EVALUATION OF ATTRACTANTS AND MONITORING FOR SAP BEETLE CONTROL IN STRAWBERRIES

By

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This document is dedicated to my mother Shelly Colloredo

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Sap beetles (Coleoptera: Nitidulidae) are important pests of strawberries in north-central Florida. During 2004, field monitoring studies were conducted to track the movement of sap beetles into strawberry fields. Treatments included 1) traps located on the periphery of the field, 2) traps located between strawberry plots, and 3) traps located within plots. Results showed that traps located on the periphery of the field captured significantly more sap beetles than other treatments.

Additional studies were conducted in 2005 to evaluate attractants to be used in traps for monitoring sap beetles. Treatments included traps baited with 1) pollen dough, 2) pollen dough fed on by larvae of *L. insularis,* 3) ripe strawberries, 4) ripe strawberries fed on by *L. insularis*, and 5) control (unbaited traps). Traps were also placed in harvested and un-harvested plots to study sap beetle response in the different micro-environments.

Results indicated that all treatments evaluated captured significantly more sap beetles than control treatments. Furthermore, traps placed in un-harvested strawberry plots captured significantly more sap beetles than traps placed in harvested plots.

In studies to examine sap beetle response to different stages of strawberries we sampled sap beetles on 1) dried strawberries [on the ground], 2) ripe strawberries, 3) over-ripe strawberries, and 4) ground litter. During both years over-ripe strawberries had consistently more sap beetles than all other treatments.

Volatiles of dry, ripe, and over-ripe strawberries were collected separately and analyzed using GC Mass Spectrometry. Volatile profiles of ripe and over-ripe strawberries had significantly larger peak areas relative to internal standards. Further studies showed that volatile profiles of strawberries and strawberries fed upon by *L. insularis* also had significantly larger peak areas relative to internal standards.

Finally, we investigated reduced-risk chemical tactics for control of sap beetles. Conventional and reduced-risk insecticides were evaluated in laboratory assays for their effectiveness against sap beetles. Five treatments were investigated including one conventional standard Malathion 5EC, two reduced-risk insecticides, spinosad (SpinTor 2SC) and thiamethoxam (Actara 25G), as well as imidacloprid (Provado 1.6 F), and control [untreated plots]. Malathion consistently killed more sap beetles than any other treatment. The results indicate that reduced-risk insecticides rarely kill sap beetles when used to control other pests, which may account for recent increases in sap beetle populations.

CHAPTER 1
INTRODUCTION

Florida is the second largest producer of strawberries in the United States and provides 15% of the total U.S. crop, and 100% of the domestically produced winter crop. Approximately 95% of Florida's commercial production acreage is located in Manatee and Hillsborough counties with the other 5% occurring in both north and south Florida. Strawberry is one of the most expensive crops to produce. During the 1998-1999 strawberry season production costs averaged $17,000 per acre. Nevertheless, the Florida strawberry industry is undergoing an increase in acreage and profits. In the past 15 years, Florida has increased its acreage by 40% and income has increased by 300% (Mossler and Nesheim 2003). During the 1999-2000-production season, 6178 acres of strawberries were grown producing a profit exceeding $167 million.

Despite the recent surge in Florida's strawberry acreage and profits, production trends may be threatened due to several key pests that cause significant damage in strawberries. Sap beetles (Coleoptera: Nitidulidae) are one of several key pests associated with strawberry production (Fig. 1-1). They are listed in the 2004 Florida Crop Profile as a major pest of strawberries (Mossler and Nesheim 2004). Sap beetles cause direct as well as indirect injury to fruit and can significantly reduce marketable yields. Direct feeding causes large cavities in ripe and over-ripe fruit rendering it unmarketable. Sap beetle damage to strawberry fruit can cause secondary infections by plant pathogens. They vector mycotoxin-producing fungi that expedite the natural

fermentation process (Dowd 1994). Also, sap beetles leave the fruit vulnerable to other pathogens such as botrytis fruit rot (gray mold), the most important strawberry disease in Florida (Mossler and Nesheim 2003).

Sap beetles have a wide host range and feed on a variety of rotting fruits and decaying plant matter (Myers 2001, Peng and Williams 1990). There are more than 2,500 species of nitidulids described and over half of the genera are cosmopolitan (Rondon et al. 2004). Twenty-one genera have been reported in Florida. According to Potter (1995), nine nitidulid species can be found on strawberry fruit in east Hillsborough County including; *Carpophilus freemani* Dobson, *C. fumatus* Boheman, *C. humeralis* F., *C. mutilatus* Erichson, *Colopterus insularis* (Castelnau), *Stelidota geminata* (Say), *S. ferruginea* Reitter, *Haptoncus luteolus* (Erichson), and *Lobiopa insularis* (Castelnau).

One important species of sap beetle found in Florida is the strawberry borer, *L. insularis.* This beetle is distributed throughout the southeastern U.S. from Alabama and Texas to Florida. *Lobiopa insularis* is one of the most abundant species of sap beetle found in eastern Hillsborough County (Rondon et al. 2004). It can also be found throughout Central America and the West Indies to Columbia and Brazil (Parsons 1943). In Brazil, *L. insularis* has severely damaged up to 70% of strawberry plantings in some areas (Fornazier et al. 1986).

Another important species is the strawberry sap beetle, *S. geminata*. This species has been found to damage strawberries in Delaware, Indiana, Illinois, Ohio, Virginia, and Maryland. In the past, estimated losses of strawberries in Michigan attributed to the strawberry sap beetle exceeded $3 million (Miller and Williams 1982).

Florida strawberries are grown on raised beds with plastic mulch (Fig. 1-2). Winter grown strawberries are characteristic of a relatively short growing season usually ranging from late November to early April. The cost of labor, water supply, pesticide use, and other inputs can be economically costly to growers. On average, growers harvest fruit 2-3 times per week. Continual harvesting puts restrictions on pesticides since many have re-entry periods longer than three days.

Standard production practices utilize large amounts of pesticides, including organophosphate insecticides that are potentially harmful to the environment, people, and non-target organisms found in the ecosystem. In Florida, several varieties of strawberries are grown each year, including Camarosa, Carmine, Earlibrite, Gaviota, Oso Grande, Strawberry Festival, Sweet Charlie, and Treasure (Duval et al. 2004). Transplants are planted from late September to early November. Because of Florida's ideal environment for insect, mite, disease, and nematode development, the use of clean transplants is crucial to production. Transplants are often treated with fumigants such as methyl bromide, and fungicides are applied continuously up to the point of harvest since fruit are highly perishable.

The introduction of the 1996 Food Quality Production Act (FQPA) has targeted many pesticides including organophosphates and carbamates to be phased out over a 10-year period. One compound that is targeted to be phased out is methyl bromide. With the phase out of methyl bromide and the high dependency on fungicides, there will be a need for alternative methods of reducing pest and pathogen populations to tolerable levels. An effective integrated pest management program may reduce sap beetle

populations, as well as the incidence of fungal pathogens allowing growers to rely less heavily on pesticides.

Monitoring and trapping for sap beetles is an important tactic in an integrated pest management program in strawberries. An effective trap design along with development of attractive baits for sap beetles may be important in monitoring sap beetle populations, consequently improving timing of control tactics.

Our hypothesis is that development of an effective trap and lure system and improvements in sampling methods will improve detection of sap beetle populations in strawberry fields. In addition, identification of reduced-risk insecticides will provide an effective tool for controlling high populations of sap beetles without destroying important natural enemies.

The development of cultural practices is important to provide farmers with integrated pest management techniques that will reduce pest populations and will have a positive impact on the environment.

Figure 1-1. Sap Beetle. *Lobiopa insularis* (adult).

Figure 1-2. Strawberry plants, Citra, FL (2004).

CHAPTER 2
LITERATURE REVIEW

In developing an IPM program, it is crucial to know the biology, behavior, and population dynamics of the pests involved. Without this background information it is more difficult to interpret the data.

Sap Beetles

Habitats of sap beetles are relatively variable. Larvae of the subfamily Cateretinae live in the seed capsules of various plants and adults feed on pollen and petals of the same plants or others. Other subfamilies of sap beetles are saprophagous and mycetophagous and feed on decaying fruits and on fermenting plant juices (Potter 1994). Many sap beetles have a wide host range feeding on flowers, fruits, sap, fungi, stored products, and fermenting tissue from many fruit and vegetable crops while others are extremely host specific.

Semiochemicals

Relationship Between Sap Beetles, Fungi, and Volatile Constituents

Mycetophagous beetles vector fungi that are thought to expedite the fermentation process and therefore attractiveness of the decaying fruit. Fungi increase the release of volatiles common to fruit substrates by a combination of increased fruit-cell lysis and/or fungal catabolism of fruit constituents that parallels the process of fruit ripening (Phelan and Lin 1991, Lin and Phelan 1992). Zilkowski et al. (1999) studied the attractiveness of oranges fed upon by *C. humeralis* (Coleoptera: Nitidulidae) in wind-tunnel bioassays and found that oranges fed upon by either sex of the beetle were consistently more attractive

than volatiles from beetle-free oranges. The reason for the increase in attraction was not very clear but it is was hypothesized that the oranges may have been inoculated with fungi from the beetles. This study supported previous findings, which showed that fungal inoculation of food substrates enhanced host location for two nitidulid species, *C. hemipterus* F. and *C. lugubris* Murray (Blakmer and Phelan 1991).

Host and sap beetle associated fungal-induced volatile components have been identified for many sap beetle species. For example, the sap beetle *C. humeralis* is attracted to the volatiles released by the fungus *Fusarium verticillioides* (Saccardo) (Bartelt and Wicklow 1999). Volatiles produced by the fungus included a blend of five alcohols (ethanol, 1-propanol, 2-methyl-1propanol, 3-methly-1 butanol, and 2-methyl-1-butanol), acetaldehyde, and ethyl acetate. Four phenolics were produced along with unidentified hydrocarbons and a ketone. These authors identified the attractive components for the sap beetle to comprise primarily of alcohols, acetaldehyde, and ethyl acetate, rather than phenolics, which were also present in the volatiles. Alternatively, when *C. humeralis* feeds on oranges, the fungal-induced volatiles that elicit attraction from conspecifics of the beetle are different from those released by *F. verticillioides*. They comprise 2,5, diisopropylpyrazine, 2-phenylethanol and an unidentified product (Zilkowski et al. 1999), suggesting that *C. humeralis* is a generalist insect.

Volatile profiles of insect dependent fungi have been found to contain many of the same components as profiles of host volatiles. Sixteen components identified from the headspace profile of the fungus *Ceratocystis fagacearum* (Bretz) have been found previously in the odors from various food substrates, and eleven of the components tested on a number of nitidulid species elicited attractive responses from some of the beetles.

The 16 components included one aldehyde (acetaldehyde), one ketone (2- butanone), five alcohols (ethanol, 1-propanol, 2-methylpropanol, 3-methylbutanol, and 2-methylbutanol) and nine esters (methyl acetate, ethyl acetate, ethyl propionate, propyl acetate, methyl butyrate, isobutyl acetate, methyl isovalerate, butyl acetate, and isopentyl acetate), which were previously identified as common fruit constituents attractive to sap beetles. They concluded that *C. fagacearum* attracts nitidulids by mimicking food odors (Lin and Phelan 1992).

Acquisition and Identification of Fungal Spores

Mycetophagous sap beetles can acquire fungi by feeding on fungal spores or by the fungus coming in contact with depressions on different sclerites of the body surface. Fungal spores are then propagated through the alimentary canal of the sap beetle suggesting that they can be dispersed through the insect's excrement. *C. truncatus* Randall, the primary sap beetle vector of oak wilt pathogen, is attracted to oak wilt fungal fruiting mats where it feeds, mates, and oviposits (Kyhl et al. 2002). The fungal propagules are ingested and accumulate on cuticular surfaces of both the adults and larvae.

Little work has been done on the identification of fungi vectored by sap beetle pests of strawberries although fungi of other nitidulids have been identified. Species in the genera *Aspergillus*, *Penicillium*, and *Fusarium* have been found in association with maize sap beetles such as the corn sap beetle *C. dimidiatus* F. and the dusky sap beetle, *C. lugubris*. Some species including *C. lugubris* and beetles in the genus *Glischrochilus* are vectors of tree diseases such as oak wilt, *C. fagacearum* (Myers 2004).

Aggregation Pheromones

The importance of male-produced aggregation pheromones has been well documented among the family Nitidulidae (Cosse and Bartelt 2000, Pena et al. 1999). Sap beetle aggregation pheromones of a particular species have been found to attract related species. In a study examining the structure, electrophysiology, and behavior of the male-produced aggregation pheromone of *C. truncatus,* it was found that the pheromone could be used as a cross attractant for other sap beetle species including *C. lugubris*, *C. antiquus* Melsheimer, and *C. brachypterus* Say (Cosse and Bartelt 2000). These pheromones may be important in the attraction of large numbers of sap beetles to a particular strawberry field and may increase damage considerably.

