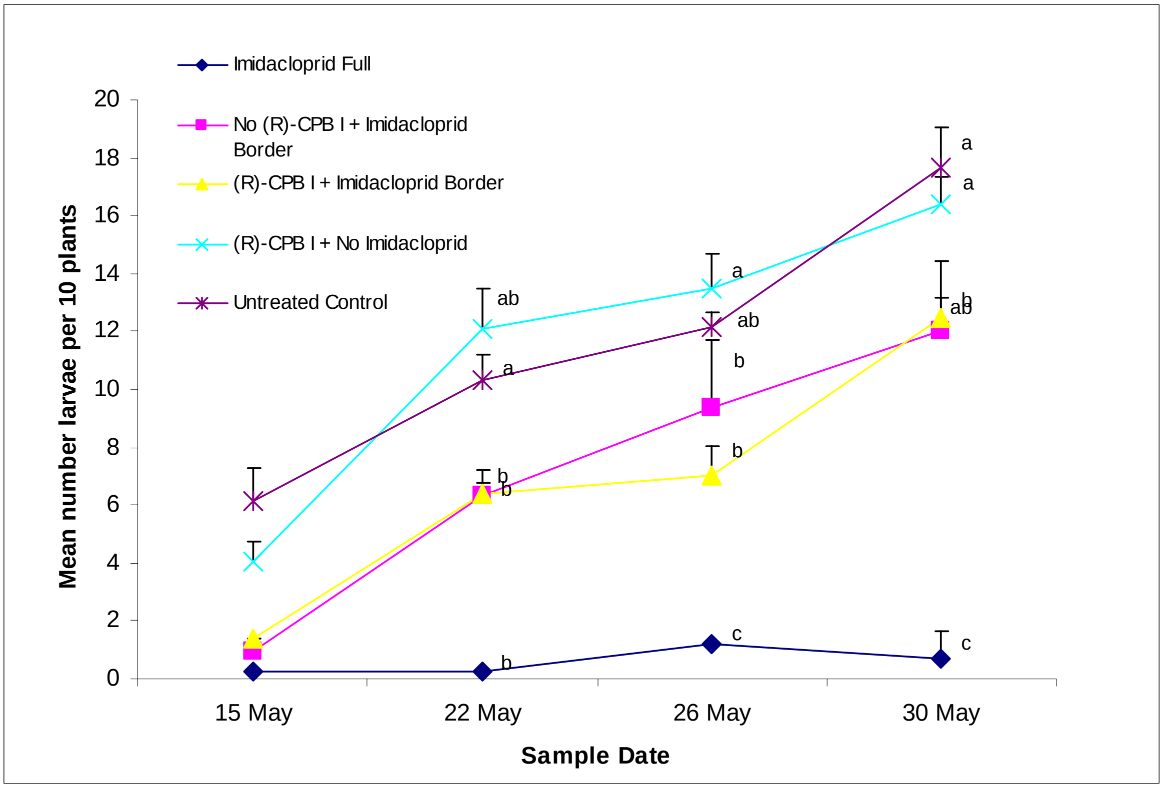
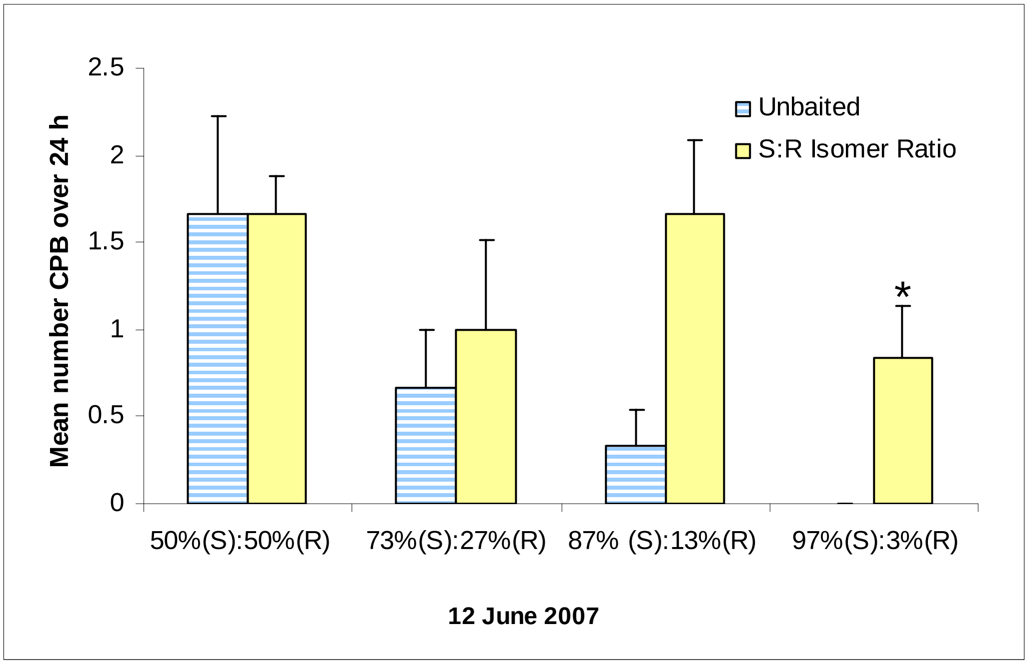
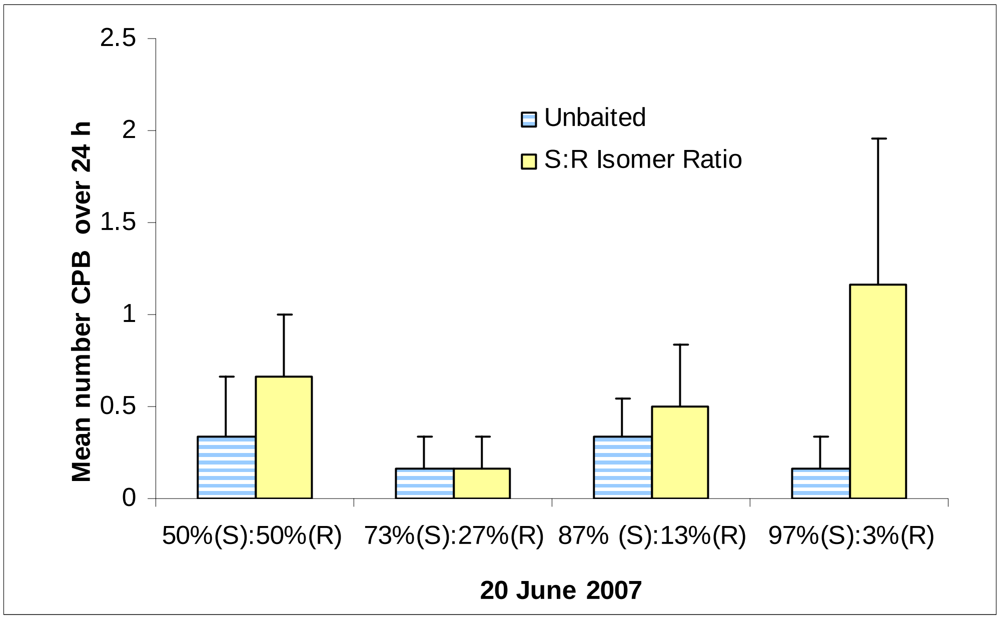


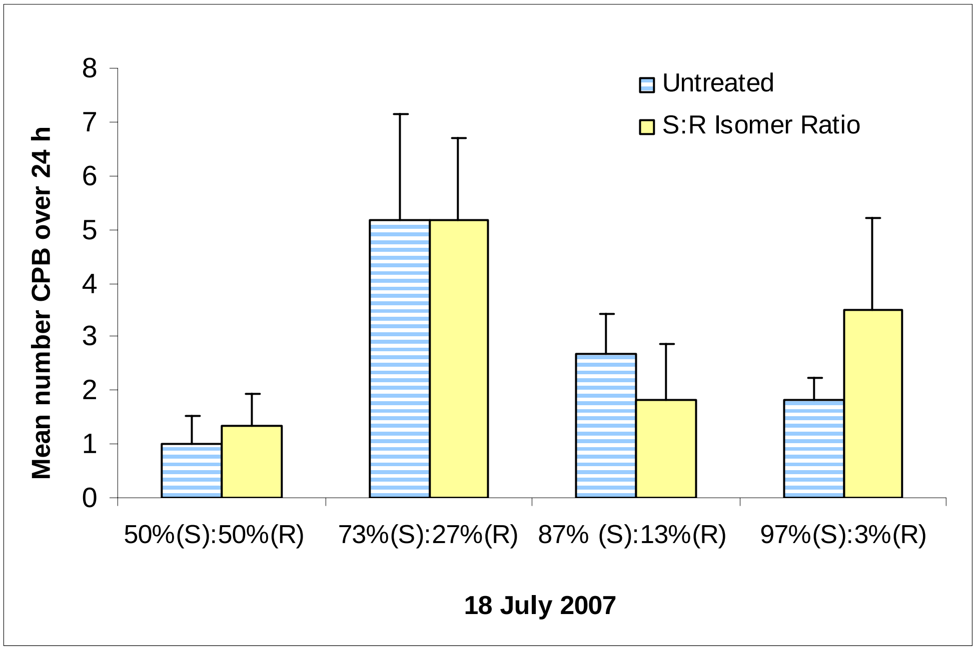
Fig. 3.3. Densities of CPB larvae per ten plants (mean ± SE) in (*R*)-CPB I pheromone study for selected sample dates in Painter, VA 2006. Following a significant date x treatment interaction, data for each date were analyzed separately by ANOVA and means were separated using Fisher's protected least significance difference at the $P \leq 0.05$ level of significance. Bars with a letter in common are not significantly different.


2.5
2
1.5
1
0.5
0
Mean number CPB over 24 h
Unbaited
S:R Isomer Ratio
*
50%(S):50%(R)
73%(S):27%(R)
87% (S):13%(R)
97%(S):3%(R)
12 June 2007

(A)


2.5
2
1.5
1
0.5
0
Mean number CPB over 24 h
Unbaited
S:R Isomer Ratio
50%(S):50%(R)
73%(S):27%(R)
87% (S):13%(R)
97%(S):3%(R)
20 June 2007

(B)



(C)

Fig. 3.4. Mean (± SE) No. of CPB adults caught in pitfall traps unbaited and baited with various ratios of (*S*)- and (*R*)-enantiomers of the CPB I aggregation pheromone after 24 h, Painter, VA 2007, (A) 12 June, (B) 20 June and (C) 18 July.

