Biology and chemical ecology of the sugarcane beetle and integrated pest management of sweet potato soil insects in Louisiana

Tara Parker Smith
Louisiana State University and Agricultural and Mechanical College, tsmit46@lsu.edu

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BIOLOGY AND CHEMICAL ECOLOGY OF THE SUGARCANE BEETLE
AND INTEGRATED PEST MANAGEMENT
OF SWEET POTATO SOIL INSECTS IN LOUISIANA

A Dissertation

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Louisiana State University and
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in

The Department of Entomology

by

Tara P. Smith
B.S., University of Louisiana at Monroe, 2000
M.S., Louisiana Tech University, 2001
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ABSTRACT

Host plant preference and chemical ecology of the sugarcane beetle, *Euetheola humilis* were evaluated in greenhouse and laboratory studies. Sweet potato, *Ipomoea batatas*, was significantly preferred over all other plant species evaluated in a host plant preference test. Corn, *Zea mays*, and sugarcane, *Saccharum* spp., were the next most chosen plant species in the study. In olfactometer experiments, sugarcane beetles responded significantly more to beetle injured and mechanically injured roots vs. uninjured roots. Male and female beetles were also significantly more attracted to female conspecifics. Differences were not detected between sweet potato cultivars in olfactometer trials. Cultivar preference studies suggested that some cultivars may be more attractive than others.

Susceptibility of sugarcane beetle and sweetpotato weevil, *Cylas formicarius*, to selected insecticides was evaluated in laboratory bioassays. Sugarcane beetles were significantly more susceptible to z-cypermethrin than to chlorpyrifos and bifenthrin. Sweetpotato weevils from two cohorts were most susceptible to methyl parathion and the cohorts were differentially susceptible to selected insecticides. Reduced susceptibility of a reference cohort of sweetpotato weevil was noted for all insecticides evaluated.

A planting date study was conducted over two years in two locations in Louisiana. Damage from soil insects in sweet potato can be affected by many factors, such as insect abundance and life stage, and stage of the crop. A representative early, middle, and late planting date were used to assess soil insect abundance and damage throughout the sweet potato production season. Planting date affected damage from soil insects in sweet potato. Significantly more total insect damaged roots were sampled from late planting dates compared to early and middle planting dates. Cucumber beetle, *Diabrotica* spp., damage was greater in late
planting dates, relative to early and middle planting dates. Late planting dates also had an increased probability of sugarcane beetle damage compared to early and middle planting dates. The majority of adult insects sampled were *Diabrotica* beetles and *Diabrotica* abundance was variable throughout the season and was positively correlated with percent larvae damaged roots at various seasonal intervals.

Sweet potato soil insect abundance and damage were also investigated at various herbicide regimes in a two year study. Differences in soil insect damage or adult insect abundance were not detected between various herbicide regimes. U.S. No. 1 and 2 yield was significantly higher in herbicide treated plots vs. untreated control plots and weed densities were significantly reduced in some treated plots compared to untreated control plots.

Sugarcane beetle studies have provided information on the biology, chemical ecology and possible management options for this insect in sweet potato. In addition, these studies have examined the importance of an integrated pest management system in sweet potato. Integrated pest management involves manipulating the crop as well as careful management of insect species.
CHAPTER 1
INTRODUCTION AND REVIEW OF LITERATURE

Sweet Potato

Sweet potato, *Ipomoea batatas* (L.), originated near northwestern South America around 8000-6000 B.C. (Austin 1988). Sweet potatoes belong to the family *Convolvulaceae* and were first discovered and grown by Proto-Chibhuan, Chibhuan, or Chibhuan influenced people around 3000 B.C. (Austin 1988, O’Brien 1972). Sweet potatoes are a resilient crop and can be grown in high and low technology agricultural systems (Jansson & Raman 1991). The crop is drought tolerant and can be grown in tropical and temperate agricultural regions (Bouwkamp 1985). With the exceptions of China, Japan, Korea and Taiwan, sweet potatoes are used primarily for human consumption (70-100%) (Lin et al. 1985). Sweet potatoes are a widely grown and valuable crop in many areas of the world (Horton & Ewell 1991). Sweet potatoes are grown in over 100 countries and among root crops are second in production to white potatoes (*Solanum tuberosum* L.) (Horton 1988). Sweet potatoes are grown not only as a staple food crop, but also as vegetable, snack food, animal food and raw material for industrial produce (Bouwkamp 1985).