Biology

Sap beetles are believed to overwinter as adults or pupae in woodlands on the periphery of strawberry fields. As strawberries form on plants, sap beetles are able to detect chemical cues that are emanated to the wooded periphery and surrounding areas (Rhainds and English-Loeb 2002). Adults migrate from peripheries only when temperatures reach 16ºC (67ºF) (Myers 2004). Rhainds and English-Loeb (2002) studied the location of sap beetles within the field and recorded more sap beetles on fruit that was on the ground compared with fruit in the canopy, suggesting differential suitability of strawberry fruits as a food source for sap beetles (Rhainds and English-Loeb 2002).

Life Cycle

Although the life cycle varies for different species, it is possible to make some generalizations for the family Nitidulidae. After females deposit eggs on rotting fruit it takes approximately two to five days for eggs to hatch. After hatching, larvae feed on available material. Sap beetles are characterized by three or four larval instars. The

entire larval period lasts approximately 1.5 weeks, after which larvae burrow into the surrounding soil and pupate. For the dusky sap beetle, it takes approximately 28 to 30 days from egg deposition to adult emergence. Luckmann (1963) collected *Glischrochilus quadrisignatus* (Say) adults from April through October and found that only females collected in the spring had functional ovaries and produced eggs.

Most sap beetles exhibit two generations per year although in tropical climates, multiple generations can occur if resources are available throughout the year (Myers 2004). Adults are characterized by a relatively long life span and can live for approximately 2 to 2.5 months. This may explain the relatively wide host range of most sap beeltes. A longer adult period allows sap beetles to adapt to several different types of substrates (Myers 2004).

Monitoring

Monitoring is the 'cornerstone' for many IPM programs that allows for heightened awareness of pest density. Regular monitoring allows the grower to predict pest outbreaks, and gives sufficient time to implement management programs. This ultimately reduces the need for harmful pesticides. For sap beetles, monitoring can be performed by visual counts or with the use of baited traps (Foott and Hybsky 1976).

Baits

Baits have been shown to increase captures for sap beetles. Previous studies have shown that whole wheat bread dough is effective in attracting nitidulids. Williams et al. (1994) studied the efficacy of four trap baits for monitoring beetles, including whole wheat bread dough, fermenting brown sugar, a mixture of fermenting malt/molasses, and vinegar. Twenty species in nine different genera were collected and the majority showed preference to baits in the following order although all baits proved to be attractive: wheat

dough, brown sugar, malt and molasses, and vinegar. Only a few species deviated from this pattern.

Laboratory studies have shown that modified bread dough is an effective bait in catching *L. insularis* and other sap beetles (B. Torto unpublished data). The dough incorporates autoclaved bee-collected pollen, commercial pollen substitute, and honey. The ingredients are intermixed and are allowed to harden to a dough-like consistency.

The effectiveness of food baits combined with male-produced aggregation pheromone has also been well documented. Many sap beetles exhibit cross-attraction to pheromones. However, beetles which are not attracted to the pheromone may be lured by coattractants. Williams et al. (1993) showed that traps baited with pheromone and bread dough caught at least five times more adult beetles than bread dough alone. In another study involving the effectiveness of pheromone bait stations on the attraction of nitidulid pollinators and subsequent fruit set of *Annona* spp., Pena et al. (1999) found that trees containing traps with pheromones combined with host volatile odor produced more fruit than did untreated trees. James et al. (1996b) studied the effect of pheromone baited traps (with fermenting bread dough as a coattractant) on reducing *Carpophilus* spp. in stone fruit orchards. They found that populations in ripe fruit were significantly reduced compared with non pheromone baited traps.

James et al. (2000) found that multispecies pheromone lures for *Carpophilus* spp. were effective as attractants in areas containing more than one damaging species of sap beetles. Captures of *C. davidsoni* Dobson and *C. mutilatus* Erichson in traps baited with aggregation pheromones (of both species or a three way lure that also included the

pheromone of *C. hemipterus* (L.)) were not significantly different from captures in traps baited with conspecific pheromones (James et al. 2000).

Trap design and position also affect monitoring efficiency and overall trap captures. In two separate experiments, Peng and Williams (1991) studied the effect of trap design and height on captures of sap beetles. In the first experiment, nine different trap designs were compared in an apple orchard where beetles were present. Traps were baited with whole-wheat bread dough, and results showed that the Lindgren funnel trap (funnel-shaped trap) was the most effective and the McPhail trap (glass invaginated trap) caught the fewest beetles. In the second experiment, the number of beetles collected per trap decreased as trap height increased. Also, trap height differed for the same species in two different habitats suggesting that sap beetle presence varied with the nature of the habitat (Peng and Williams 1991).

In a study investigating trap design and attractants, James et al. (1996a) found that water-based funnel traps with aggregation pheromone and fermenting bread dough caught 3-7 fold as many *Carpophilus* spp. beetles than wind-oriented pipe traps or dry funnel traps. In another study comparing trap design Williams et al. (1993) studied the effectiveness of three trap types including the wind-oriented pipe trap (T-shaped trap made of PVC in which insects are collected in a plastic cup at the bottom of the pipe), the Japanese beetle plastic trap and the nitidulid inventory technique trap (consisting of a canning jar with a plastic cone inserted in the mouth of the jar) and found that the nitidulid inventory trap was the most effective (Williams et al. 1993).

Although several different trap types and pheromones have been found to be effective against sap beetles, many of these experimental traps and pheromones are not

commercially available. In a study comparing commercial and experimental traps with pheromones, Dowd (2005) found that commercial traps and pheromones were as effective as experimental counterparts.

Sap beetles over-winter in woodland peripheries; therefore, the use of mass trapping may be effective at disrupting the migration of adults from woodland and surrounding areas to strawberry fields when fruits begin to ripen (Rhainds and English-Loeb 2002). Rhainds and English-Loeb (2002) placed traps baited with bread dough in the center of strawberry rows and on the periphery, and recorded similar captures of beetles early in the season from both locations. However, captures of sap beetles were highest in peripheral traps after strawberry fruits began to ripen. Traps placed at the periphery of the strawberry field captured many adults but did not reduce infestation of fruits by larvae in the field, indicating that mass trapping of adults with food baited traps is not a viable management strategy but may be used for monitoring purposes (Rhainds and English-Loeb 2002).

Control

Sap beetle population control in strawberries includes biological, cultural, and chemical management strategies that when used together make up an effective integrated pest management program for strawberries.

Biological

A few parasitic wasps have been found to effectively parasitize sap beetles in the family Nitidulidae. In laboratory assays, Weiss and Williams (1980) explored host-parasite relationships of an endoparasitic wasp, *Microctonus nitidulidis* Loan (Hymenoptera: Braconidae) and the strawberry sap beetle, *S. geminata.* Strawberry sap beetle egg production was greatly reduced by parasitism, while fertility of beetle males

was not affected. High rates of parasitism reduced oviposition rates by 2/3 suggesting that *M. nitidulidis* may be a promising biocontrol agent for sap beetle management that needs to be further explored. Other parasitic wasps include *Brachyserphus abruptus* (Say), *M. nitidulidis*, and *Zeteticontus insularis* (Howard). *Z. insularis* can successfully parasitize the sap beetle *L. insularis, which* is a common pest of strawberries in Florida (Coler et al. 1986).

A new species of nematode may be a promising biological control agent for sap beetles. *Psammomermis nitiduesis* was found in 80 percent of the sap beetles taken from a field in Peoria, Illinois in early spring (Lyons-Johnson 1997). Subsequently, biological control involving nematodes may be a plausible strategy in the future.

Although biological control for sap beetles is a promising component of an IPM program, there are no biological control agents commercially available at this time.

Cultural

The most important cultural strategy for managing sap beetle damage in strawberries is to harvest all fruit as soon as they are mature. Another management tactic is dropping ripe and fermenting fruits into center rows where they decay and allow beetles to complete their life cycle (Mossler and Nesheim 2004). This strategy works well when populations of sap beetles are relatively low but it is ineffective when populations have surpassed a threshold density.

Chemical

Currently, five insecticides are used to control sap beetles although several other classes of insecticide are labeled for use in strawberries. These include bifenthrin (Capture 2E), diazinon, pyrethrins, carbaryl (Sevin), and Malathion (Mossler and Nesheim 2004). Malathion has been the standard material of choice for control of sap

beetles and has been used successfully in commercial fields for several years. However, sap beetle resistance to Malathion may be on the rise (Williams et al. 1984). Kehat et al. (1976) reported that stronger concentrations of Malathion were necessary for moderate control. Applying more than the recommended rate of organophosphate insecticides is especially detrimental to non-target organisms. Therefore, it is important to find less harmful insecticides to control this pest.

In another study, Dowd et al. (2000) compared the efficacy of two conventional compounds aerially applied for the control of sap beetles in high amylose corn. Results showed that Malathion granules can be as effective as commercial formulations in controlling sap beetles. Numbers of beneficial coccinellid beetles and predators were significantly higher after treatment with Malathion granules compared with chlorpyrifos granules. Low levels of active ingredients and low toxicity of Malathion granules provides environmental advantages over commercial formulations of other insecticides (Dowd et al. 2000).

Rhainds and English-Loeb (2002) studied the impact of insecticide application on infestation of strawberry sap beetles. Five treatments were evaluated but they found that treatments with fenpropathrin reduced larval infestation but did not affect the capture of adults in food-baited traps. Results also indicated that insecticide treatment reduced larval infestation for fruits on the ground when compared with infestation in the plant canopy, suggesting that the dense leaf cover of strawberry plants may prevent insecticide from reaching the ground.

Several new classes of insecticides have been registered for use in small fruit crops. With the potential loss and/or restriction of organophosphate insecticides through the

1996 Food Quality Protection Act (FQPA), there is an increasing need to expand the spectrum of control for these newer insecticides to cover as many pests as possible. Three relatively new insecticides include: 1) imidacloprid, Provado 1.6 F (Bayer Cropscience Kansas City, MO), 2) thiamethoxam, Actara 25 WG (Syngenta Crop Protection Wilmington, DE) and 3) spinosad, SpinTor 2SC (Dow, Agrosciences, Carmel, IN). These insecticides are effective in controlling a broad range of insects in a wide variety of crops giving them the potential to replace many conventional chemicals. Thiamethoxam and spinosad are classified as reduced risk and may conserve natural enemies that regulate other key pests in the strawberry ecosystem.

Imidacloprid is a neonicotinoid in the chloronicotinyl subclass. It provides protection to a broad range of crops and suppresses damage of sucking insect pests. It is effective against many insects that are resistant to commercially used insecticides and provides immediate and residual control on contact and systemically through the plant. The short duration of residue on the leaf surface results in low activity of this insecticide on parasitoid natural enemies (Wilkinson 2002).

Thiamehoxam is a second-generation neonicotinoid compound with stomach and contact activity. It belongs to the subclass thianicotinyl of the neonicotinoid insecticides, which interferes with the nicotinic acetylcholine receptors in the insect nervous system (Maiensfisch et al. 2001). As a foliar, soil application, or as seed treatment, it has strong systemic and translaminar activity that allows it to kill insects on the underside of leaves. It can be used on several plants for a broad range of commercially important sucking pests and chewing pests such as beetles and lepidopteran larvae (Torres et al. 2003).

Spinosad is a broad-spectrum insecticide used in a variety of crops. Its new mode of action allows for low human toxicity. It is very compatible with IPM and resistance management programs. It is derived from the actinomycete *Saccharopolyspora spinosa* and acts in conjunction with the neurotransmitter acetylcholine to prolong insect neural responses, which are often witnessed as twitching and paralysis (Salgado 1997).

Although there is sufficient information on the interaction of sap beetles and host and non-host volatile compounds and their chemical compositions for many species, there is not much information available on those sap beetles that are pests of strawberry crops in Florida. These gaps will now be investigated in this thesis.

CHAPTER 3
FIELD EFFICACY AND CHEMICAL COMPOSITION OF HOST AND NON-HOST VOLATILES ATTRACTIVE TO SAP BEETLE PESTS OF STRAWBERRIES

Strawberries are an important high value crop in Florida. The state is the second largest producer of strawberries in the nation with over 6,178 acres and an annual profit exceeding $168 million. Currently, several key arthropod pests threaten strawberry production. These include twospotted spider mites and sap beetles. Biological control programs are currently being developed for twospotted spider mites. In this chapter, our aim is to investigate the potential to monitor sap beetles using host and non-host volatile attractants. In addition, a subsequent objective is to gain a better understanding of sap beetle ecology in strawberries. Sap beetles cause direct and indirect damage to strawberry fruit (Liburd and Finn 2004).

Currently, production practices utilize large amounts of pesticides including organophosphates to control sap beetles and other key pests. This practice has been effective for some time; however increasing pressure from environmentalists and the general public coupled with the development of resistance against "mainstay" organophosphate insecticides have resulted in new research initiatives to investigate potential alternatives to broad-spectrum insecticides.