Chapter Four

Evaluation of the (*S*)-CPB I pheromone and synthetic host volatiles in a trap crop strategy and development of a trap prototype design to manage Colorado potato beetle populations

One approach to reducing the amount of insecticides needed to control an insect pest such as a leaf-feeding beetle, is through the use of an attractant, which can draw beetles to a point source (Brust and Foster 1995, Hossain et al. 2007). Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) (CPB), is an ideal target for such a strategy because adults typically walk from overwintering sites to new potato fields, and thus can easily be intercepted (Blom et al. 2002). In addition, chemicals that are highly-attractive to CPB have been identified and synthesized. A three component kairomonal blend consisting of (*Z*)-3-hexenyl acetate, (+)-linalool, and methyl salicylate attracted both larvae and adults of CPB (Dickens 2000, 2002). The potential of the kairomone in a stimulo-deterrent strategy for CPB management was demonstrated in laboratory and greenhouse experiments as well as preliminary field tests (Martel et al. 2005a). The effectiveness of the kairomone in a trap-crop strategy was shown in field tests where yields for conventionally managed plots and plots bordered by attractant-treated trap crops did not differ, and 44% less insecticide was applied to plots bordered by attractant-treated trap crops (Martel et al. 2005b). More recently, a male-produced aggregation pheromone, (*S*)-3,7-dimethyl-2-oxo-oct-6-ene-1,3-diol [(*S*)-CPB I], was found to be highly attractive to CPB adults (Dickens et al., 2002) and larvae (Hammock et al. 2007). The compound has been synthesized (Dickens et al. 2002, Mori and Tashiro 2004), and shown to be effective in the field (Kuhar et al. 2006). The combination of pheromone and plant volatiles including the 3-component plant attractant is even more attractive than either by itself (Dickens 2006). The objectives of this study were to evaluate the effectiveness of the (*S*)-CPB I aggregation pheromone + synthetic host volatiles in a trap crop strategy in potatoes, and to determine the relative effectiveness of a novel CPB trap for catching colonizing adults in the field.

Materials & Methods

Evaluation of (S)-CPB I aggregation pheromone + synthetic host volatiles in a trap crop.

Field studies were conducted in 2005 at the Eastern Shore Agricultural Research & Extension

Center in Painter, VA. A total of 20 spatially isolated plots of 'Superior' potatoes, separated by 7.6 m of bare ground, were planted on 7 April. Each plot consisted of ten 7.6 m rows planted on 91 cm row centers with potato seed pieces spaced 30 cm apart within the row (Fig. 4.1). The experiment was arranged in a randomized complete block with five treatments replicated four times. Treatments included the following: 1) "conventional insecticide" in which all ten rows were treated at-planting with an in-furrow application of imidacloprid at a rate of 255.5 g[AI]/ha, 2) "imidacloprid border" in which the middle six rows were untreated and the outer two rows per side were treated with imidacloprid as described previously, 3) "attractant border" in which the middle six rows were untreated and the outer two rows per side contained (*S*)-CPB I pheromone + synthetic host volatile lures, 4) "attractant + insecticide border" where the middle six rows were untreated and the outer two rows per side contained the (*S*)-CPB I pheromone + synthetic host volatile lures + imidacloprid treatment, and 5) "untreated control" in which all ten rows were left untreated.

The (*S*)-CPB I pheromone was released from red rubber septa obtained from Fisher Scientific Co., Cat. No. 03-215-5, while the synthetic host volatiles were released from cigarette filters made from cellulose acetate (Filtrona Richmond, Inc., Richmond, Virginia). For the pheromone lure preparations, (*S*)-3,7-dimethyl-2-oxo-oct-6-ene-1,3-diol [(*S*)-CPB I] was dissolved in a 1:1 mixture of hexane and ethyl acetate. (*S*)-CPB I was 96.5% optically pure (Mori and Tashiro, 2004). Septa were washed several times in hexane and then individually treated with 50 µl total of 10-µg/µl solutions of (*S*)- and (*R*)-CPB I in 50% hexane:50% ethyl acetate to give 500 µg of CPB I enantiomers per septum. The solvent was allowed to evaporate prior to wrapping each septum in aluminum foil and placing them in plastic freezer bags in an ice chest for transport to the field.

The three component kairomonal blend was comprised of (*Z*)-3-hexenyl acetate (98% purity), (+)-linalool (97% purity), and methyl salicylate (99% purity). Three microliters of each attractant component were diluted in 0.5 ml of a slow release formulation comprising polyethylene glycol (20%), methanol (42.5%), glycerol (25%) and distilled water (12.5%), and then injected into the cigarette filters (Dickens 1989, Martel et al. 2005b).

Lures were installed in the appropriate treatment rows on 12 May, soon after overwintering adult CPB had emerged. The pheromone-treated rubber septa and host volatile lure filters were fastened together to a metal flag with wire and situated so they were located 8 cm above the soil surface. Each outer row (two rows per side of each plot) of the designated plots received five lures, for a total of 20 lures per plot. The lures were spaced approximately 1.5 m apart from one another down each row. The lures were replaced with fresh lures, prepared as described above, on 22 May.

On 14, 16, 18, 23, 25, 27 May and 1, 6 and 9 June the number of CPB egg masses, larvae and adults on 10 plants in the middle rows of each plot were counted. On 16 June, the percentage defoliation was visually estimated for each plot. The two middle rows from each plot were harvested with a single-row mechanical potato harvester on 6 July. Potatoes were evaluated according to U.S. standards (Grade B, small A, large A and Chef) and then weighed to evaluate tuber yield.

To determine the effect of the (*S*)-CPB I pheromone and synthetic host volatiles, the numbers of CPB egg masses, larvae and adults over time were analyzed using repeated measures analysis of variance procedures (Analytical Software 1998). Data for CPB adults, larva and egg masses were square root (x + 0.05) transformed prior to analysis. Percentage defoliation data were arc-sine, square root transformed prior to analysis. All means were separated using Fisher's protected least significance difference at the $P \leq 0.05$ level of significance. Untransformed data were used in all figures.

Evaluation of attractant-based trap for catching CPB adults. Field experiments were conducted in June 2007 at the Eastern Shore Agricultural Research & Extension Center in Painter, VA. A CPB adult trap prototype was constructed using two yellow 530 ml Solo plastic cups (Solo Cup Co., Highland Park, IL). One cup was buried flush with the ground while the other cup was glued on top of the bottom cup so the open ends were facing one another (Fig. 4.2). The top cup had a 6 cm x 9 cm opening cut out on both sides of the cup to allow release of the lures. The lures were placed in the top cup by creating a small hole in the end of the cup and fastening a piece of 24 gauge wire which was then wrapped around the lures. The bottom cup was filled with approximately 400 ml of water and 30 ml of liquid soap detergent to collect insects falling into the trap. To evaluate the efficacy of this attractant-based trap prototype, six of the previously described traps were situated around a 4.5 m circle, with traps spaced 2.3 m apart. Every other trap prototype received the (*S*)-CPB I + synthetic host volatile lures, while the other three traps contained no lures. The pheromone lures were prepared from (*S*)-3,7-dimethyl-2-oxo-oct-6-ene-1,3-diol [(*S*)-CPB I] as previously described. Red rubber septa, also previously described, were used to release the pheromone.

Cigarette filters, 3.0 cm in length, were used to dispense the synthetic host volatiles. Host volatiles were mixed in a ratio of 2:1:1 of (*Z*)-3-hexanyl acetate, linalool, and methyl salicylate, respectively. Each wick was treated with 25% of the host volatiles in peanut oil, with a total amount of 2 ml being applied to each wick. Both synthetic host volatile treated wicks and pheromone treated septa were maintained in a freezer until use in the field.

The experiment was replicated in the field in three locations, and was conducted twice, on 12 and 20 June. For each experiment, CPB adults were collected from surrounding potato fields, held in containers for 24 h, and then released at densities of 100 or 200 beetles every two to three days for a total of two weeks (12 June experiment) or 1 week (20 June experiment). After 24 h following each beetle release, traps were assessed for numbers of beetles caught. All beetles were collected and taken to the laboratory and sexed using the distinctive projections on the last ventral abdominal segment (Rivnay 1928). To determine the attractiveness of the lures over time, the 12 June experiment collected data using the same lures and releasing CPB adults periodically for approximately two weeks, while the 20 June experiment collected data for approximately one week. The two experiments were analyzed using a Student's *t*-test (n = 3) at the $P \leq 0.05$ level of significance.

Results

***Evaluation of (S)-CPB I aggregation pheromone + synthetic host volatiles in a trap crop*.** Adult CPB were active in potato plots throughout the month of May, and immediately after installing pheromones in the appropriate potato plots (Fig. 4.3). Due to substantial defoliation of the untreated control plots in the experiment and the subsequent dispersal of CPB adults into plots with more lush foliage, the sample counts of CPB adults and egg masses after 27 May were misleading, and therefore not analyzed. There was a significant effect of treatment on numbers of CPB adults for all sampling dates except 25 May (Table 4.1, Fig. 4.3). The imidacloprid full plot treatment had the fewest number of beetles for all sample dates. For four of the five significant sample dates, there were no differences for beetle numbers between the untreated control and center of the attractant border. The center of the imidacloprid border plot had significantly fewer CPB adults than the untreated control for three of the five significant sample dates. The center of the imidacloprid + attractant border treatment had significantly fewer CPB adults compared to the control for all but one sample date. There were no significant differences in adult numbers between the center of the imidacloprid border treatment and the center of the imidacloprid + attractant border treatments (Fig. 4.3). However, beetle numbers between the imidacloprid full plot treatment and the center of the imidacloprid + attractant border treatment did not differ significantly for four of the five sample dates.