Some studies have successfully used baited traps to capture and monitor sap beetle adults (Foot and Hybsky 1976). However, there is still conflicting information on whether trapping is an effective management tool to control sap beetles. Studies have suggested that baited traps may increase the number of sap beetles in the production

system (Rhainds and English-Loeb 2002), while others have shown that mass trapping may be an effective control measure for sap beetle pests (Foot and Hybsky 1976). A better understanding of sap beetle responses to baited traps is essential to develop effective management strategies to control this pest.

A few host and non-host volatiles for sap beetle pests have been identified. Volatile components varied among species and host; however, general components and blends that are attractive to sap beetles include alcohols, esters, and acids. Although some of these volatiles have been identified, little research has been conducted on host and non-host volatile attractiveness to sap beetles of strawberries in Florida. Determination of bait attractiveness in the field as well as identification of attractive components of these baits is important when developing an effective monitoring system for sap beetle control in strawberries.

Specific objectives for this research were to compare the attractiveness of host and non-host baits in the field, as well as to investigate trapping location within the field and in adjacent woodland areas. Differences in sap beetle captures among baits inoculated with a fungus of sap beetles were compared with aseptic baits. Finally, a comparison of the chemical components of these baits was made to identify candidate attractants.

Materials and Methods

Field Site and Experiments

Field research was conducted at the University of Florida, Plant Science Research and Education Unit located in Citra, Florida. Strawberries, *Fragaria x ananassa* Duschene., cv. 'Strawberry Festival' were planted on raised beds. Research was conducted in two fields each consisting of 24 plots in 2004, and 28 plots in 2005. Each

plot contained six rows of strawberry plants spaced approximately 0.3 m apart. Plots measured 7.3 X 6.1 m with a buffer of 7.3 m between plots.

Two types of field experiments were conducted. An initial experiment was designed to track the movement of sap beetles into strawberry fields and a second experiment was designed to evaluate attractants (host and non-host compounds).

2004 tracking the movement of sap beetles

This experiment was conducted early in the field season (1/26/04-2/23/04) when strawberry fruit were beginning to ripen and fermented fruits were absent from the field. The trap used to track the movement of sap beetles was designed based on a model from the USDA-ARS laboratory in Gainesville, Florida. The trap consisted of two white 1.0 L plastic buckets (one inside of the other) with the bottom cut out of the inner bucket (Fig. 3-1). Two windows approximately 10.2 x 5.1 cm were cut on each side of the trap and fitted with an aluminum wire mesh to allow sap beetles to enter. The inside of each trap contained a wire mesh funnel approximately 14 cm in diameter, which directs sap beetles to the bait and keeps larger organisms from entering into the trap. A string was glued to the trap to allow for hanging and a plastic top was placed on the trap to keep water from entering. Holes were created at the bottom of the outer bucket to allow for drainage.

Each trap was baited with a pollen dough mixture (B. Torto unpublished data) that has been shown to attract sap beetles in previous studies. Approximately 80 g of pollen dough was placed inside a cotton stockinette and tied off with rubber bands on each end. The stockinette containing pollen dough was placed inside the trap (Fig. 3-2). A water source (15 ml of deionized water) was also provided in small plastic vials with a 1-cm hole punched through the snap cap. Cotton dental gauze (Richmond Dental, Charlotte,

NC) was placed through the hole of the cap into the water source. Baits and water sources were changed weekly.

Treatments for this experiment included: 1) traps located on the periphery of the field [0.2 m from the woods], 2) traps located between strawberry plots, and 3) traps located within plots. Treatments were replicated three times and were set up in a completely randomized block design. Traps were placed at least 30.5 m apart and were anchored inside the soil. Traps were checked weekly for the presence of sap beetles. Total sap beetle numbers were recorded and sap beetle adults were identified at the Department of Plant Industry in Gainesville, Florida.

2005 evaluation of attractants

Experiments to evaluate attractants were conducted from early February through March 2005. Experiments consisted of four treatments that included traps baited with 1) pollen dough, 2) pollen dough fed upon by larvae of *L. insularis,* 3) ripe strawberries, 4) ripe strawberries fed upon by *L. insularis*, and 5) control (unbaited traps). Treatments were arranged in a completely randomized block design among strawberry plants and were replicated 4 times (Fig. 3-3). Traps identical to those used in 2004 were used in this study. Also, traps were baited by placing the bait into a cotton stockinette as described above. Water was provided as described above. Baits and water sources were changed weekly. Traps were checked weekly and the number of sap beetles was counted and recorded for eight weeks during the growing season.

Sap beetle response to attractants in harvested and un-harvested strawberries

After four weeks traps containing the most attractive treatment (pollen dough fed upon by larvae of *L. insularis)* were placed in harvested and un-harvested strawberry

plots. Trap catches from harvested plots were compared with un-harvested plots to track sap beetle response.

Statistical analyses

All data from sap beetle field counts were analyzed by repeated measures Analysis of Variance using the SAS GLM procedure. All data were square-root transformed to stabilize variances, and means separated with least significant differences (TUKEY) $\alpha = 0.05$ (SAS Institute Inc. 2002). Data reported in the tables and figures represented untransformed means ± standard errors.

Laboratory Experiments

Rearing protocol

Since sap beetles propagate fungi, field-collected larvae or adults (already exposed to fungi) can be used to inoculate substrates through feeding. Adults of *L. insularis* were collected from traps near bee hives in High Springs, FL. A colony was reared for inoculation purposes and was kept in an insect growth chamber at 26.7° C with 14:10 L:D and 70% RH (Fig. 3-4A). All stages of the insect were fed on a mixture of fresh strawberries and pollen dough. A water source was also provided using a small plastic vial with a hole punched through the cap. Cotton dental gauze was placed through the hole and deionized water was added to the container. Adults were kept separate in mason jars. Eggs were laid on wax paper (Fig. 3-4B) and were removed every two days. Eggs were then placed in larval containers with food and water (Fig 3-4C) When larvae reached the 'wandering stage' they were set onto autoclaved soil. One hundred to 300 larvae were placed in rearing boxes containing autoclaved soil (Fig 3-4D) (approximately 9% moisture), a food source, and a water source. At the end of the pupal stage, they were harvested from soil and sexed (Fig. 3-4E). Fifty to 75 insects were placed in Petri dishes

lined with filter paper. A small piece of dental gauze, moistened with deionized water, was taped to the top of the Petri dish to provide moisture (Fig. 3-4F). After adults eclosed they were placed in mason jars. Virgin males and females were sexed using standard procedures based on their abdominal tip characters.

Inoculation

Approximately 1,500 larvae of *L. insularis* approximately one day old were used for fermentation of pollen dough and strawberries. Buckets were filled with approximately 6 L of each treatment. Two buckets containing 6L of pollen dough and 2 buckets containing 6 L of strawberries were used. The weight of the bucket of pollen dough and strawberries was determined prior to inoculation (strawberry ~ 2.3kg, pollen dough ~6.4 kg). Strawberries were cut in quarters to maximize surface areas exposed to fermentation and leaves were removed from fruit. The contents of each bucket to be fermented were divided in half and spread around the bottom of a 22-gallon Rubbermaid container with a screened top. A water source was provided only in pollen dough containers. This water source was not needed in strawberry containers. All containers were then placed in a growth chamber maintained at 26°C and 90% RH.

Larvae were used for fermentation instead of adults because of their ability to swim and move through the substrate. Twenty-four hours after inoculating treatments with larvae, aluminum foil with six holes punched with a paper clip was placed under the lid of each container to provide darkness and allow for maximum fermentation. Pollen dough was sprayed with 5 mL of distilled water. Larvae were allowed to feed for one week and then they were removed from the substrate.

Treatment preparation

Bait bags were then prepared with one ice cream scoop of treatment that was placed into the cotton stockinette and tied off with rubber bands. Each bag weighed approximately 50-80 g. Treatment bags were prepared and frozen at -68 °C) until used for the field trapping experiments. The frozen treatments were removed to thaw overnight before use. Control traps contained only a water source, 15 ml in a vial.

Volatile collection and analysis

Volatiles were collected and analyzed using equipment located at the USDA-ARS CMAVE in Gainesville, Florida (Fig. 3-5). Volatiles were collected from the same attractants evaluated in the field and analyzed. Treatments included: 1) pollen dough, 2) pollen dough fed upon by larvae of *L. insularis,* 3) ripe strawberries, 4) ripe strawberries fed upon by *L. insularis*, and 5) control. Each treatment was replicated three times. Treatment bags (as described above) were placed into quick-fit glass chambers (30-cm-long x 3-cm-OD). Volatiles were collected on Super Q filters by pulling charcoal-filtered and humidified air through the traps at 500 ml/min for 2 h. Each filter was eluted with 250µl of GC/GC-MS-grade dichloromethane (Burdick and Jackson, Muskegon, MI), and the eluents stored at -68 °C prior to analysis by coupled gas chromatography (GC)-mass spectrometry (MS). This was done on a HP-6890 GC coupled to a HP5973 mass spectrometer (Electron impact mode, 70eV, Agilent, Palo Alto) equipped with a HP-1 column (30 m x 0.25 mm ID x 0.25 µm, J & W Scientific, Folsom, CA). The volatiles from pollen dough fed upon by a field-collected sap beetle were also analyzed for comparison. For the analysis, 40 ng of octane and 80 ng of nonyl acetate were added to 40 µl of each volatile extract and 1 µl was analyzed. Peak areas of the components were

integrated and the total areas of volatiles analyzed compared with that of the internal standards. The components in the volatiles were identified by comparing their mass spectral data with those in the library (NIST, 98K) of the mass spectrometer.

Statistical analyses

All data from the volatile collections were analyzed by repeated measures Analysis of Variance using the SAS GLM procedure. All data were log (x+1)-transformed to stabilize variances, and means separated with least significant differences (TUKEY) $\alpha = 0.05$ (SAS Institute Inc. 2002). Data reported in the tables and figures represent untransformed means ± standard errors.

Results

2004 Tracking the Movement of Sap Beetles

For the first three weeks there were no significant differences among treatments. In week 4 there were significant differences among all the treatments evaluated. Traps located on the periphery (near the woods) captured the highest number of sap beetles (Table 3-1 and Fig. 3-6). This treatment was significantly different from all other treatments that consisted of traps located within the plots and between the plots ($F = 36.5$; df = 2:4; $P = 0.0027$). Traps located within rows also captured significantly more sap beetles than traps located between plots. Throughout the trapping period (1/26/04-2/27/04) traps located on the periphery (near the woods) captured significantly more beetles than other treatments ($F = 5.8$; df = 2:28; $P = 0.0081$) [Table 3-1]. Overall, traps on the periphery captured 5-fold more beetles than any other treatment.

Among the sap beetles captured, *Urophorus humeralis* (F.) was the most dominant species ~ 30% of the beetles caught. Other species captured include,

Colopterus truncatus (Randall), *Epuraea luteolus* (Erichson), *Cryptarcha ampla* Erichson and *Carpophilus* sp.

2005 Evaluation of Attractants

Throughout the trapping period (2/9/05-3/30/05) all of the treatments evaluated captured significantly more sap beetles than the control ($F = 9.8$; df = 4:145; $P < 0.0001$) [Table 3-2]. There were no significant differences in captures among the different types of bait used for the overall trapping period. Sap beetle capture varied among weeks (Fig. 3-7). During the first week, traps baited with ripe strawberries captured significantly more sap beetles than other treatments ($F = 27.9$; df = 4:12; $P < 0.001$) [Table 3-3]. During week 2, traps baited with ripe strawberries and ripe strawberries fed upon by *L. insularis* captured significantly more sap beetles than the control ($F = 5.7$; df = 4:12; $P = 0.009$). During week 4 traps baited with strawberry fed upon by *L. insularis* larvae were weakly attractive ($F = 3.0$; df = 4:12; $P = 0.0618$). During weeks 3, 5 and 7 none of the treatments were significantly different. During week 6 there were no significant differences in the captures of sap beetles between baited traps. However, baited traps captured significantly more sap beetles than the control ($F = 11.2$; df = 4:12; $P = 0.0005$). During week 8, traps baited with pollen dough captured significantly more sap beetles than strawberries fed upon by *L. insularis*, fresh strawberries, and the control ($F = 14.3$; df = 3:12; $P = 0.0002$) [Table 3-4]. There were no significant differences between strawberries fed upon by *L. insularis*, ripe strawberries, and the control.

Harvested Versus Un-harvested Plots

Traps placed in un-harvested strawberries and baited with pollen dough captured 2.2-times more sap beetles than those placed in plots that were harvested weekly.

Overall, these traps captured significantly more sap beetles than traps placed in harvested plots ($F = 4.5$; df = 3:24; $P = 0.04$) [Figure 3-8].

Volatile Collection and Analysis

Overall, the total areas of the volatile profiles of baits that contained strawberries either fed upon or not by *L. insularis* were higher than that of the control treatment ($F = 4.4$; df = 4:8; $P = 0.0355$) [Fig 3-9]. There were no significant differences in the peak areas of volatiles between other treatments evaluated.