There were significant differences between treatments for all sampling dates analyzed for egg mass numbers (Table 4.1, Fig. 4.4). The imidacloprid full plot had the fewest number of egg masses for all sampling dates. Egg mass numbers between the untreated control and center of the attractant border treatment did not differ significantly for five of the six sample dates (Fig. 4.4). Egg mass numbers between the center of the imidacloprid border treatment and the untreated control were significantly different for three of the six sampling dates. The center of the attractant + imidacloprid border treatment had significantly fewer egg masses than the untreated control for all but one sample date. There were no significant differences in egg mass numbers between the center of the imidcloprid border treatment and the center of the attractant + imidacloprid border treatments for all but one sample date.

CPB larvae did not appear in the field until early June (Fig. 4.5). There were significant differences between treatments for all sample dates analyzed (Table 4.1). On 1 June, only the imidacloprid full plot treatment had significantly fewer larvae than the untreated control (Fig. 4.5). Larval counts between the untreated control and the center of the attractant border treatment did not differ significantly for all three sample dates. Both the center of the imidacloprid border treatment and the center of the attractant + imidacloprid border treatment had significantly fewer CPB larvae than the untreated control for the last two sample dates. They also did not differ from one another in egg mass numbers for these two sample dates. The imidacloprid full plot treatment had the fewest number of larvae for all sample dates.

There was a significant treatment effect on percentage defoliation ($F = 53.92$; d.f. = 4, 12; $P < 0.0001$) and marketable yield ($F = 15.12$; d.f. = 4, 12; $P < 0.0001$) with the imidacloprid full plot, the center of the imidacloprid border and attractant + imidacloprid border treatments having less defoliation than the untreated control and the center of the attractant border treatments (Table 4.2). The imidacloprid full plot treatment had significantly higher yield than all other treatments. There were no significant differences in yields for all other treatments (Table 4.2).

Evaluation of attractant-based trap for catching CPB adults. The sex ratio of male to female beetles caught in traps was approximately 50%, thus catches of both were combined for data analysis and reporting. For the 12 June experiment, there were no significant differences in numbers of CPB in the baited versus unbaited traps for all sample dates (Table 4.3; Fig. 4.6). For the 20 June experiment, there were no significant differences in numbers of CPB in the baited versus unbaited traps for sample dates 21 June, 23 June, and 26 June (Table 4.3; Fig. 4.7). However, there were significantly more CPB found in the baited traps compared to the unbaited traps for 28 June sample date (Table 4.3; Fig. 4.7).

Discussion

Studies have demonstrated the potential use of both the synthetic host volatiles (Martel et al. 2005a, 2005b, 2007) and the (*S*)-CPB I aggregation pheromone (Kuhar et al. 2006) for manipulation of CPB populations. Laboratory and greenhouse studies utilizing potato plants treated with synthetic host volatiles found that attractant-treated plants were significantly more attractive than untreated potato plants (Martel et al. 2005a). In addition, field studies investigating use of these synthetic host volatiles in a trap crop found more post-diapause, colonizing adults, egg masses and small larvae in attractant-treated trap crops than in untreated trap crops (Martel et al. 2005b). Furthermore, studies combining the synthetic plant volatiles and a pyrethroid insecticide proved effective against small larvae in the field (Martel at al. 2007).

Field studies utilizing the CPB synthetic aggregation pheromone in a trap crop demonstrated its potential for controlling CPB populations (Kuhar et al. 2006). Moreover, field and laboratory bioassays have revealed CPB prefer the mixture of synthetic host volatiles and pheromone than just the host volatiles alone (Dickens 2006). The first study presented here, where the combination of the (*S*)-CPB I aggregation pheromone and synthetic host volatiles were used in a trap crop strategy, indicates that this type of experimental design failed to reduce CPB numbers in untreated middle rows of potatoes. The lure density used for this experiment may account for the experiments ineffectiveness at reducing CPB populations. In Kuhar's et el. (2006) trap crop study, a total of 80 lures were placed around the perimeter of potato plots similar in size to those used in this study. Due to limited pheromone availability, only 20 lures were available for use in each plot for the experiment described here, which may have failed to provide enough of the aggregation pheromone to elicit beetle response. There were no differences in CPB adults, larvae and egg masses between the untreated control and center rows of the untreated attractant border treatment in the study presented here. There was a reduction in adults, larvae and egg masses in the trap crop centers for both the imidacloprid border and imidaclorpid + attractant border treatments. However, there were no significant differences found between these two treatments, indicating that the pheromone + synthetic host volatiles did not increase the efficacy of an imidacloprid border treatment for reducing CPB numbers in my experimental design. The use of imidacloprid as a perimeter treatment has previously been investigated and found to reduce densities of CPB (Blom et al. 2002). However, in that study, as well as in this study, whole-field imidacloprid treatments still had significantly higher yields than imidacloprid perimeter treatments (Blom et al. 2002). Although imidacloprid perimeter treatments can reduce CPB densities, refinement of the border width may need to be investigated further to produce yields similar to whole-field imidacloprid applications.

Combining sex pheromones and host plant volatiles for mass trapping has proven to be effective in controlling major agricultural pests such as *Carpophilus spp*, and *Rhynchophorus ferrugineus* Olivier (James et al. 1996, Hallett et al. 1999, Baker and Heath 2004). There may be potential for this management approach for CPB, but the attractant trap design used for the experiment presented herein did not appear to effect catches of CPB adults, as the number of beetles caught in the (*S*)-CPB I + synthetic host volatile traps did not differ from unbaited traps. Although the (*S*)-CPB I + synthetic host volatiles used to bait the traps tested here did not affect CPB catch, the traps did appear to be effective at trapping CPB adults. Taking into considerations that the area the traps accounted for in the circle arena was 0.04 m^2 (six traps) compared to the entire area circle area of 16.42 m^2, it does not appear that the beetles were caught in the traps by chance alone. On one sample date following the release of 200 beetles, 48 CPB adults were caught in baited traps, while 41 were caught in unbaited traps. The lack of difference between beetles caught in baited traps versus unbaited traps may be attributed to the amount of (*S*)-CPB I + synthetic host volatiles released in the area tested. To date, little research has been conducted to determine the appropriate amount of (*S*)-CPB I + synthetic host volatiles required to elicit a positive response by CPB, and in the case presented here, beetles may have very well been overwhelmed by the amount of (*S*)-CPB I + synthetic host volatiles used in the study. The amount of (*S*)-CPB I + synthetic host volatiles used here may have disoriented the beetles, which may explain why beetles were caught in traps, but differences were not observed between baited versus unbaited traps. The attraction of the traps may also be related to the color of the traps (yellow) and their vertical orientation in the field. Also important for this study, are the age and sexual status of the beetles tested. In Dickens et al. (2002) studies, where the pheromone was first identified and described, CPB adults were obtained from a colony were they were isolated upon emergence. Furthermore, those beetles were unmated at the time of testing and their age was known. In the case of my field experiments, adults were collected from unsprayed fields, and although relatively newly-emerged, their age and mating status were unknown. These factors likely affected their response to the pheromone and plant volatiles present in the traps. Additional research needs to be conducted to determine the optimal spacing and amount of pheromone + synthetic host volatiles necessary to attract and trap CPB adults.

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Table 4.1. ANOVA results for densities of Colorado potato beetle adults, egg masses and larvae for (*S*)-CPB I + synthetic plant volatile treatments across all sampling dates, Painter, VA, 2005. Lures were placed in the field on 12 May and replaced with fresh lures on 22 May.

Sampling Date	Adults[b]			Egg masses[b]			Larvae[c]		
	F	df	*P*[a]	*F*	df	*P*[a]	*F*	df	*P*[a]
14 May 2005	5.40	4, 12	0.0101*	3.38	4, 12	0.0451*		-	
16 May 2005	15.38	4, 12	0.0001*	5.69	4, 12	0.0083*		-	
18 May 2005	4.35	4, 12	0.0210*	12.72	4, 12	0.0003*		-	
23 May 2005	3.34	4, 12	0.0466*	43.80	4, 12	<0.0001*		-	
25 May 2005	2.82	4, 12	0.0732	26.25	4, 12	<0.0001*		-	
27 May 2005	4.87	4, 12	0.0145*	20.16	4, 12	<0.0001*		-	
1 June 2005		-			-		5.75	4, 12	0.0080*
6 June 2005		-			-		21.71	4, 12	<0.0001*
9 June 2005		-			-		38.87	4, 12	<0.0001*

[a] Source of variation significant at α = 0.05

[b] Due to defoliation of untreated plots, and adult movement into plots with more foliage, sample counts of CPB adult and egg masses after 27 May were misleading, and therefore not analyzed.

[c] Larval activity did not begin until 1 June.

Table 4.2. Percentage defoliation and marketable yield of potatoes produced with various pheromone + synthetic host volatiles strategies for management of Colorado potato beetle in Painter, Virginia 2005. Lures were placed in the field on 12 May and replaced with fresh lures on 22 May.

Treatment	Percentage defoliation	Marketable yield (cwt/acre)
Imidacloprid Full Plot	1.25 ± 1.25^{c}	113.80 ± 10.8^{a}
Center of Imidacloprid Border	65.0 ± 10.8^{b}	63.56 ± 10.5^{b}
Center of Imidacloprid + Attractant Border	65.0 ± 10.8^{b}	65.96 ± 9.70^{b}
Center of Attractant Border	92.5 ± 2.5^{a}	52.49 ± 9.0^{b}
Untreated Control	90.0 ± 5.0^{a}	60.62 ± 6.1^{b}

Table 4.3. Student's *t*-test results for CPB adults caught in attractant-based traps 24 h following release of CPB adults on various dates, Painter, VA 2007.

Sample Date	Exp 1 (12 June)			Exp 2 (20 June)		
	t	df	*P*	*t*	df	*P*
15 June	1.07	2	0.3971		-	
19 June	0.49	2	0.6718		-	
21 June	0.63	2	0.5932	1.00	2	0.4227
23 June	0.14	2	0.8995	0.97	2	0.4336
26 June	0.64	2	0.5885	0.59	2	0.6127
28 June	0.50	2	0.6689	7.00	2	0.0198*

*****Source of variation significant at $P \leq 0.05$

\- Sampling did not begin until 21 June.