GC-MS identified components in the volatiles of the attractive treatments as mainly alcohols, fatty acids and esters (Table 3-5). Additionally, aldehydes, ketones, hydrocarbons, terpenoids, and nitrogenous and sulfur derivatives were present in these volatiles. Overall, the compositions of the volatiles released by the baits fed upon by *L. insularis* were compositionally richer than those that were released by the baits not fed upon by the sap beetle (Table 3-5). Forty-one components were identified in the volatiles from the pollen dough fed upon by *L. insularis*, 32 components were identified in the volatiles released by the pollen dough only. Similarly, 47 components were identified in the volatiles of strawberries fed upon by the beetle, while 44 components were identified in the volatiles of the ripe strawberries. From the volatiles of pollen dough fed upon by the field collected sap beetle, 35 components were identified.

Discussion

Tracking the Movement of Sap Beetles

During the 2003-2004 field season, results from trap catches showed that traps near the woods, along the periphery of the strawberry field caught more sap beetles compared with other treatments. These results are consistent with those obtained by Rhainds and English-Loeb (2002) who reported larger numbers of sap beetles in traps along the border

of the field compared with traps within the field. Since sap beetles are believed to over-winter in wooded peripheries, it is possible that traps placed adjacent to wooded areas may intercept over-wintering sap beetles moving into strawberry fields. Therefore, it is possible that traps located on the periphery of the fields near the woods may be effective in reducing sap beetle populations by disrupting migration to strawberry fields.

During weeks 1-3 there were no significant differences among the number of sap beetles caught in different locations. However, in week 4 baited traps placed by the woods caught significantly more sap beetles than other treatments. These results suggest that there is a positive interaction between timing and trap location. Sap beetles are probably responding to increases in the concentration or the amount of host volatile as the strawberries mature. Rhainds and English-Loeb (2002) showed similar results in which traps located on the border of strawberry fields and within strawberry fields captured similar numbers of sap beetles early in the season but were highest in border traps once the fruit began to ripen. Low overall captures early in the season suggest that beetles do not inhabit strawberry fields before fruit begin to ripen. This may be because fruit fermentation odors are not strong enough early in the season to elicit sap beetle migration from wooded areas.

Five different species of nitidulids were recorded from trap catches. The dominant species was *U. humeralis*. Our findings were different to those of Potter (1995) who recorded 9 different species. Differences in locality (Hillsborough County, versus Marion County, Potter 1995), may account for our findings. The only species we found that Potter (1995) recorded was *Carpophilis* spp.

Evaluation of Attractants

Overall, all treatments evaluated during the 2004-2005 field season were more effective in capturing sap beetles than the control. However, there were no differences in trap captures among other treatments. Blackmer and Phelan (1995) found similar results when three out of four predominant sap beetle species were attracted to all baits tested. In their study, they found minimal preference among baits including: strawberry, banana, tomato, maize, and whole wheat bread dough. Weekly data were inconsistent throughout the trapping period. Traps containing strawberries captured more sap beetles in week 1 than any other treatment while traps baited with pollen dough fed upon by *L. insularis* caught the most sap beetles in week 8.

As in the 2003-2004 field season, phenology seems to play an important role in attractant choice. During the first week of trapping, ripe and fermenting fruits were unavailable and traps containing strawberries captured most of the sap beetles. It is possible that when the fruit began to ripen the beetles were more attracted to their natural host. These results are consistent with Blackmer and Phelan (1995) who found that when maize kernels were full, attraction to maize baits virtually ended in three out of four cases.

Although there were no significant differences in the attractiveness of the different lures, it is possible that the lure prepared from pollen dough may be more suitable for use in the field since the presence of honey slows the decomposition process which may prolong the release of attractants for a longer period than the fresh fruit.

Although previous data had suggested that hosts fed upon by sap beetles were more attractive than treatments that were not fed upon (Zilkowski et al. 1999, and Bartelt and Wicklow 1999), the attractiveness of the different treatments fed upon by *L. insularis*

to sap beetles did not differ significantly from those of aseptic treatments in the field. It is possible that treatments, which were fed upon fermented too quickly and did not last very long in the field. However, we cannot rule out the possibility that different species of sap beetles may prefer different stages of host and non-host fermentation. There were no significant differences in attractiveness of pollen dough fed upon by *L. insularis* (colony reared) or that of the field collected sap beetle suggesting that if baits were to be inoculated either source of sap beetles could be used.

Harvested Versus Un-harvested Plots

Un-harvested plots had consistently more sap beetles than harvested plots. These results were expected since un-harvested plots contained more fermenting fruits (an abundance of host volatiles) and many sap beetles are attracted to fermenting plant juices (Potter 1994).

Volatile Collection and Analysis

The composition of volatiles of treatments fed upon by *L. insularis* and the field collected sap beetle were different from the volatiles of the treatments without the beetles. They differed both in quantity and quality. Generally, a larger number of esters were released in the volatiles of the treatments fed upon by the beetles compared with asceptic counterparts. Clearly, the major difference between the volatiles released by the pollen dough treatments was the presence of fermentation-related products, including 3- and 2-methyl-1-butanol and fatty acids in the volatiles of pollen dough fed upon by the different sap beetles.

Many of the same components or classes of compounds found in our study have been found to be attractive to nitidulids in previous studies including alcohols, ketones, esters, hydrocarbons, and nitrogenous compounds (Chapter 2). 3- and 2-methyl-1-

butanol were found in our analysis in treatments fed upon by larvae of *L. insularis* and the field-collected sap beetle, as well as ripe strawberries but not in pollen dough treatments. These same components were found in volatile profiles of the fungi *C. fagacearum and* F. *verticillioides* (Lin and Phelan 1992, Zilkowski et al. 1999). This implies that our treatments containing these compounds may have been inoculated by a fungus. Also, it is possible that ripe strawberries were inoculated by beetles in the field before they were collected for analysis.

Our hypothesis was that treatments inoculated via feeding by *L. insularis* would have larger profile areas and thus be more attractive in baited traps. However, the results showed that this appeared not to be the case. We cannot rule out the possibility that fresh strawberries taken from the field could have been previously inoculated by field insects causing the release of volatiles which might have contributed to the overall profile area. Also, all the baits were prepared at the beginning of the season and then frozen. Baits were thawed weekly which could have expedited the fermentation process. This is especially the case with the strawberry treatments since the fruit is more prone to fermentation than pollen dough. Also, strawberries and strawberries fed upon had larger quantities of fatty acids, alcohols, and esters that could serve as the candidate attractants in the bait treatments, consistent with previous results obtained for other sap beetles (Bartelt and Wicklow 1999).

Also, timing of volatile release from bait treatments may have played a role in bait attractiveness. Baits were changed weekly, however there may have been differences in volatile release among bait treatments. For example, since pollen dough treatments contain honey, which slows down the fermentation process release of volatiles may have

been prolonged in these treatments while volatile release of strawberry treatments may have occurred within just a few days after placement in the field. Less frequent replacement of baits may be important in ascessing the effectiveness of attractants so that volatiles from all attractants are released before renewal. James et al. (1998) found that renewing multispecies pheromone lures every 2 weeks instead of weekly is an effective method of trapping for sap beetles.

In summary, there were no significant differences overall in the attractiveness among bait treatments in the field. However, all treatments were significantly different from the control and therefore may have potential for development for use in strawberry pest management programs for sap beetles. Additionally, since significant differences among treatments were inconsistent weekly, phenology including fruit development, geography, and weather conditions may be important in bait selection and placement. Volatile analysis showed that many of the same components found to be attractive to sap beetles in previous studies were present in bait attractants.

Figure 3-1. Sap beetle trap used in experiments to evaluate tracking and movement of sap beetles and evaluation of attractants.

Figure 3-2. Trap containing treatment bag and water source.

Figure 3-3. Trap placed among strawberry plants.

Figure 3-4. Maintaining the colonies. A) sap beetle colonies, B) mason jar with wax paper egg collectors, C) larval rearing container, D) autoclaved soil, E) separation of pupae from soil, and F) sexed pupae in Petri dishes.

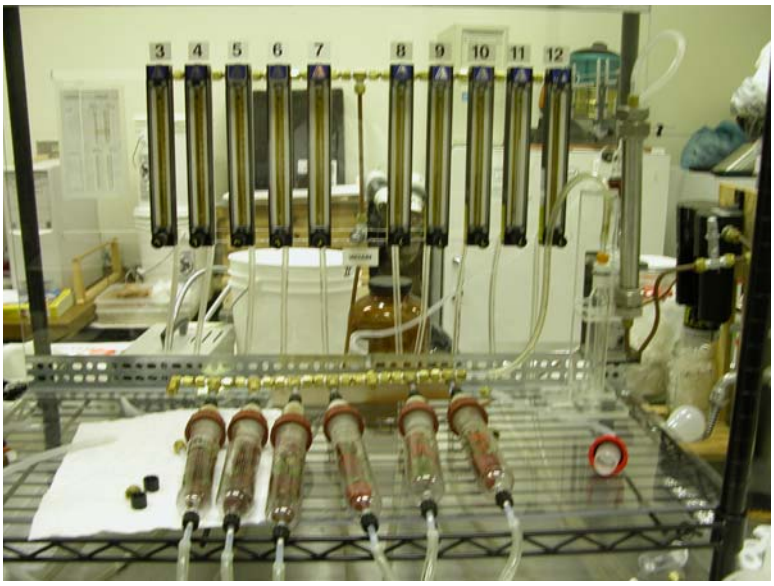

Figure 3-5. Volatile collection system used for volatile collection and analysis of bait treatments.

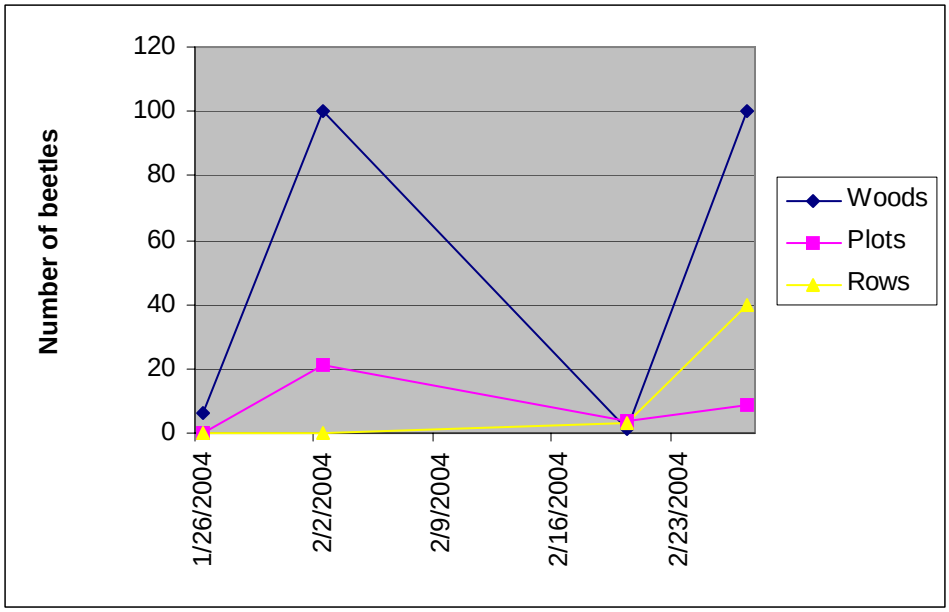

Figure 3-6. Total number of sap beetles captured in a strawberry field, Citra, FL (2005).