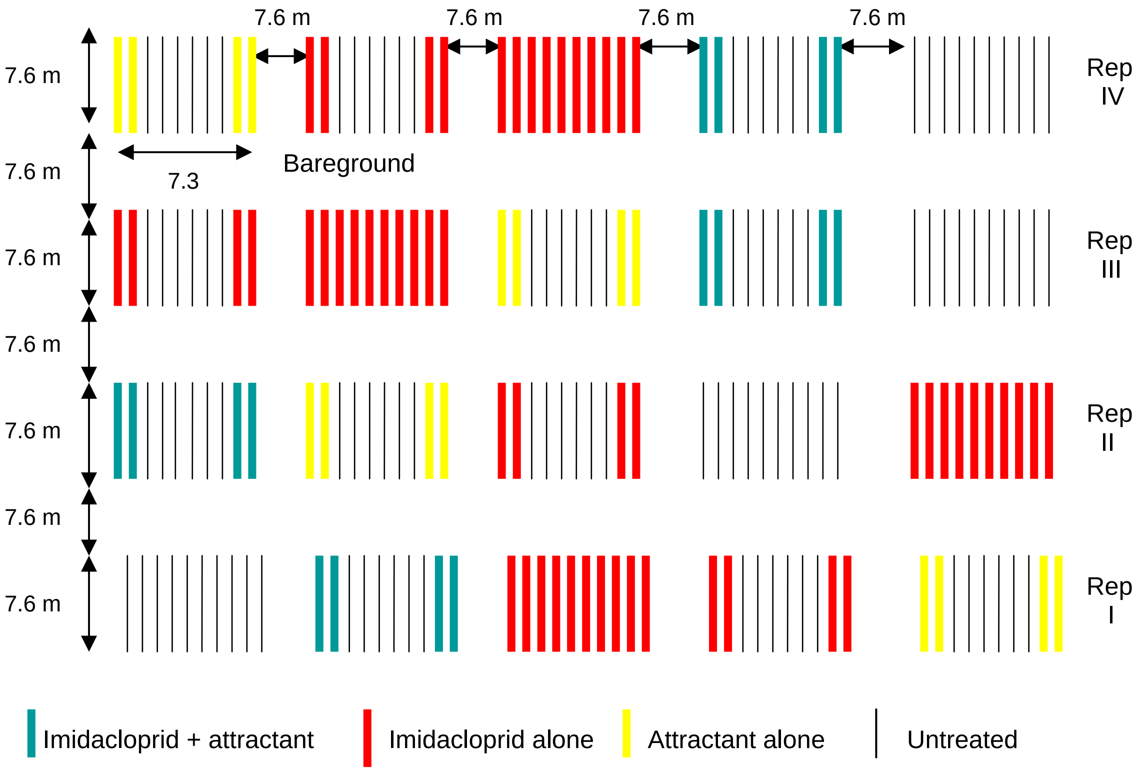

Fig. 4.1. Diagram of trap crop study experimental design. The experiment was arranged in a randomized complete block with five treatments replicated four times. Each plot consisted of ten 7.6 m rows planted on 91 cm row centers with potato seed pieces spaced 30 cm apart within the row.

Fig. 4.2. Attractant-based trap with (*S*)-CPB I pheromone + synthetic plant volatile lures.

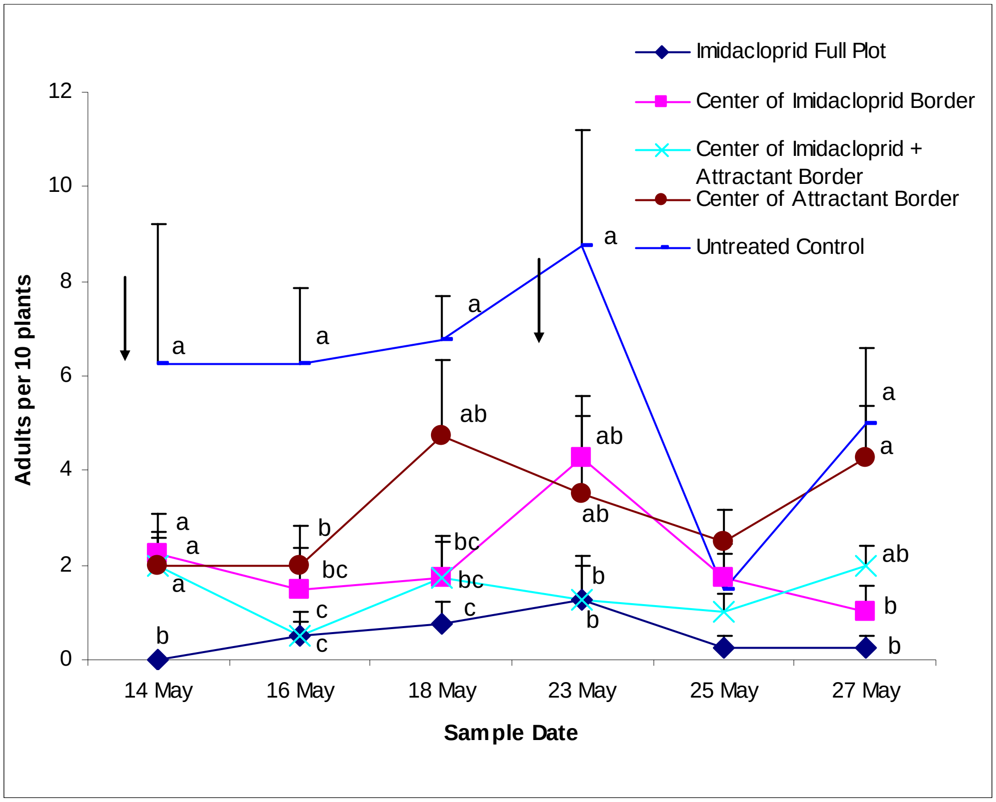

Fig. 4.3. Densities of CPB adults per ten plants (mean ± SE) in CPB attractant study in Painter, VA 2005. Following a significant date x treatment interaction, data for each date were analyzed separately by ANOVA and means were separated using Fisher's protected least significance difference at the $P \leq 0.05$ level of significance. Bars with a letter in common are not significantly different. Arrows indicate lure placement in the field on 12 and 22 May.

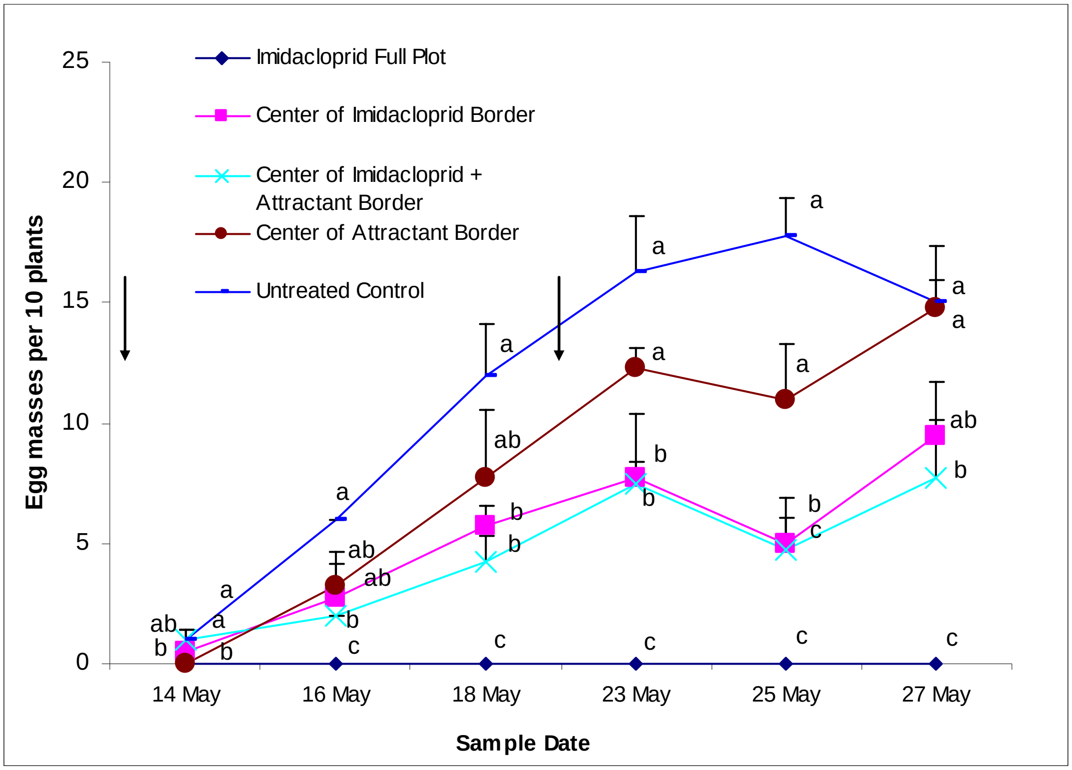

Fig. 4.4. Densities of CPB egg masses per ten plants (mean ± SE) in CPB attractant study in Painter, VA 2005. Following a significant date x treatment interaction, data for each date were analyzed separately by ANOVA and means were separated using Fisher's protected least significance difference at the *P* ≤ 0.05 level of significance. Bars with a letter in common are not significantly different. Arrows indicate lure placement in the field on 12 and 22 May.