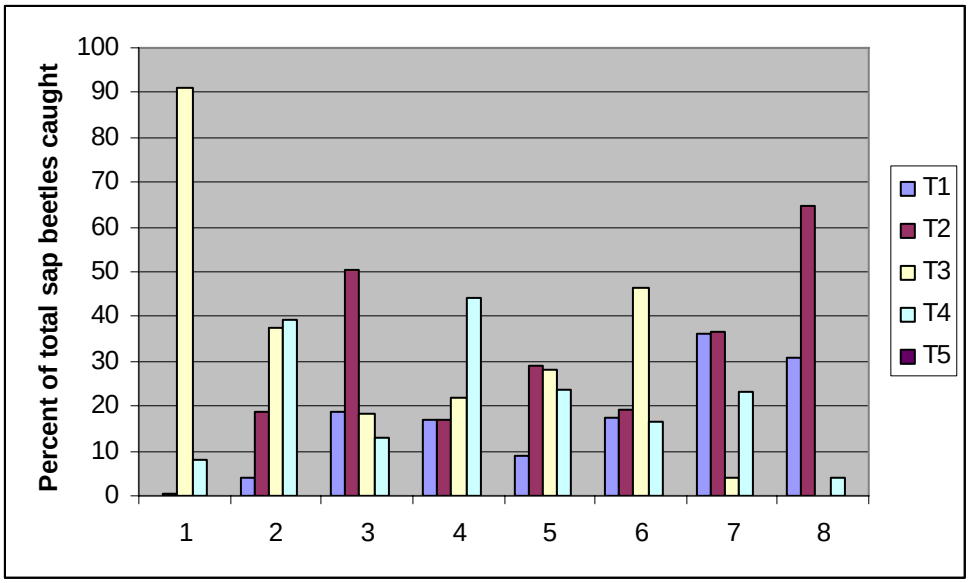

Figure 3-7. Percent of total sap beetles captured in a strawberry field, Citra, FL (2005). (T1 = pollen dough, T2 = pollen dough fed upon by larvae of *L. insularis*, T3 = ripe strawberries, T4 = ripe strawberries fed upon by larvae of *L. insularis*, and T5 = control)

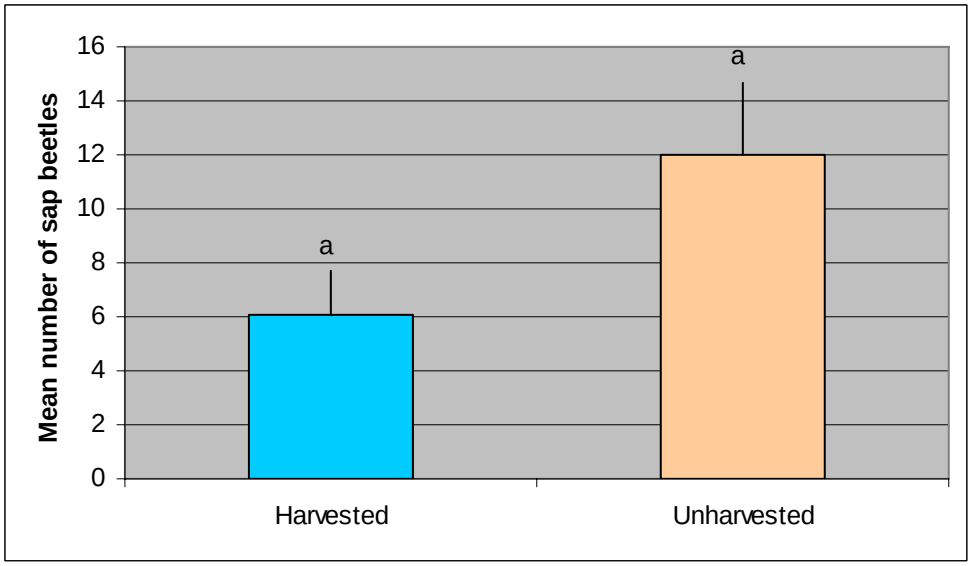

Figure 3-8. Mean ± SEM number of sap beetles in harvested and un-harvested plots of strawberries, Citra, FL (2005).

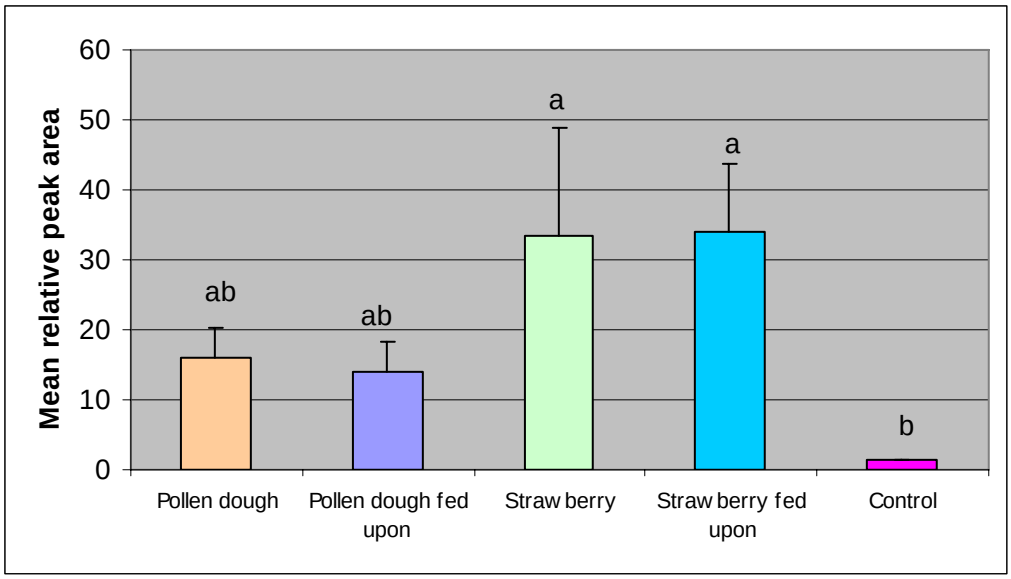

Figure 3-9. Mean ± SEM relative peak areas for bait treatments. Mean peak areas were calculated relative to internal standards octane and nonyl acetate. Means followed by the same letter are not significantly different ($P = 0.05$, TUKEY test).

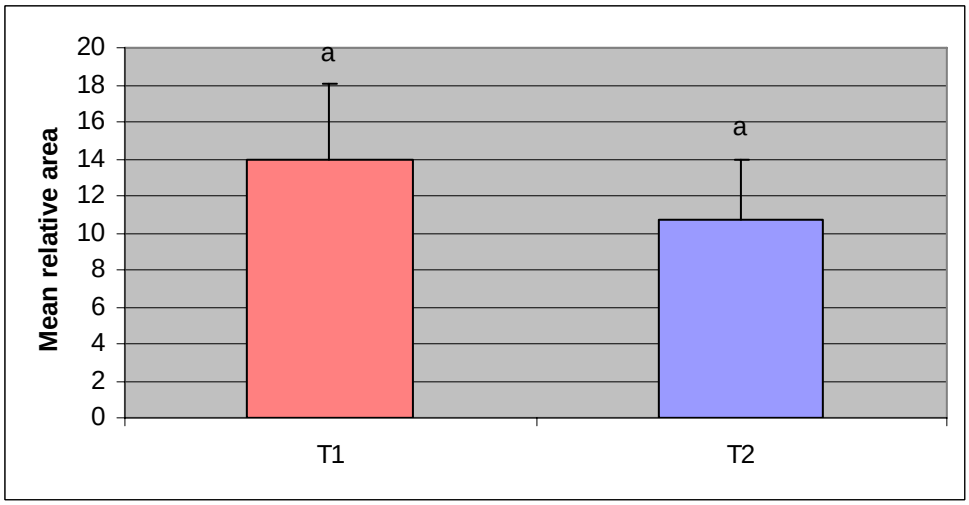

Figure 3-10. Mean ± SEM relative peak areas for treatments 1 and 2. Mean areas were calculated relative to areas of internal standards octane and nonyl acetate. (T1 = pollen dough fed on by larvae of *Lobiopa insularis* and T2 = pollen dough fed on by larvae of a field collected sap beetle).

Table 3-1. Mean ± SEM number of sap beetle adults in strawberries, Citra, FL (2004).

Treatments	Week 1	Week 2	Week 3	Week 4	Total Captures
Woods, Periphery	2.0 ± 1.2	27.0 ± 17.6	0.3 ± 0.3	33.3 ± 4.4 a	15.7 ± 5.9 a
Between Plots	0.0 ± 0.0	7.0 ± 3.6	1.7 ± 0.7	0.3 ± 0.6 b	2.9 ± 1.1 b
Within Plots	0.0 ± 0.0	0.0 ± 0.0	1.0 ± 0.6	13.3 ± 1.8 c	3.6 ± 1.8 b

Means followed by the same letter are not significantly different ($P = 0.05$, TUKEY test)

Table 3-2. Mean ± SEM number of sap beetle adults in strawberries, Citra, FL (2005).

Treatments	Total Captures
Pollen Dough	5.6 ± 1.3 a
Pollen Dough fed upon by *L. insularis* larvae	9.5 ± 2.3 a
Strawberry	14.1 ± 4.5 a
Strawberry fed upon by *L. insularis* larvae	5.8 ± 1.3 a
Control	0.0 ± 0.0 b

Means followed by the same letter are not significantly different ($P = 0.05$, TUKEY test)

Table 3-3. Mean ± SEM number of sap beetle adults in strawberries, Citra, FL (2005) weeks 1-4.

Treatments	Week 1	Week 2	Week 3	Week 4
Pollen Dough	0.0 ± 0.0 b	1.0 ± 1.0 ab	8.2 ± 4.6	1.8 ± 0.9 ab
Pollen Dough fed upon by *L. insularis* larvae	0.3 ± 0.3 b	4.5 ± 4.5 ab	22.6 ± 12.9	1.8 ± 1.4 ab
Strawberry	69.0 ± 18.0 a	9.0 ± 1.4 a	8.0 ± 3.6	2.3 ± 1.7 ab
Strawberry fed upon by *L. insularis* larvae	6.8 ± 2.8 b	9.5 ± 2.8 a	5.8 ± 1.9	4.5 ± 2.0 a
Control	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0	0.0 ± 0.0 b

Means followed by the same letter are not significantly different ($P = 0.05$, TUKEY test)

Table 3-4. Mean ± SEM number of sap beetle adults in strawberries, Citra, FL (2005) weeks 5-8.

Treatments	Week 5	Week 6	Week 7	Week 8
Pollen Dough	0.8 ± 0.8	7.5 ± 1.9 a	14.0 ± 6.2	11.3 ± 3.8 ab
Pollen Dough fed upon by *L. insularis* larvae	2.5 ± 1.3	8.3 ± 3.7 a	14.3 ± 6.4	22.3 ± 3.9 a
Strawberry	3.3 ± 1.4	20.3 ± 9.6 a	1.5 ± 0.7	0.0 ± 0.0 c
Strawberry fed upon by *L. insularis* larvae	2.0 ± 2.0	7.0 ± 2.2 a	9.0 ± 9.0	1.5 ± 1.5 bc
Control	0.0 ± 0.0	0.0 ± 0.0 b	0.0 ± 0.0	0.0 ± 0.0 c

Means followed by the same letter are not significantly different ($P = 0.05$, TUKEY test)

Table 3-5. Volatiles present in bait attractants.

	T1	T2	T3	T4	Field SB	T5
ALCOHOL						
3-methyl-1-butanol		+	+	+	+	
2-methyl-1-butanol		+	+	+	+	
2-methyl-3-pentanol			+			
2-methoxy-4-penten-2-ol					+	
1,3-butaediol	+	+			+	
2,3-butanediol	+	+			+	
phenylethanol	+			+	+	
ALDEHYDE						
benzaldehyde	+	+	+	+	+	
hexanal					+	
nonanal	+	+			+	trace
decanal	+				+	
phenylacetaldehyde		+				
Lilac aldehyde A					+	
Lilac aldehyde B					+	
Lilac aldehyde C					+	
KETONE						
acetophenone			+	+		
3-hydroxy-2-butanone		+				
3-hydroxy-2-methyl-2-butanone					+	
2-nonanone	+		+			
2-heptanone			+	+		
2-undecanone		+	+	+		
(E,E)-3,5-octadien-2-one	+	+				
FATTY ACID						
propionic acid		+	+	+		
butanoic acid				+		
2-methylpropionic acid				+		
3-methylbutanoic acid		+	+	+	+	
2-methylbutanoic acid		+	+	+	+	
4-hydroxybutanoic acid					+	
cyclopropanecarboxylic acid			+			
pentanoic acid		+		+	+	
hexanoic acid			+	+		
2-methylpentanoic acid					+	
ESTER						

Table 3-5. Continued.

	T1	T2	T3	T4	Field SB	T5
ethyl butanoate			+	+		
ethyl-2-methyl propanoate		+				
ethyl-2-methyl butanoate			+	+		
ethyl hexanoate	+	+	+	+		
ethyl heptanoate		+				
ethyl tiglate				+		
ethyl 2-butenoate				+	+	
ethyl octanoate	+	+	+	+		
ethyl nonanoate	+	+				
ethyl decanoate	+	+				
ethyl dodecanoate		+	+	+		
ethyl hexadecanoate		+				
ethyl phenylpropanoate			+	+		
ethyl benzoate	+					
ethyl phenylacetate	+	+		+		
ethyl cinnamate			+	+		
ethyl-2-hydroxy propanoate	+	+		+	+	
2-methylpropanyl acetate			+	+		
3-methyl-1-butanyl acetate			+	+		
2-methyl-1-butanyl acetate			+	+		
3-methyl-1-butanyl propanoate			+			
methyl butanoate				+		
methyl hexanoate			+	+		
n-propyl acetate			+	+		
propyl hexanoate			+	+		
hexyl acetate			+	+		
2-phenylethyl acetate		+	+	+		
3-phenyl-1-propanyl acetate			+			
methyl phenylpropanoate				+		
Lilac acid formate D					+	
diethyl butanedioate	+	+				
LACTONE						
butyrolactone	+	+			+	
5-ethyldihydro-2(3H)furanone		+		+	+	
2, 5 dimethyl-4-methoxy-3(2H) furanone			+	+		
HYDROCARBON						
4-methoxy-1-butene	+	+			+	
3-hydroxy-2-butene	+					

Table 3-5. Continued.