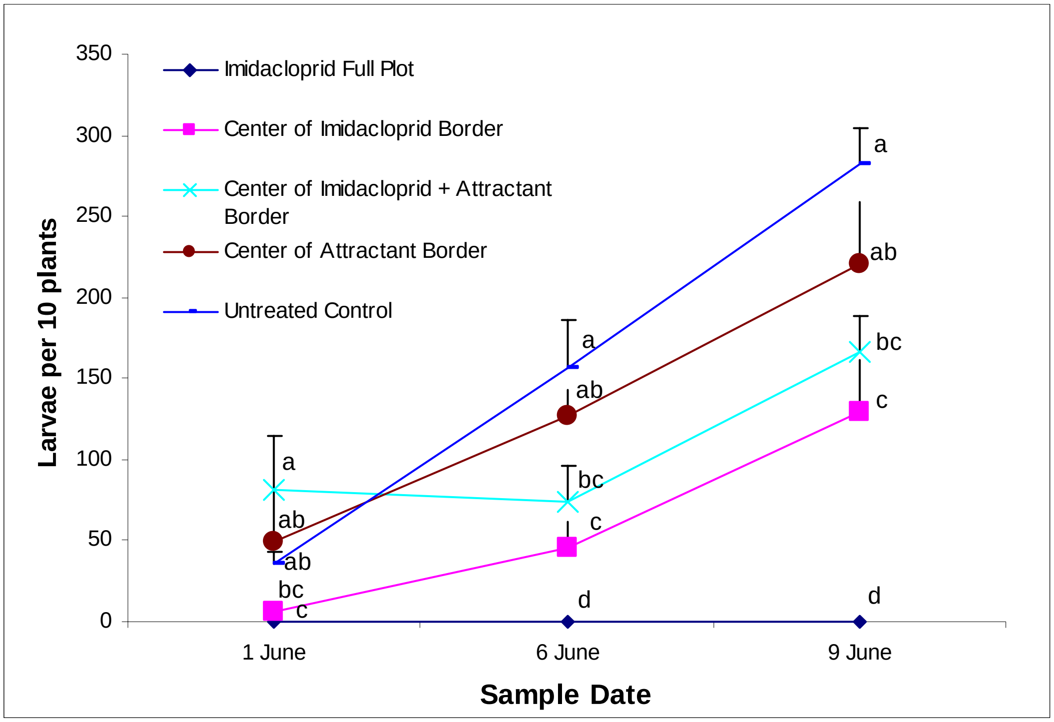

Fig. 4.5. Densities of CPB larvae per ten plants (mean ± SE) in CPB attractant study across all sample dates in Painter, VA 2005. Following a significant date x treatment interaction, data for each date were analyzed separately by ANOVA and means were separated using Fisher's protected least significance difference at the $P \leq 0.05$ level of significance. Bars with a letter in common are not significantly different. Lures were placed in the field on 12 and 22 May.

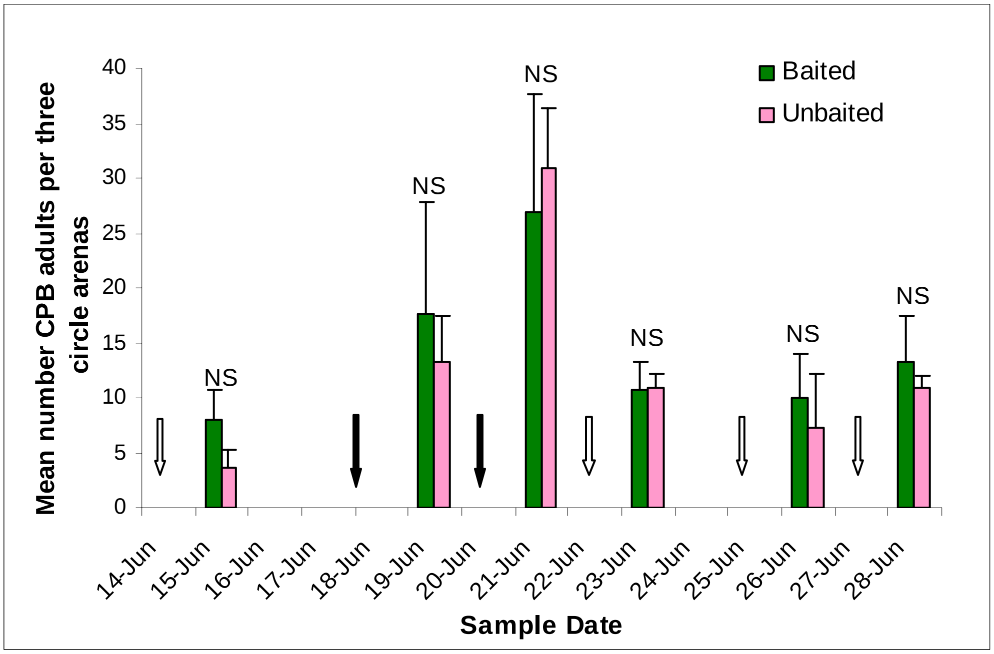

Fig. 4.6. Mean ± SE catch of adult CPB in (*S*)-CPB I + synthetic host volatile baited and unbaited prototype trap design in Painter, VA 2007. Lures were placed in the field on 12 June. White arrows indicate the release of 100 beetles on that date while black arrows indicate the release of 200 beetles for that date. Data for each date were analyzed separately using a Student's *t*-test at the 0.05 level of significance.

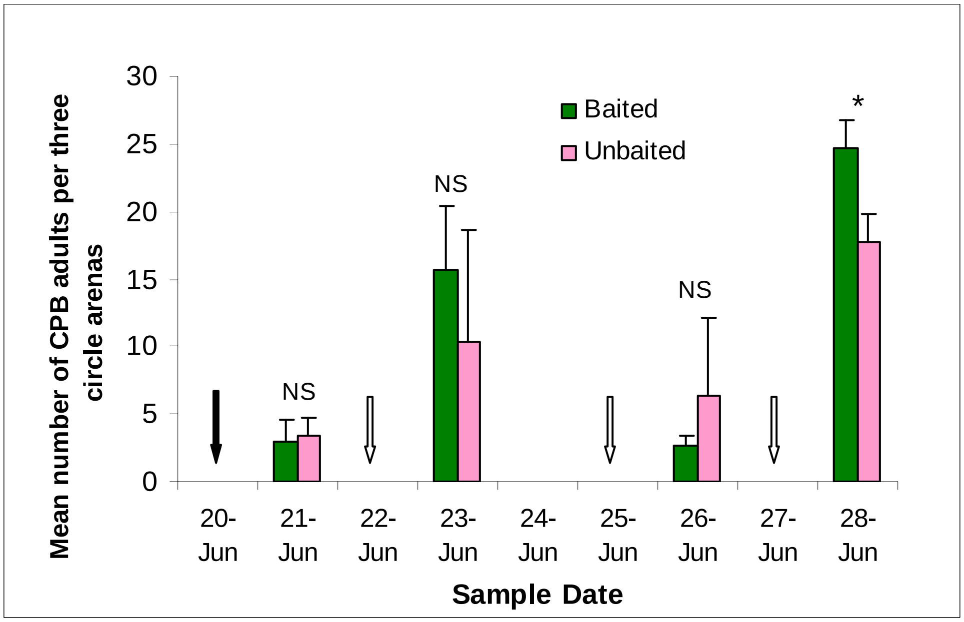

Fig. 4.7. Mean ± SE catch of adult CPB in (*S*)-CPB I + synthetic host volatile baited and unbaited prototype trap design in Painter, VA 2007. Lures were placed in the field on 20 June. White arrows indicate the release of 100 beetles on that date while black arrows indicate the release of 200 beetles for that date. Data for each date were analyzed separately using a Student's *t*-test at the 0.05 level of significance.

Chapter Five

Synergistic activity of binary mixtures of metaflumizone and esfenvalerate on Colorado potato beetle

The Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae), is the most important insect pest of potatoes (*Solanum tubersosum* L.*)* in both the United States and Europe (Hare 1990). In the absence of control measures, the beetle can completely defoliate plants resulting in crop loss in potato, eggplant (*Solanum melongena* L.) and other solanaceous crops (Casagrande 1987). The beetle has an extensive history of developing resistance to insecticides with the most recent data indicating development of resistance to at least 49 different active ingredients and all major insecticide classes (Whalon et al. 2007). New insecticides are needed for insecticide resistance management.

A novel semicarbazone insecticide, metaflumizone (BAS 320I, BASF) was recently introduced and shown to have positive toxicological and environmental profiles as well as low toxicity to beneficial arthropods, making it a useful insecticide for resistance management programs. In addition to activity against lepidopteran pests (Taylor and Riley 2007), metaflumizone has also shown promising activity against CPB (Cutler et al. 2006). Kuhar et al. (2006) achieved greater than 95% control of CPB in the field with foliar applications applied at 80 g AI ha^{-1}. Metaflumizone has a novel mode of action that blocks voltage-dependent sodium channels directly without requiring bioactivation (Vandecoevering 2007). However, since pyrethroid insecticides also act on sodium channels there is interest in how this might impact efficacy of semicarbazone insecticides. Since many populations of CPB in the U.S. have developed resistance to pyrethroid insecticides (Roush et al. 1990, Tisler and Zehnder 1990, Heim et al. 1992, Miyo et al. 1999, Olson et al. 2000). The mode of action of pyrethroids, however, is much different than that of semicarbazones which block sodium channels. Pyrethroids act on insect nervous systems by modulating sodium channels. They prevent sodium channel deactivation and inactivation, which leads to continuous stimulation of the nerve. Pyrethroids, unlike metaflumizone, require bioactivation to work. Currently it is unknown how the combination of the two chemistries would affect insecticidal efficacy. Herein, I report laboratory and field tests of the interaction of metaflumizone and esfenvalerate in mortality to various life stages of CPB.

Materials & Methods

Chemicals. Metaflumizone (Alverde ® 2SC, 240 g[AI]/ha) was supplied by BASF of North America Inc. (Research Triangle Park, NC, USA). Esfenvalerate (Asana ® XL, 79 g[AI]/ha) was supplied by DuPont ™ (Wilmington, DE, USA).

Insects. Colorado potato beetle adults, egg masses, and larvae were collected from insecticide-free potato fields near Painter, VA. Egg masses were placed in Petri dishes and maintained at 25 ± 5 ° C with a photoperiod of 16:8 (L:D) to obtain larvae for bioassays. Fresh potato leaves were provided as a food source for larvae and adults.

Bioassays. Residual leaf-dip bioassays were conducted to determine the susceptibility of CPB to metaflumizone, esfenvalerate, and metaflumizone + esfenvalerate. Separate bioassays were conducted on small larvae (first and second instars), large larvae (third and fourth instars), and adults. Serial dilutions were prepared using distilled water. Preliminary tests were conducted to determine what concentrations (ppm of active ingredient) caused 0-100% mortality. Based on these preliminary tests, constant doses of esfenvalerate (equivalent to half of the LC_{50}) were used as a synergist with varying concentrations of metaflumizone. For each test, five doses (serial dilutions) plus a control were replicated four to five times on 10 adults, 10 small larvae, and 10 large larvae. Potato leaves were obtained from insecticide-free potato plants grown in the field. Leaves used for the experiment averaged 54 cm^2 in size. Leaves were dipped in the test solutions with gentle agitation for 4s and then placed on plastic drying racks under a fume hood for one hour. One leaf was inserted into a moistened piece of Aquafoam (Syndicate Sales, Inc., IN, USA) and placed in a 9 cm diam. Petri dish. For adult bioassays, ten adults were placed in two Petri dishes (five adults to each dish) and mortality was assessed at 1, 3, and 5d. Beetles were considered dead or moribund if they were unable to right themselves or walk a distance equal to their own body length when disturbed (Mota-Sanchez et al. 2006). For larval bioassays, 10 larvae were placed in each Petri dish and mortality was assessed at 24h and 48h. Larvae that were shriveled and unable to respond to nudging from a probe were considered dead. Tests were repeated if control mortality exceeded 20%. All tests were conducted under ambient laboratory conditions at 25 ± 5 º C with a photoperiod of 16:8 (L:D).