	T1	T2	T3	T4	Field SB
2-ethoxy-butane			+		
butyl cyclohexane	+				
hexyl cyclohexane	+	+	+		
(Z)-1,4-dimethylcyclohexane					
decane	+		+		
dodecane	+		+	+	
tetradecane	+				+
TERPENE					
linalool			+	+	
(Z)-linalool oxide	+	+			+
α-terpineol			+	+	
(E)- nerolidol			+	+	
(E)-geranyl acetone				+	
BEZENOID					
styrene			+	+	
guaiacol				+	
phenol			+		
FURAN					
furfural		+			+
1(2-furanyl)ethanone	+				+
2,5-dimthyltetrahydrofuran	+	+			
SULFUR COMPOUND					
benzothiazole	+		+	+	+
NITROGENOUS					
2,5-dimethylpyrazine	+	+			+
2,3-dimethylpyrazine	+	+			+
2-ethyl-6-methylpyrazine	+	+			+
2,3,5-trimethylpyrazine	+	+			+
3-ethyl-2,5-dimethylpyrazine					+
MISCELLANEOUS					
2,4,5-trimethyl-1,3-dioxolane		+	+	+	
4-methyl-1,3-dioxolane		+	+	+	
methyl antranilate		+			+

KEY: + -detected in the volatiles captured on Super Q filter. (T1 = pollen dough, T2 = pollen dough fed upon by larvae of *L. insularis*, T3 = ripe strawberries, T4 = ripe strawberries fed upon by larvae of *L. insularis*, T5 = control, and Field SB = field-collected sap beetles).

CHAPTER 4
ATTRACTIVENESS OF DIFFERENT STAGES OF STRAWBERRY FRUIT

Connell (1980) reported that strawberry sap beetle adults, *S. geminata,* are attracted to ripe, over-ripe and injured fruits of many plants. Beetles migrate from the periphery of strawberry plots and oviposit into rotting strawberries, although some eggs may be laid in fresh strawberries (Mossler and Nesheim 2004).

Sanitation is often considered the most important control method in deterring sap beetle pests. This involves the removal of rotting fruit from the field. However, removing rotted fruit from the field may not be practical since harvesting usually occurs two to three times per week. A better understanding of sap beetle preference to different stages of strawberry fruit is important when developing control measures for sap beetles.

Regular sampling is an important monitoring tool that gives information on insect population or pest status. Based on the number of samples taken from a field, conclusions can be drawn regarding pest status. Currently, there is little information on sampling for sap beetles in strawberries although the importance of sampling for sap beetle pests is well established. A closer examination of the chemical composition of different stages of the fruit will allow for better understanding of why sap beetles may prefer to feed and lay eggs in certain types of fruit. This may allow for further development of monitoring and trapping techniques.

Specific objectives of this research were to determine the attractiveness of different stages of strawberry fruit to sap beetles in the field. Additionally, I wanted to compare

volatile composition of these different stages of strawberries to identify candidate components for the sap beetles.

Methods

Field research was conducted at the University of Florida, Plant Science Research and Education Unit located in Citra, Florida. Strawberries, *Fragaria x ananassa* Duschene., cv. 'Strawberry Festival' were planted on raised beds. Each plot contained six rows of strawberry plants spaced approximately 0.3 m apart. Plot size was 7.3 X 6.1 m. The spacing between plots was 7.3 m.

In Situ Counts

In order to evaluate the attractiveness of sap beetles to different stages of strawberries, 8 plants from two center rows in each plot were examined for sap beetles. Sampling was done by visually counting the number of sap beetle adults on all 8 plants (per plot) on a weekly basis. Four treatments were evaluated. In each treatment, sap beetles were counted on 1) dried strawberries on the ground, 2) ripe strawberries, 3) over-ripe strawberries, and 4) ground litter. For the purposes of this study, dry strawberries were those that were completely void of moisture and were brown, ripe strawberries were 80% bright red with visible decay, and over-ripe strawberries were 80% dark red with at least 25% decay. The experiment was set up using a completely randomized block design with 24 replicates.

Statistical Analyses

All data from sap beetle field counts were analyzed by repeated measures Analysis of Variance using the SAS GLM procedure. All data were square root transformed to stabilize variances, and means separated with least significant differences

(TUKEY) $\alpha = 0.05$ (SAS Institute Inc. 2002). Data reported in the tables and figures represent untransformed means ± standard errors.

Volatile Collection and Analysis

Volatiles from the following treatments 1) dry, 2) ripe and 3) over-ripe strawberries were collected and analyzed as previously described in Chapter 3 using facilities available at the USDA-ARS CMAVE laboratory in Gainesville, Florida. Forty grams of strawberries were used from each treatment. Treatments were replicated three times.

The leaves from the strawberries were removed, while keeping the fruits intact. Volatiles were collected using Super Q filters for approximately 2 h then eluted using 250µl of dichloromethane. Samples were then analyzed using GC-Mass Spectrometry. The total profile area relative to sum of the areas of the internal standards octane and nonyl acetate was used to assess the quantity of components in each treatment. Our hypothesis was that profiles with larger areas relative to internal standards parallel the most attractive fruit stages in the field

Statistical Analyses

All data from volatile collections were analyzed by repeated measures Analysis of Variance using the SAS GLM procedure. All data were log transformed to stabilize variances, and means separated with least significant differences (TUKEY) $\alpha = 0.05$ (SAS Institute Inc. 2002). Data reported in the tables and figures represent untransformed means ± standard errors.

Results

In Situ Counts

2004

Throughout the experimental period (4/7/04-4/20/04) over-ripe strawberries attracted significantly more sap beetles than those found in ground litter ($F = 95.4$; df = 3, 259; $P < 0.0001$) [Table 4-1]. Significantly more sap beetles were found in ground litter than in dry or ripe strawberries. There were no significant differences between dry and ripe strawberries. Data for individual weeks indicated that over-ripe strawberries had significantly more sap beetles than all other treatments (all weeks, $P < 0.0001$) [Table 4-1]. Generally, over-ripe strawberries had 3-times more sap beetles than any other treatment.

2005

Similar findings were recorded in 2005. Over-ripe strawberries attracted significantly more sap beetles than all other treatments throughout the trapping period ($F = 154.6$; df = 3, 354; $P < 0.0001$) [Table 4-2]. Significantly more sap beetles were found in ground litter than in dry or ripe strawberries. There were no significant differences between the number of sap beetles found in dry and ripe strawberries. Data for individual weeks varied. During the first week over-ripe strawberries had significantly more sap beetles than any other treatment ($F = 36.2$; 3, 69; $P < 0.0001$) [Table 4-2]. There were no significant differences among ripe and dry strawberries and ground litter. For week 2, over-ripe strawberries had significantly more sap beetles than ripe and dry strawberries ($F = 11.2$; df = 3:69; $P < 0.0001$) [Table 4-2]. There were no significant differences between over-ripe strawberries and ground litter. Significantly more sap beetles were found in ground litter than dry strawberries (Table 4-2). There were no significant

differences between ground litter and ripe strawberries. Also, there were no significant differences between dry and ripe strawberries. For weeks 3 and 4, over-ripe strawberries had significantly more sap beetles than any other treatment (For week 3, $F = 111.2$; df = 3, 69; $P < 0.0001$) and (for week 4, $F = 125.7$; df = 3, 69; $P < 0.0001$) [Table 4-2]. Significantly more sap beetles were found in ground litter than in dry and ripe strawberries. There were no significant differences among dry and ripe strawberries.

Volatile Collection and Analysis

Overall, the volatile profiles of ripe and over-ripe strawberries had significantly larger areas relative to internal standards than those of dry strawberries and control treatments ($F = 38.2$; df = 3:6; $P = 0.0003$) [Fig. 4-1]. The volatile profiles of dry strawberries had significantly larger areas relative to internal standards than control treatments. There were no significant differences between the total areas of volatiles released from ripe and over-ripe strawberries.

GC-MS identified components in the volatiles of the attractive treatments as mainly alcohols, fatty acids and esters (Table 4-3). Additionally, aldehydes, ketones, hydrocarbons terpenoids, lactone, furan, and nitrogenous and sulfur derivatives were present in these volatiles. Overall, the composition of the volatiles released by over-ripe strawberries was compositionally richer than those that were released by dry and ripe strawberries (Table 4-3). Twenty-three components were identified in the volatiles from dry strawberries, 46 components were identified in the volatiles released by ripe strawberries. Fifty six components were identified in the volatiles of over-ripe strawberries.

Discussion

During both field seasons (2003 - 2005), over-ripe strawberries had significantly more sap beetles than dry and ripe strawberries. Also with the exception of week 2 (2005), over-ripe strawberries had significantly more sap beetles than ground litter. It is believed that over-ripe strawberries have a high percentage of fermenting fruit emitting volatile and sap-beetles responded by accumulating on these fruit. Sap beetles have been known to accumulate on fermenting fruit (Potter 1994). This data is consistent with Warner (1990) who found sap beetles in later ripening berries but none in berries that mature early.

Ground litter treatments had significantly more sap beetles than dry and ripe strawberries. Two reasons may account for the high numbers of sap beetles in the ground litter. First, over-ripe strawberries fall to the ground and beetles may respond to volatile cues by moving to the ground to take advantage of fermenting fruits. Another theory is that sap beetles play dead and drop off fruit into the ground litter when disturbed. Neumann and Patti (2004) found similar results with the related sap beetle, *Aethina tumida* (Murray).

Cavities characteristic of sap beetle injury were abundant in ripe (marketable) strawberries. This type of injury renders the fruit unmarketable, highlighting the importance of this pest in fresh marketable fruits. Secondly, small cavities from sap beetle injury leaves the fruit vulnerable to secondary infection from pathogens. Also, invasion by fungal pathogens may cause aggregation of sap beetles and possibly increase the incidence of sap beetle damage. Sap beetles tended to congregate under fruit axils, where they are difficult to detect.

Volatile profiles of dry, ripe, and over-ripe strawberries showed that peak areas relative to internal standards were highest for over-ripe fruit. Profiles of ripe strawberries were richer than those of dry strawberries. Over-ripe fruits consistently had more sap beetles than any other treatment in the field, which is correlated to the high peaks recorded in the GC Mass spectrometry results. Profiles of dry strawberries had significantly higher areas relative to internal standards. However, they were completely void of sap beetles in the field during both field seasons. Chemical analysis showed that dry strawberries contained many of the same components as ripe and over-ripe strawberries, but contained fewer esters. Dry strawberries also produced several fermentation components such as alcohols and fatty acids. This may be due to previous feeding when fruit was ripe. Therefore, it is possible that esters may be an attractive component in ripe and over-ripe strawberry fruits. Volatile profiles of ripe strawberries lacked fermentation-related products such as 3- and 2 methyl-1-butanol and fatty acids while these compounds were abundant in dry and over-ripe fruit. These are the same compound found in fungal volatile profiles of the fungi *C. fagacearum* and *F. verticillioides* (Lin and Phelan 1992, Zilkowski et al. 1999). Therefore, it may be possible that these components were present in over-ripe and dry fruits as a result of sap beetle inoculation with fungus.

Table 4-1. Mean ± SEM number of sap beetle adults in strawberries, Citra, FL (2004).

Treatments	Week 1	Week 2	Week 3	Total Captures
Dry Strawberries	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 c
Ripe Strawberries	0.0 ± 0.0 b	0.4 ± 0.4 b	0.0 ± 0.0 b	0.5 ± 0.1 c
Over-ripe Strawberries	5.0 ± 1.0 a	6.7 ± 1.7 a	7.5 ± 1.8 a	6.4 ± 0.9 a
Ground Litter	1.7 ± 1.0 b	0.8 ± 0.3 b	0.6 ± 0.2 b	1.1 ± 0.3 b

Means followed by the same letter are not significantly different ($P = 0.05$, TUKEY test)

Table 4-2. Mean ± SEM number of sap beetle adults in strawberries, Citra, FL (2005).

Treatments	Week 1	Week 2	Week 3	Week 4	Total Captures
Dry Strawberries	0.1 ± 0.1 b	0.0 ± 0.0 c	0.1 ± 0.1 c	0.0 ± 0.0 c	0.1 ± 0.0 c
Ripe Strawberries	0.3 ± 0.1 b	0.3 ± 0.1 bc	0.4 ± 0.1 c	0.2 ± 0.1 c	0.3 ± 0.1 c
Over-ripe Strawberries	8.0 ± 1.5 a	6.0 ± 2.0 a	15.0 ± 2.2 a	14.1 ± 1.9 a	10.8 ± 1.0 a
Ground Litter	0.5 ± 0.2 b	1.8 ± 0.6 ab	6.8 ± 0.9 b	2.4 ± 0.5 b	2.9 ± 0.4 b

Means followed by the same letter are not significantly different ($P = 0.05$, TUKEY test)

Table 4-3. Volatiles present in ripe, over-ripe, and dry strawberry fruit.