Field efficacy experiments. Field bioassays were conducted at the Virginia Tech Eastern Shore Agricultural Research Extension Center in Painter, VA in 2007. Potato seed pieces ('Superior') were planted on 15 March. Individual plots consisted of two rows x 6 m long, separated by 0.9 m with plants spaced 30 cm down the row (due to space constraints and loss of plants some plots were only 4.6 m long; however, spray rates and yield were adjusted accordingly. Seven treatments were replicated four times in a randomized complete block design. Suggested field rates as well as rates lower than the field recommended rates (one tenth of the labeled esfenvalerate rate and one quarter of the labeled metaflumizone rate) were tested. In addition, a rate of esfenvalerate equivalent to one tenth of the lowest field rate was used as a synergist with metaflumizone. The seven treatments tested were as follows: 1) 'metaflumizone ¼ rate low' (20 g[AI]/ha), 2) 'esfenvalerate $^1/_{10}$ rate low' (3.3 g[AI]/ha), 3) 'metaflumizone full' (80 g[AI]/ha), 4) 'esfenvalerate full' (33.6 g[AI]/ha), 5) 'esfenvalerate $^1/_{10}$ rate low + metaflumizone $^1/_{10}$ rate low' (3.3 g[AI]/ha and 8 g[AI]/ha respectively), 6) 'esfenvalerate $^1/_{10}$ rate low + metaflumizone ¼ rate mid' (3.3 g[AI]/ha and 20 g[AI]/ha respectively), and 7) 'untreated control'. On 21 May, following observations of newly-hatched larvae in the field, foliar sprays were applied to all plots. Applications were made using a CO_2 backpack sprayer equipped with a boom that contained four extended-range flat spray nozzles, which delivered 355 l/ha at 3.40 atm. A second application was made on 31 May. Post spray counts of CPB were made on 29 May and 1, 4, and 11 June by examining 10 randomly selected plants per plot and counting numbers of CPB adults, egg masses, small larvae (first and second instars) and large larvae (third and fourth instars). Percent defoliation was visually estimated in each plot on 6 June. On 29 June both rows of each plot were harvested with a single-row mechanical potato harvester. Tuber yield was evaluated according to U.S. standards (Grade B, small A, large A and Chef).

Data analysis. Abbott's formula was used to correct the data for control mortality (Abbott 1925). Polo Plus Software was then used to calculate the lethal concentrations (LC_{50} and LC_{90}) along with the 95% confidence limits and the slope (±SE) of the log dose-probit line (LeOra Software 2002). The lethal concentrations were considered significantly different if their 95% confidence limits (CL) did not overlap. To determine the degree of synergism, synergistic ratios were calculated by dividing the LC_{50} value for metaflumizone by the LC_{50} value for metaflumizone + esfenvalerate.

To determine the effect of insecticides in the field, the effect of treatment was analyzed for each CPB life stage, percentage defoliation, and marketable tuber yield using ANOVA procedures (Analytical Software 1998). To meet normality, data were transformed using a square root (x + 0.05) transformation prior to analysis. Proportion defoliation data were arc-sine, square-root transformed prior to analysis. All mean comparisons were conducted using Fisher's protected least significant differences at the $P \leq 0.05$ level of significance. To simplify the summarization of treatment effects on CPB life stages, the efficacy data for each life stage was reported by using the date with the peak density. Untransformed data were used in all figures.

Results

Laboratory bioassays. The LC_{50} values of esfenvalerate for small larvae, large larvae, and adults were 0.82, 1.03, and 7.05 ppm, respectively (Table 5.1). Metaflumizone LC_{50} values were 0.28, 0.31, and 0.92 ppm for small larvae, large larvae, and adults. When metaflumizone was combined with esfenvalerate (0.39 ppm), the LC_{50} values were 0.08 ppm for small larvae, 0.27 ppm for large larvae, and 0.23 ppm for adults. Esfenvalerate caused synergism of metaflumizone for both adults and small larvae, (SR = 4.0 and SR = 3.5, respectively). Esfenvalerate did not cause synergism of metaflumizone for large larvae at the LC_{50} (SR = 1.2).

Field efficacy experiment. There was a significant effect of treatment on small larvae, which peaked on 4 June ($F = 12.39$; df = 6, 18; $P < 0.0001$) (Fig. 5.1A). Fewer small larvae were found in the 'metaflumizone low', 'metaflumizone full', 'esfenvalerate + metaflumizone low' and 'esfenvalerate + metaflumizone mid' treatments than the 'untreated control' treatment. There was no difference in the 'esfenvalerate full' and the 'esfenvalerate low' treatments from the 'untreated control'. Large larvae also peaked on 4 June with a significant treatment effect on numbers ($F = 25.33$; df = 6, 18; $P < 0.0001$) (Fig. 5.1B). Fewer large larvae were found in the 'metaflumizone low', 'metaflumizone full', 'esfenvalerate low', 'esfenvalerate + metaflumizone low' and 'esfenvalerate + metaflumizone mid' treatments compared to the 'untreated control' treatment. There was no difference in the 'esfenvalerate full' and the 'untreated control' treatments. At peak adult activity on 11 June, there was no statistically significant effect of insecticide treatment on CPB adult numbers observed ($F = 2.30$; df = 6, 18; $P = 0.0796$) (Fig. 5.1C). Counts of egg masses for all sample dates were not analyzed because the numbers were too low to be meaningful.

Defoliation was also affected by treatment ($F = 27.40$; df = 6, 18; $P < 0.0001$) (Fig. 5.2). There was significantly less defoliation in all treatments compared to the 'untreated control'. There was also less defoliation in the 'metaflumizone low', 'metaflumizone full', 'esfenvalerate + metaflumizone low' and 'esfenvalerate + metaflumizone mid' treatments compared to the 'esfenvalerate low' and 'esfenvalerate full' treatments. Finally, there was a significant treatment effect on yield ($F = 4.58$; df = 6, 18; $P = 0.0055$) with the 'metaflumizone low' and 'metaflumizone full' treatments having significantly higher yields than all other treatments (Fig. 5.3).

Discussion

Pyrethroid resistance in CPB is well documented (Roush et al. 1990, Heim et al. 1992, Miyo et al. 1999, Olson et al. 2000), including on the Eastern Shore of Virginia (Tisler and Zehnder 1990). This variation in susceptibility to pyrethroids on the Eastern Shore appeared to be related to planting dates and frequency of insecticide applications (Tisler and Zehnder 1990). Variation in insecticide resistance among populations is not uncommon for CPB and has been reported among fields on the same farm (Huang et al. 1994) and even from different areas within the same field (Vencill and Zehnder 1993, Weisz et al. 1994). Three quantitative trait loci (QTL) contributing to esfenvalerate resistance were identified for CPB (Hawthorne 2003). One QTL had a large effect while the other two QTL had smaller effects which may explain some of the variation in tolerance to esfenvalerate observed in susceptible populations (Hawthorne 2003).

CPB adults and larvae tested in my laboratory bioassays were highly susceptible to esfenvalerate; however, field populations of CPB were not susceptible to either the low or full rate of esfenvalerate. Toxicity is expected to be considerably higher for lab bioassays where contact with the insecticide is more frequent and degradation of the toxicant is unaffected by environmental factors. Pyrethroid applications on commercial potato fields in Virginia and Maryland in 2007 also failed to effectively control CPB populations, indicating that the beetle is resistant to pyrethroids, although susceptibility may vary by location (T.P. Kuhar, personal observations).

Synergism between insecticides has been reported in many studies. For example, organophosphates have been shown to synergize with pyrethroids against numerous insect pests (All et al. 1977, Asher et al. 1986, Martin et al. 2003, Bonnet et al. 2004). It has also been demonstrated that low rates of carbamates can be used to synergize pyrethroids (Corbel et al. 2003, Bielza et al. 2007).

Synergism, was observed here with both CPB adults and small larvae in the laboratory, but not with large larvae. The percentage of LC_{50} to the recommended field spray concentration for both adults and small larvae was reduced for metaflumizone by approximately seventy-five percent when esfenvalerate was used as a synergist. Potato foliar trials conducted in 2006 in Painter, VA as well as the field study presented here with metaflumizone indicate a high level of efficacy against CPB (Kuhar et al. 2006). It is interesting to note that one fourth rate of the standard dose of metaflumizone provided control equivalent to the proposed recommended rate from the manufacturer. When esfenvalerate was combined with one tenth or one fourth the full field rate of metaflumizone, CPB adults and larvae were controlled as effectively as with the full dose. Although the mortality attributed to the combination of esfenvalerate and metaflumizone could be considered additive, the extremely low rate of metaflumizone used with esfenvalerate and the ineffectiveness of esfenvalerate alone lead us to believe that this combination is in fact synergistic. Given the relatively inexpensive cost of pyrethroids, combining these insecticides with metaflumizone at low doses may reduce the cost of controlling CPB. This combination also has important implications for insecticide-resistance management of CPB. Given that imidacloprid-resistant beetles have shown cross-resistance to other neonicotinoids (Mota-Sanchez et al. 2006, Alyokhin et al. 2007) it is imperative that growers alternate insecticides with different modes of actions. Since growers have predominately relied on neonicotinoids at planting as an in-furrow treatment, they are limited on choices for effective non-neonicotinoid foliar insecticide treatments later in the season. The novel mode of action of metaflumizone and excellent control of CPB provides an additional tool for resistance management. In addition, the combination of metaflumizone and pyrethroids may prevent selection for resistance and prove to be an effective rotational selection for resistance management.

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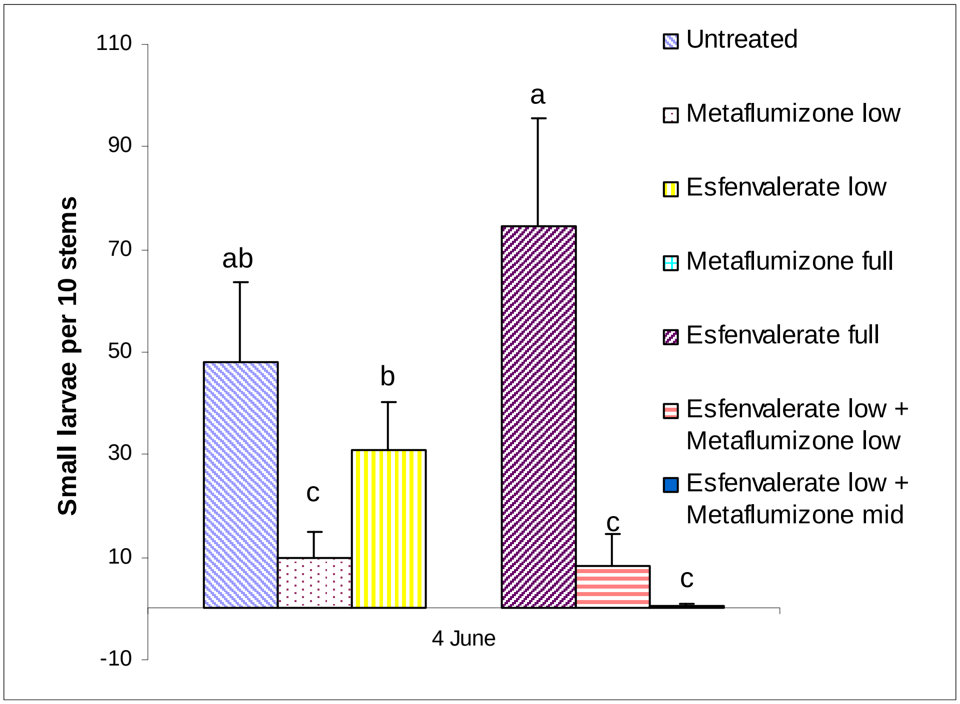
Table 5.1. Lethal concentrations (LC$_{50}$), slopes and synergistic ratios (SR) of esfenvalerate and metaflumizone with and without a synergist esfenvalerate to Colorado potato beetle adults and larvae from Painter, VA.

Insecticide	*n*	LC$_{50}$ (ppm)	95% FL (ppm)	Slope ± SE	SR[a]	Percentage of LC$_{50}$ to the field spray concentration[b]
		Adults LC$_{50}$ (ppm)				
Esfenvalerate	300	7.049	5.679-8.511	2.289 ± 0.269	-	7.4
Metaflumizone	300	0.917	0.690-1.177	1.558 ± 0.215	-	0.41
Metaflumizone + Esfenvalerate (0.39 ppm)	240	0.232	0.080-0.469	1.262 ± 0.231	4.0*	0.10
		1st & 2nd instar larvae LC$_{50}$ (ppm)				
Esfenvalerate	300	0.816	0.292-1.412	2.000± 0.252	-	0.85
Metaflumizone	300	0.284	0.236-0.341	2.346 ± 0.254	-	0.13
Metaflumizone + Esfenvalerate (0.39 ppm)	300	0.081	0.031-0.125	1.952 ± 0.302	3.5*	0.04
		3rd & 4th instar larvae LC$_{50}$ (ppm)				
Esfenvalerate	300	1.030	0.613-1.571	2.102 ± 0.248	-	1.08
Metaflumizone	300	0.312	0.254-0.383	2.032 ± 0.234	-	0.14
Metaflumizone + Esfenvalerate (0.39 ppm)	240	0.265	0.208-0.334	1.994 ± 0.262	1.2ns	0.12

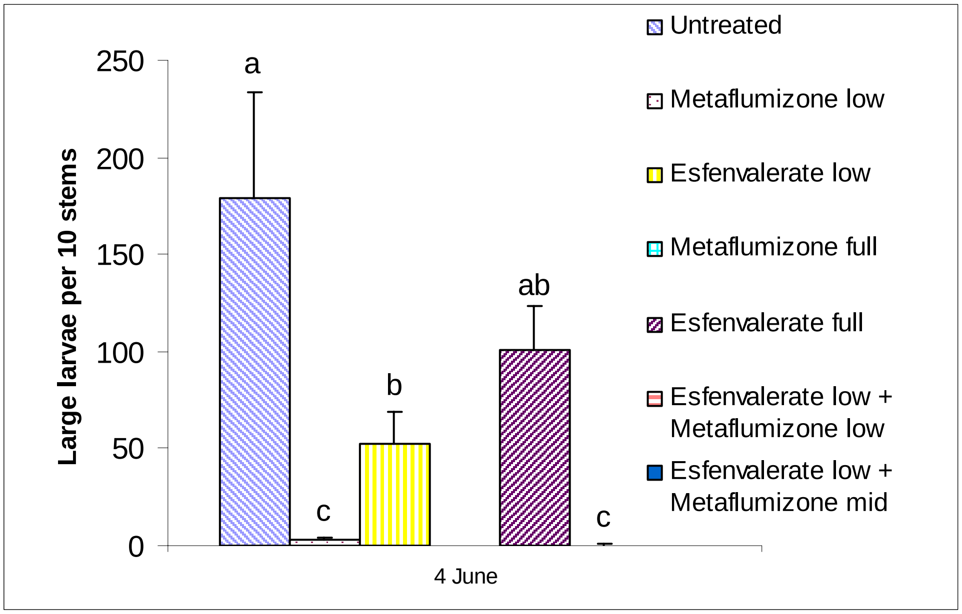
[a] Synergistic ratio was calculated as LC$_{50}$ of insecticide/ LC$_{50}$ of insecticide + synergist. A ratio followed by ns (no significant synergism) an asterisk (*) (significant synergism) is based on the overlap or nonoverlap of the LD50 95% CIs of metaflumazone alone and metaflumizone + esfenvalerate.

[b] Based on lowest recommended rate on potatoes and a spray volume of 355 l/ha.

(A)


Small larvae per 10 stems
110
90
70
50
30
10
-10
4 June
ab
c
b
a
c
c
Untreated
Metaflumizone low
Esfenvalerate low
Metaflumizone full
Esfenvalerate full
Esfenvalerate low + Metaflumizone low
Esfenvalerate low + Metaflumizone mid

(B)


Large larvae per 10 stems
250
200
150
100
50
0
4 June
a
c
b
ab
c
Untreated
Metaflumizone low
Esfenvalerate low
Metaflumizone full
Esfenvalerate full
Esfenvalerate low + Metaflumizone low
Esfenvalerate low + Metaflumizone mid

(C)

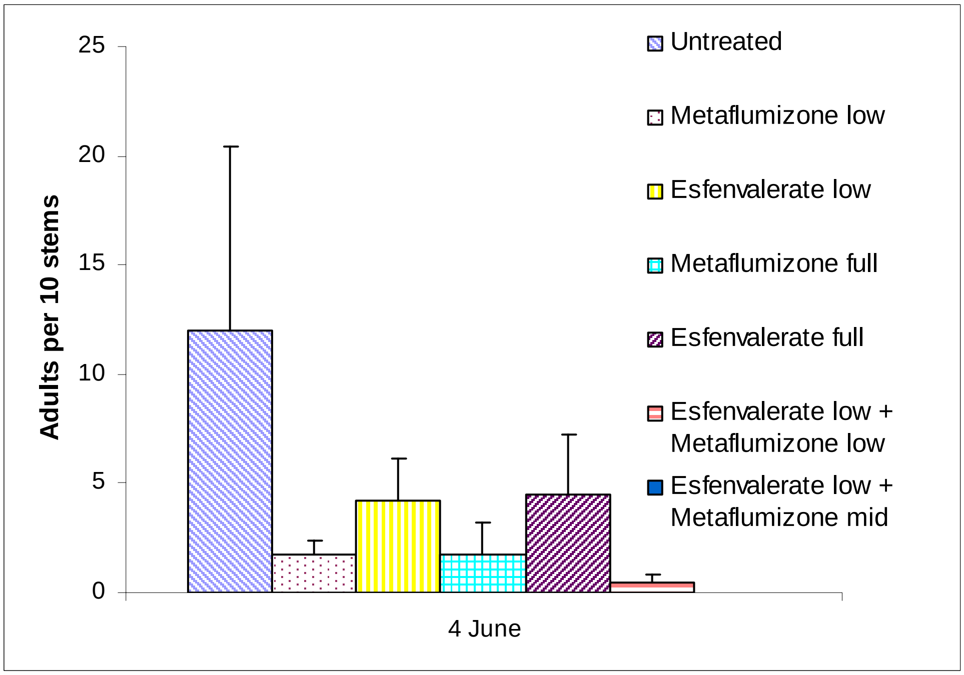


Fig. 5.1. Mean numbers (± SEM) of (A) small larvae, (B) large larvae and (C) CPB adults at peak levels on potatoes with various rates of metaflumizone, esfenvalerate and metaflumizone + esfenvalerate in Painter, VA. Error bars indicated standard errors.