	T1	T2	T3	T4
ALCOHOL				
3-methyl-1-butanol	+		+	
2-methyl-1-butanol	+		+	
2-ethyl-butanol			+	
hexanol	+			
1,3-butanediol	+			
2,3-butanediol	+			
ALDEHYDE				
hexanal			+	
(*E*)-2-hexenal			+	
heptanal	+			
nonanal	+		+	trace
decanal	+		+	
benzaldehyde	+			
KETONE				
2-heptanone		+		
6-methyl-5-hepten-2-one			+	
3-octanone			+	
1 (4-ethylphenyl)-ethanonoe		+		
LACTONE				
butyrolactone	+			
FATTY ACID				
butanoic acid			+	
3-methylbutanoic acid	+			
2-methylbutanoic acid	+		+	
2-methylpropanoic acid	+		+	
4-hydrobutanoic acid	+			
ESTER				
ethyl propionate		+	+	
ethyl butanoate	+	+	+	

Table 4-3. Continued.

	T1	T2	T3	T4
ethyl hexanoate	+	+	+	
ethyl 2-butenoate		+	+	
ethyl pentanoate		+	+	
ethyl 2-methylpropionate			+	
ethyl 2-methylbutanoate	+	+	+	
ethyl 3-methylbutanoate		+	+	
ethyl tiglate			+	
ethyl benzoate		+	+	
ethyl 2-hexenoate			+	
ethyl octanoate		+	+	
hexyl 2-methylbutanoate			+	
isopropyl hexanoate		+		
methyl 3-methylbutanoate		+		
methyl 2-methylbutanoate		+	+	
2-methyl propanoate			+	
2-methylpropyl butanoate		+		
2-methylbutyl propanoate			+	
2-methylbutyl butanoate		+		
3-methylbutyl butanoate			+	
1-methylethyl butanoate		+	+	
methyl butanoate		+	+	
methyl pentanoate		+		
1-methylethyl hexanoate		+	+	
2-methylbutyl hexanoate			+	
methyl hexanoate		+	+	
methyl octanoate		+	+	
methyl salicylate			+	
2-methylpropyl acetate			+	
3-methyl-1-butanyl acetate		+	+	
2-methyl-1-butanyl acetate		+	+	
methyl hexanoate			+	
octyl acetate			+	
pentyl acetate		+	+	
pentyl butanoate			+	
pentyl 2-methylpropionate		+		
phenylmethyl acetate		+		
2-phenylethyl acetate	+			
propyl acetate			+	
propyl butanoate		+	+	
propyl hexanoate		+		
butyl butanoate		+		
butyl acetate		+	+	
(Z)-3-hexenyl acetate			+	
(E)-3-hexenyl acetate		+		

Table 4-3. Continued.

	T1	T2	T3	T4
(E)-2-hexenyl acetate			+	
(Z)-3-hexenyl butanoate		+		
(E)-3-hexenyl butanoate		+		
hexyl butanoate		+		
hexyl propanoate		+		
hexyl acetate	+	+	+	
2-methyllbutyl propionate		+		
HYDROCARBON				
1-methyl-2propylcyclohexane		+		
1,4-diethyl-1,4-dimethyl-2,5-cyclohexadiene			+	
(Z)-1,4-dimethylcyclohexane				trace
BENZENOID				
Styrene	+		+	
p-xylene		+		
1-ethyl-2methylbenzene		+		
1,3,5-trimethylbenzene		+		
FURAN				
2,5-dimethyl-4 methoxy-2 (3H) furanone	+	+	+	
5-ethenyl-dihydro-5-methyl-2 (3H) furanone	+			
TERPENE				
α-terpineol		+		
(*E*)-nerolidol		+	+	
D-limonene	+			
SULFUR COMPOUNDS				
S-methyl butanethioate		+		
ethyl(methylthio)acetate			+	
MISCELLANEOUS				
2,4,5-trimethyl-1,3-dioxolane			+	

KEY: + -detected in the volatiles captured on Super Q filter. (T1 = dry strawberries, T2 = ripe strawberries, T3 = over-ripe strawberries and T4 =blank)

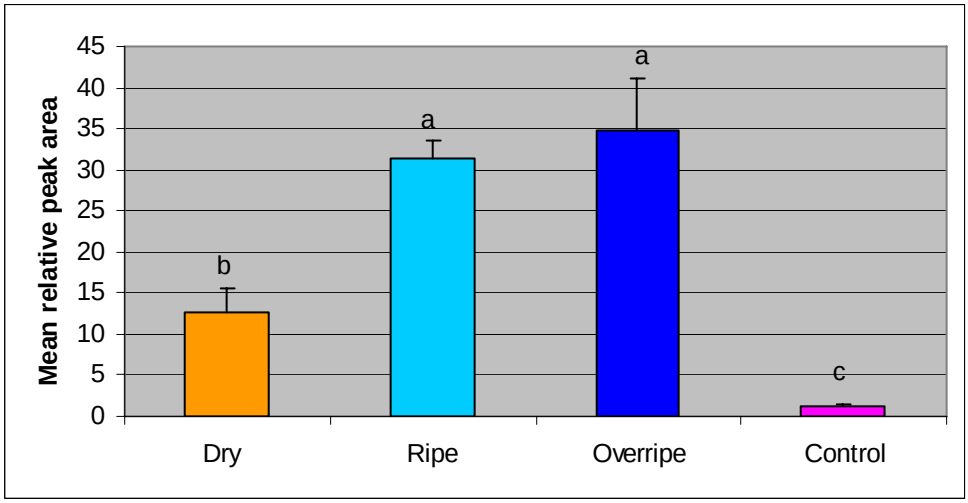

Figure 4-1. Mean ± SEM relative peak area for different stages of strawberry fruit. Means were calculated relative to internal standards octane and nonyl acetate. Means followed by the same letter are not significantly different ($P = 0.05$, TUKEY test)

CHAPTER 5
EFFECTS OF REDUCED RISK AND CONVENTIONAL INSECTICIDES ON SAP BEETLE PESTS OF STRAWBERRIES

Although several insecticides have been registered for use on strawberries for the control of sap beetles, application of organophosphate insecticides has been the standard for several years. Many of these insecticides have a long re-entry period and do not allow for frequent harvest. Insecticides with long re-entry periods allow for an abundance of over-ripe strawberries to accumulate in the field. Volatile cues from these over-ripe fruits facilitate the movement of sap beetles into strawberry fields. Recently, growers have been applying large amounts of insecticides to reduce high populations of sap beetles to tolerable levels. Frequent application of broad-spectrum insecticides increases the selection pressure and encourages development of resistant genes. Furthermore, these insecticides pose a threat to natural enemies and non-target organisms in the environment.

Effective control of sap beetles in strawberries requires good sanitation. This can be labor intensive when working with perishable commodities like strawberries. Biological control agents for sap beetle control are currently being investigated but these products are not yet commercially available. Recently, several new classes of reduced-risk insecticides have been developed for use on fruit crops. Some of these insecticides are registered for use in strawberries (not for sap beetle control). Laboratory and field assays need to be conducted to evaluate their potential to be used in sap beetle IPM programs. Promising compounds could be identified and be used with effective

monitoring, as well as with improved sanitation to better effectively manage sap beetle populations. Specific objectives of this research were to evaluate the effectiveness of conventional and reduced-risk insecticides on sap beetle pests of strawberries.

Materials and Methods

Laboratory assays to evaluate conventional and reduced-risk insecticides for control of sap beetles were conducted at the Small Fruit and Vegetable IPM Laboratoy in Gainesville, FL. Five treatments were evaluated including, 1) Malathion 5EC, 2) imidacloprid, Provado 1.6 F (Bayer Cropscience Kansas City, MO), 3) thiamethoxam, Actara 25 WG (Syngenta Crop Protection Wilmington, DE) and 4) spinosad, SpinTor 2SC (Dow, Agrosciences, Carmel, IN), and 5) control [untreated Petri dishes]. Treatments were replicated four times in a randomized complete block design (Fig. 5-1). Insecticides were applied at the recommended (scaled down) rate to filter paper (15 cm diameter) using 0.5 L hand-atomizers. Filter paper was allowed to air-dry for 30 minutes. Each filter paper was then placed in a glass Petri dish (150 x 20 mm) with 3-4 g of over-ripe strawberries.

Petri dishes were maintained at 27°C and exposed to 14 L:10 D (light:dark regime) at relative humidity of 65%. Each Petri dish contained four insects. Bioassays were run three times. In the first assay newly eclosed virgin males of *L. insularis* were used. In the second assay virgin females of *L. insularis* were tested. In the third bioassay field collected adults were tested.

Preparation of Sap Beetles for Assay

Laboratory sap beetles eclosed approximately one month prior to assay (see Chapter 3 for rearing protocol). After eclosion beetles were sexed. In the case of field collected sap beetles, they were collected from an untreated strawberry field in Citra, FL.

The beetles were identified six hours prior to assay. The age of the insects was unknown. Three adults were used per Petri dish (3 beetles in each dish for field-collected sap beetles and 4 beetles in each dish for *L. insularis*). All treatments were replicated 4 times.

Sampling

Insects were rated using a 0-3 scale based upon average activity in each Petri dish (Liburd et al. 2003). A score of 3 indicated uninhibited mobility (the status of beetles in nature). A score of 2 indicated decreased mobility (limited movement-grooming). A score of 1 indicated no responsiveness but movement was stimulated only by touch. A score of 0 indicated mortality (death). Data was recorded at 2, 6, 24, and 48 hours after treatment.

Statistical Analysis

Mean rating per replicate was calculated and analyzed by repeated measures analysis of variance using the SAS GLM procedure. All data were square-root transformed to stabilize variances, and means separated with least significant differences (TUKEY) $\alpha = 0.05$ (SAS Institute Inc. 2002). Data reported in the tables and figures represent untransformed means ± standard errors.

Results

Lobiopa insularis

Overall, Malathion was the only treatment that significantly reduced the number of sap beetles in the assay ($F = 12.2$; df = 4:69; $P < 0.0001$). There were no significant differences among the other treatments evaluated.

At 2 h there were no significant differences among any of the treatments. However, at 6 h Provado 1.6 F had significantly higher mortality than the control (F = 3.9; df = 4:12; P = 0.0301) [Table 5-1]. There were no significant differences among other treatments evaluated. At 24 and 48 h significantly more beetles died in treatments exposed to Malathion compared with other treatments (for 24 h F = 3.8; df = 4:12; P = 0.0323, for 48 h F = 99.2; df = 4:12; P <0.0001) [Table 5-1].

***Lobiopa insularis* males**

Overall, Malathion was the only treatment that significantly reduced the number of sap beetles in the assay (F = 9.1; df = 4:69; P < 0.0001) [Table 5-2]. There were no significant differences among other treatments evaluated.

At 24 h there were no significant differences among treatments evaluated. However, at 48 h significantly more beetles died in treatments exposed to Malathion compared with other treatments (F = 145.9; df = 4:12; P < 0.0001) [Table 5-2]. There were no significant differences among other treatments.

***Lobiopa insularis* females**

Overall, Malathion was the only treatment that significantly reduced the number of sap beetles in the assay (F = 11; df = 4:69; P < 0.0001) [Table 5-3]. Unlike males at 6 h, SpinTor 2 SC, Provado 1.6 F, and Actara 25G were not significantly different to treatments of Malathion. There were no significant differences among other treatments evaluated.

At 2 h there were no significant differences among treatments. However, at 6 h Malathion was the only treatment that had significantly more dead sap beetles compared with the control (F = 3.1; df = 4:12; P = 0.0553). All other treatments were not significantly different from each other. At 24 and 48 h Malathion was the only treatment

that significantly reduced the number of sap beetles in the assay (for 24 h, $F = 4.7$; df = 4:12; $P = 0.0159$) and (for 48 h, $F = 34.9$; df = 4:12; $P < 0.0001$) [Table 5-3]. There were no significant differences among other treatments evaluated.

Field Collected Sap Beetles

Overall, Malathion was the only treatment that significantly reduced the number of sap beetles in the assay ($F = 11.4$; df = 4:69; $P < 0.0001$) [Table 5-4]. There were no significant differences among the other treatments evaluated.

After 6 h there were no differences among treatments. However, at 24 and 48 h significantly more beetles died in treatments exposed to Malathion compared with other treatments (for 24 h, $F = 6.5$; df = 4:12; $P = 0.0051$) and (for 48 h, $F = 13$; df = 4:12; $P = 0.0003$) [Table 5-4].

Discussion

Our results indicate that Malathion was the only effective insecticide in killing sap beetles. The results confirmed a potential reason for the recent increase in sap beetle numbers in strawberry fields. Recently, extension agents have been encouraging growers to use more reduced-risk insecticides in order to conserve natural enemies and the environment. These reduced-risk insecticides may be effective against primary pests such as thrips and Lepidopterans but leave secondary pests like sap beetles unharmed. Females appear to be more susceptible than males. This information became clear with treatments of Malathion. Females of *L. insularis* were affected by Malathion at 6 h post-treatment while males were not affected until 48 h post-treatment. The same was true for some of our reduced-risk insecticides. SpinTor 2 SC, Actara 25 WG and Provado 1.6 F caused a slight reduction in female populations at 6 h. These same insecticides did not

affect males *L. insularis* males. The reason for the observed difference in susceptibility of females and males is unclear.