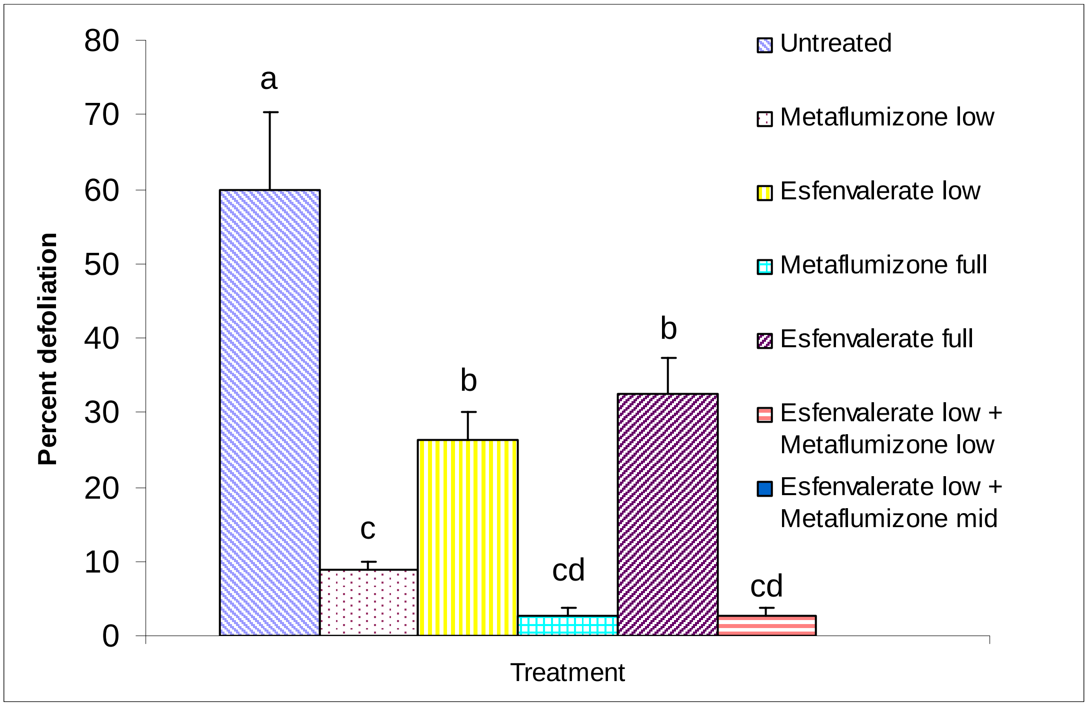


Fig. 5.2. Defoliation of potato plants treated with various rates of metaflumizone, esfenvalerate and metaflumizone + esfenvalerate in Painter, VA. Error bars indicate standard errors.

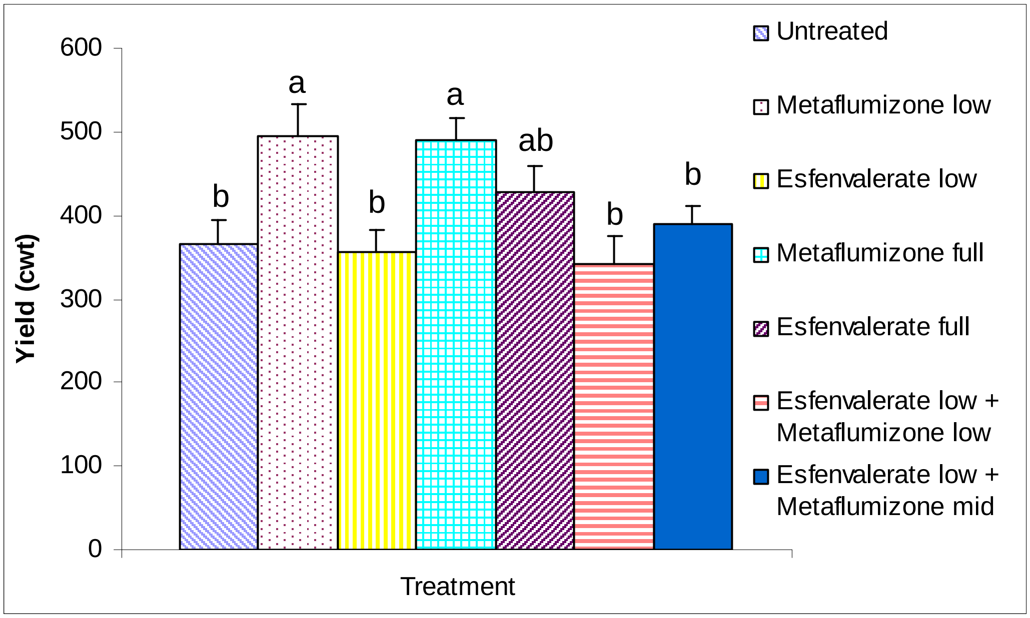


Fig. 5.3. Tuber yields on plots treated with various rates of metaflumizone, esfenvalerate and metaflumizone + esfenvalerate in Painter, VA. Error bars indicate standard errors.

Conclusions

The Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae), is a major pest of solanaceous crops, and is considered the most destructive insect pest of potato, *Solanum tuberosum*, L., in North America, Europe, and Asia (Hare 1990, Jacques 2005). In-furrow applications of neonicotinoids have effectively controlled beetle populations for more than a decade; however resistance to this chemistry has developed in numerous locations (Grafius and Bishop 1996, Mota-Sanchez et al. 2000, Olson et al. 2000, Zhao et al. 2000, Tolman et al. 2005, Alyokhin et al. 2006, Mota-Sanchez et al. 2006). Metaflumizone, a novel semicarbazone insecticide, was recently introduced and shown to have positive toxicological and environmental profiles, as well as efficacy against CPB, making it a useful insecticide for resistance management programs. To assess the efficacy of metaflumizone alone and in combination with a type II pyrethroid, esfenvalerate, laboratory and field trials were conducted in Virginia (Chapter 5). In laboratory bioassays, the combination of metaflumizone and a low concentration (0.39 ppm) of esfenvalerate was slightly synergistic on adults and early (1st-2nd) instar larvae. The combination of a low rate of esfenvalerate and metaflumizone at one tenth the field rate controlled beetles as well as the full rate of metaflumizone in the field. Synergism between metaflumizone and pyrethroids may prevent selection for resistance and prove to be an effective rotational selection for resistance management.

Exploiting the chemical ecology of CPB may unveil novel strategies for pest management. Recent identification of a male aggregation pheromone for CPB, (*S*)-3, 7-dimethyl-2-oxo-oct-6-ene-1, 3-diol [(*S*)-CPB I] (Dickens et al. 2002) and synthesis of host volatiles based on the potato plant [(*Z*)-3-hexenyl acetate, (+)-linalool, and methyl salicylate] (Dickens 1999, 2000, 2002), present an alternative strategy for managing CPB populations.

Host plant preference in CPB varies geographically and appears to be complex. In laboratory tests conducted in Virginia, CPB adults showed a preference for potato over both tomato and eggplant foliage and eggplant over tomato foliage (Chapter 2). Field studies conducted with eggplant and tomato revealed more CPB adults, larvae and egg masses on eggplant on tomato. Additional field studies however, found no difference between counts of live beetles and counts of dead beetles on untreated and imidacloprid-treated paired plants of potato and eggplant. Studies were conducted to determine if feeding adults played a role in host plant choice. Imidacloprid-treated eggplants and potatoes were paired with an untreated eggplant or potato plant covered in a mesh bag which contained two adult male beetles. Eggplant with feeding male beetles paired with another eggplant attracted significantly more adult beetles than any other treatment combination. The results from these studies indicated that host plant choice in CPB is influenced by the presence of other feeding beetles. A male-produced aggregation pheromone, which was recently identified for CPB, may potentially play a role in host plant choice for CPB (Dickens et al. 2002).

Laboratory studies have found that while both male and female beetles orient to the (*S*)-enantiomer of the male-produced CPB I aggregation pheromone, they exhibit no preference for the (*R*)-enantiomer or racemate blend of the pheromone (Dickens et al. 2002). Field studies, conducted to determine if (*R*)-CPB I had an effect on CPB life stages in the field, found no differences for all CPB life stages between untreated potato plots with and without plots inundated with (*R*)-CPB I lures (Chapter 3). No differences were also observed in numbers of CPB adults, larvae and egg masses between the insecticide border treatments with and without the (*R*)-CPB I lures**.** Field studies conducted to determine if a racemic mixture containing optimal amounts of the (*S*)- and (*R*)-enantiomers of (*S*)-CPB I was attractive to CPB adults. Pitfall studies, conducted with various blends of the (*S*)- and (*R*)-enantiomers, revealed that only the 97%(*S*):3%(*R*) ratio was attractive to CPB adults in the field.

The combination of the (*S*)-CPB I pheromone and synthetic plant volatiles were found to be more attractive to CPB adults than either by itself (Dickens 2006). The effectiveness of the (*S*)-CPB I aggregation pheromone + synthetic host volatiles in a trap crop strategy in potatoes was investigated and failed to reduce CPB numbers in untreated middle rows of potatoes (Chapter 4). Imidacloprid border and imidaclorpid + attractant border treatments, however, did reduce adults, larvae and egg masses in the trap crop middles. However, there were no significant differences found between these two treatments, indicating that the pheromone + synthetic host volatiles did not increase the efficacy of an imidacloprid border treatment for reducing CPB numbers in my experimental design. The effectiveness of a novel CPB trap for catching colonizing adults in the field was also investigated (Chapter 4). Although CPB adult numbers caught in the traps was high, there were no differences observed between the baited and unbaited traps, indicating the pheromone + synthetic host volatiles did not affect catch numbers.

Although conventional management of CPB is done primarily through whole-field insecticide applications, the potential exists to use perimeter-field treatments, host preferences and semiochemical strategies to manipulate CPB populations. In addition, the incorporation of metaflumizone into growers' current insecticide regimes provides a useful and much needed addition for current insecticide resistance management programs. Although the use of host preferences and semiochemicals for CPB management appears to be feasible, additional research is needed to determine the appropriate methodology necessary to implement these programs.

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Vita

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Erin M. Hitchner was born March 9, 1977 in Woodbury, New Jersey. Growing up on a vegetable farm, Erin's interest in entomology and agriculture was cultivated at a young age. She and her sisters could often be found searching under rocks and tree bark for insects, and walking through fields with their father, assisting him as he scouted for insects. Her first insect collection, which was a component of a high school Biology course, introduced her to the formal study of entomology. Erin's interest in entomology continued to grow as she took courses on the subject while pursuing her Bachelor's degree at Rutgers University. She earned her B.A. in Biology in 1999, and began working at the Rutgers Agricultural Research and Extension Center as the IR-4 Field Research Director. Erin continued to expand her entomology knowledge while growing and maintaining a variety of fruits and vegetables as part of her work at the center. In 2004, Erin entered the Ph.D. program in the Department of Entomology at Virginia Tech under the advisement of Drs. Thomas Kuhar and Rod Youngman. Erin defended her Ph.D. dissertation on November 2, 2007.