Although Malathion was the only insecticide that killed more sap beetles than the control, the reduced risk insecticides tested should not be completely discounted. There are many factors that may be responsible for these results. Isaacs et al. (2004) states that reduced-risk insecticides generally have less immediate toxic effects on pests than conventional insecticides, but their activity may have repellent or anti-feeding effects. The bioassays were run for a total of 48 h. Future studies might include longer test periods to better assess the slower acting toxic effects of reduced-risk insecticides. Also, field experiments that monitor populations of feeding insects pre-and post-spray may account for repellent and anti-feeding effects of reduced-risk insecticides.

Since strawberry production requires harvesting generally every 2 days, an insecticide with a PHI period of 2 days would be ideal. Malathion has a PHI of 3 days (Mossler and Neisham 2004) so harvesting must be delayed. Future research on chemical control of sap beetles in strawberries should focus on systemic reduced-risk insecticides with short PHI periods to allow for maximum harvesting. The incorporation of reduced-risk insecticides and frequent harvesting may be the key to sap beetle control in strawberries.

Table 5-1. Mean ±SEM rating of L. insularis males and females combined.

Treatments	2 HAT	6 HAT	24 HAT	48 HAT	Overall Rating
Malathion 5EC	2.6 ± 0.4	2.7 ± 0.1 ab	1.3 ± 0.6 b	0.1 ± 0.1 b	1.7 ± 0.3 b
SpinTor 2SC	3.0 ± 0.1	2.8 ± 0.0 ab	2.8 ± 0.1 ab	2.8 ± 0.1 a	2.8 ± 0.1 a
Provado 1.6 F	3.0 ± 0.1	2.6 ± 0.2 b	2.6 ± 0.1 ab	2.6 ± 0.1 a	2.7 ± 0.1 a
Actara 25G	3.0 ± 0.1	2.7 ± 0.1 ab	2.7 ± 0.1 ab	2.5 ± 0.5 a	2.7 ± 0.1 a
Control	3.0 ± 0.0	3.0 ± 0.1 a	3.0 ± 0.1 a	3.0 ± 0.1 a	3.0 ± 0.0 a

Means followed by the same letter are not significantly different (P = 0.05, TUKEY test)

Table 5-2. Mean ± SEM rating of *L. insularis* males.

Treatments	2 HAT	6 HAT	24 HAT	48 HAT	Overall Rating
Malathion 5EC	2.9 ± 0.1	2.8 ± 0.1	1.5 ± 0.5	0.1 ± 0.1 b	1.8 ± 0.3 b
SpinTor 2SC	2.9 ± 0.1	2.8 ± 0.0	2.8 ± 0.0	2.7 ± 0.1 a	2.8 ± 0.0 a
Provado 1.6 F	2.9 ± 0.1	2.4 ± 0.3	2.4 ± 0.2	2.4 ± 0.2 a	2.5 ± 0.1 a
Actara 25G	3.0 ± 0.0	2.7 ± 0.1	2.7 ± 0.1	2.7 ± 0.1 a	2.8 ± 0.0 a
Control	3.0 ± 0.0	2.9 ± 0.1	2.9 ± 0.1	2.9 ± 0.1 a	3.0 ± 0.0 a

Means followed by the same letter are not significantly different ($P = 0.05$, TUKEY test)

Table 5-3. Mean ± SEM rating of *L. insularis* females.

Treatments	2 HAT	6 HAT	24 HAT	48 HAT	Overall Rating
Malathion 5EC	2.9 ± 0.1	2.6 ± 0.1 b	1.1 ± 0.2 b	0.1 ± 0.1 b	1.7 ± 0.3 b
SpinTor 2SC	3.0 ± 0.0	2.8 ± 0.0 ab	2.8 ± 0.1 a	2.7 ± 0.1 a	2.8 ± 0.0 a
Provado 1.6 F	3.0 ± 0.0	2.7 ± 0.2 ab	2.7 ± 0.1 a	2.4 ± 0.2 a	2.8 ± 0.1 a
Actara 25G	2.9 ± 0.1	2.7 ± 0.1 ab	2.6 ± 0.1 a	2.7 ± 0.1 a	2.6 ± 0.1 a
Control	3.0 ± 0.0	3.0 ± 0.0 a	3.0 ± 0.0 a	2.9 ± 0.1 a	3.0 ± 0.0 a

Means followed by the same letter are not significantly different ($P = 0.05$, TUKEY test)

Table 5-4. Mean ±SEM rating of field collected sap beetles, Citra, FL.

Treatments	2 HAT	6 HAT	24 HAT	48 HAT	Overall Rating
Malathion 5EC	2.9 ± 0.1	2.7 ± 0.1	0.9 ± 0.6 b	0.5 ± 0.5 b	1.8 ± 0.3 b
SpinTor 2SC	3.0 ± 0.0	3.0 ± 0.0	2.8 ± 0.1 a	2.8 ± 0.1 a	2.9 ± 0.0 a
Provado 1.6 F	2.9 ± 0.1	2.9 ± 0.1	2.8 ± 0.1 a	2.6 ± 0.2 a	2.8 ± 0.1 a
Actara 25G	3.0 ± 0.0	3.0 ± 0.0	2.8 ± 0.1 a	2.8 ± 0.1 a	2.6 ± 0.0 a
Control	3.0 ± 0.0	3.0 ± 0.0	3.0 ± 0.0 a	3.0 ± 0.0 a	3.0 ± 0.0 a

Means followed by the same letter are not significantly different ($P = 0.05$, TUKEY test)

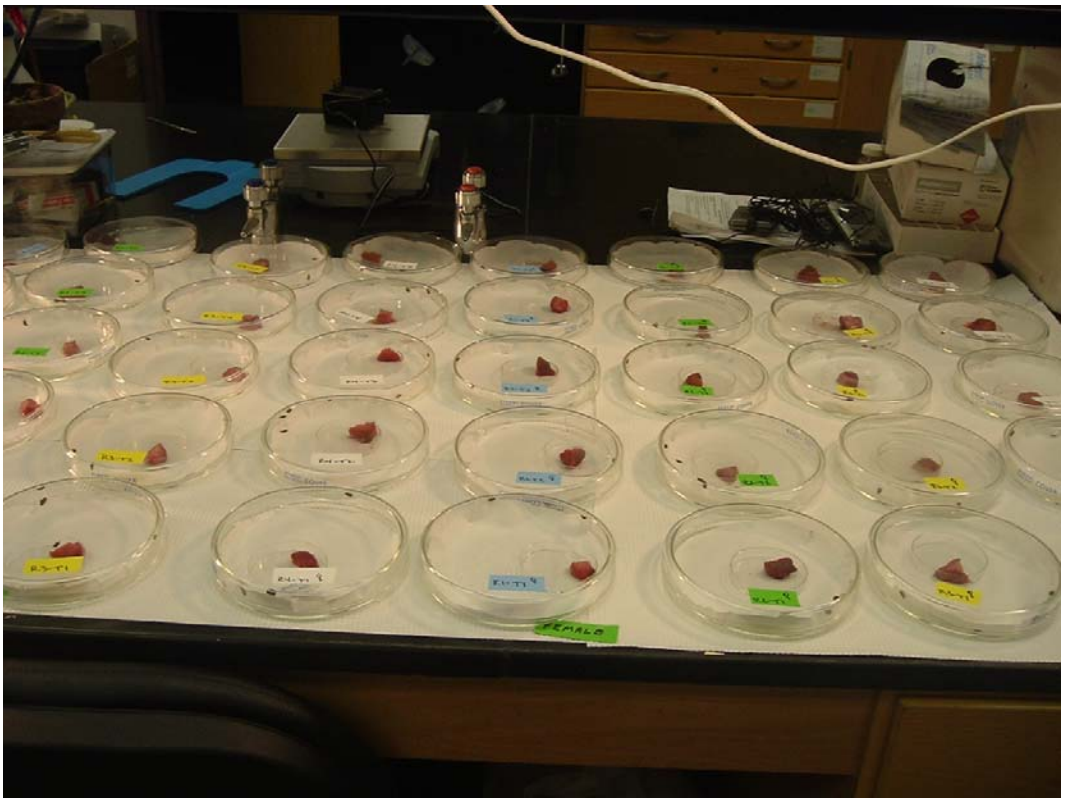

Figure 5-1. Insecticide bioassay set-up.

CHAPTER 6
SUMMARY AND CONCLUSIONS

Research on the behavior and biology of sap beetle pests has led to improvements in managing these pests. Trapping has been shown to be an effective monitoring tool and may be effective as a management tactic as well. Many baits have been shown to effectively attract sap beetles, especially whole wheat bread dough which has been used in many studies. Research on host volatile compounds has shown that sap beetles are attracted primarily to esters, fatty acids, and alcohols. Using chemical tactics is also an important component of the total strawberry pest management program. Also, advances in biological control and pheromones used as attractants are promising additions to a comprehensive integrated pest management program for strawberries. Many of these tactics must be properly integrated to achieve the most cost effective and safest pest management program to suppress sap beetle population to tolerable levels.

Unfortunately, little work has been done on a comprehensive pest management program for sap beetles found in Florida strawberries. Since many sap beetles are generalist feeders and since weather and other uncontrollable conditions may affect sap beetle behavior, it is essential to investigate several strategies. In this thesis we studied several aspects of sap beetle management including, movement into strawberries, monitoring their activities in the field and potential use of reduced-risk insecticides.

The results of this study suggest that traps placed near the woods are more effective at capturing sap beetles than traps placed within and between strawberry rows. Although

this was the case, most sap beetles found in baited traps near the woods were found early in the production season. This may indicate that border sprays or ringing the field with attract & kill traps may be a reduced-risk tactic to prevent high populations from increasing. Trapping during the 2005 field season showed that all bait treatments were significantly better than the control but not different from one another overall. A better understanding of how the bait treatment works may lead to improvements in the type of attractants used to monitor sap beetles. As in 2004, trap catches were inconsistent between weeks (Fig 3-6). This suggests that levels of fruit maturity and environmental factors can affect trap captures. Other factors that were not investigated in this thesis include trap placement and timing of bait deployment in the field. Our studies indicate that preventative trapping tactics can be implemented early in the season, before fruits begin to ferment. Once the fruit begins to ferment sap beetles are attracted to their natural host and captures in strawberry baited traps will decline. Until more research information becomes available traps can be baited with pollen dough. This bait can be easily prepared and ingredients can be quantified.

The number of sap beetles caught in traps baited with strawberries and pollen dough fed upon by *L. insularis* were not significantly different from fresh strawberries or pollen dough, respectively. Laboratory studies have shown that sap beetle hosts, which have previously been fed upon by sap beetles, attract significantly more sap beetles. This did not occur in our field studies. Field trapping contains many more variables which can affect beetle response or trap efficacy.

Results from traps placed in harvested versus un-harvested fruit showed that those traps placed in un-harvested plots captured significantly more sap beetles. This result

was expected because many sap beetles are attracted to decaying or fermenting fruit, which is typical in un-harvested strawberry fields. Therefore, timely harvesting and sanitation is crucial to reducing sap beetle populations.

Voltile profiles of baits showed that strawberries and strawberries fed upon by *L. insularis* had significantly larger areas in relation to internal standards than control treatments. This suggests that strawberries and strawberries fed upon by *L. insularis* may have more active compounds that are attractive to sap beetles. Volatiles of pollen dough fed upon by *L. insularis* and pollen dough fed upon by the field-collected sap beetle did not show any significant differences in mean relative peak areas. This suggests that treatments fed upon by either sap beetle could be used in the field. Furthermore, over-ripe strawberries had consistently higher numbers of sap beetles than all other treatments. Again, frequent harvesting and interception of sap beetles before entry into the field may help to alleviate a high population of sap beetles.

Results of insecticide bioassays showed that Malathion was the only treatment that effectively killed sap beetles compared with other treatments. Although this was the case, control provided by reduced-risk insecticides should be evaluated for sub-lethal effects on sap beetle pests. A longer evaluation period may have given a different type of result. Nevertheless, preliminary evidence indicates that some reduced-risk insecticides do not kill sap beetles, which may account for their recent high numbers in the field.

Future studies involving sap beetle pests of strawberries should include wind tunnel and olfactometer studies to test potential pheromones as well as kairomone attractants for sap beetles. The incorporation of aggregation pheromones should be investigated for sap beetles in strawberries. Traps containing insecticide strips may be useful in evaluating

the number of sap beetles caught in traps, eliminating the variable of beetle escape. Insecticide bioassays studying repellent activities of reduced-risk insecticides should also be assessed.

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BIOGRAPHICAL SKETCH

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