The United States is a major contributor to sweet potato production with potatoes grown mainly in the states of North Carolina, Louisiana, Mississippi, California, Alabama and Texas, with an average annual production valued at $309 million (USDA 2006). Sweet potato production plays a vital role in the agroecosystem of the southern United States (Curtis 2003). North Carolina, Louisiana, and Mississippi are the three leading states in sweet potato production. These states along with Alabama, the fifth largest producer of sweet potatoes in the United States, grew close to 28,000 hectares, totaling over 75% of United States production in 1999 (Curtis 2003). Sweet potatoes are an important agricultural commodity in Louisiana. In
2005, sweet potatoes were harvested from 6800 hectares in Louisiana (USDA 2006). The majority of Louisiana sweet potatoes are grown in West Carroll, Morehouse, Franklin, Richland, Avoyelles, St. Landry and Evangeline parishes (Sanders and Cannon 2000). Sweet potatoes are the most important vegetable crop grown in Louisiana with respect to acreage and economic impact (Sanders and Cannon 2000).

Sweet potato production in Louisiana begins in February with the bedding of seed potatoes, transplanting occurs from April through July depending on geographic locality and harvesting begins in July and extends until Thanksgiving (USDA 2001). The overall farm value of Louisiana sweet potatoes grown in 1999 was $72 million. Sanders and Cannon (2000) reported production of sweet potatoes exceeding 7 million pounds with a total economic value to the state of $125 million after harvesting, washing, grading, packing and shipping of potatoes.

**Insects Affecting Sweet Potato**

Many pests affect sweet potato production, such as insects, nematodes, rodents, weeds and diseases (Jansson & Raman 1991). Over 270 insect species and 17 mite species are known to feed on sweet potato worldwide (Talekar 1992), and there are 19 insect species that can affect sweet potato in the United States alone (Cuthbert 1967). Sweet potatoes are produced in the spring and summer in the United States when insect abundance is high (Cuthbert 1967). Insect damage from a variety of phytophagous pests may reach 60-90% (Chalfant et al. 1990, Jansson and Raman 1991), and all plant parts including roots, stems, and foliage can be affected (Talekar 1992).

Many insects reduce the quality and yield of sweet potatoes, either by feeding directly on the storage roots or by defoliating leaves and vine boring (Talekar 1992). Aphids, whiteflies, and leafhoppers can also transmit many of the viruses known to infect sweet potato (Talekar
Many foliage feeding insects do not cause yield reductions because sweet potato plants can often compensate for high levels of defoliation (Chalfant et al. 1990).

Soil insects that damage the root are the most harmful because they can cause significant economic losses even in low numbers (USDA 2001). Root feeders, especially the sweetpotato weevil, *Cylas formicarius* Fab., are the most destructive insects throughout tropical and subtropical production areas (Talekar 1992). *Cylas formicarius* can attack sweet potatoes in the field and in storage. Larvae feeding on sweet potato roots can result in major economic damage and yield loss (Chalfant et al. 1990). Larval tunneling causes terpene production in the storage roots, which imparts a bitter taste and leaves the sweet potatoes unsuitable for human consumption (Uritani et al. 1975). Reported yield losses worldwide due to sweetpotato weevil damage range from 5-80% (Sutherland 1986). One objective that was addressed by this research was the baseline establishment of insecticide data for potential control of the sweetpotato weevil. Susceptibility of two cohorts of sweetpotato weevil to selected insecticides was determined and resistance ratios are presented to discuss differences in susceptibility between the two populations.

Damage from a variety of other soil insects that feed on sweet potato is similar in appearance and difficult to differentiate at harvest. For this reason, damage incurred by wireworms (*Conoderus* spp.), rootworms (*Diabrotica* spp.), and flea beetles (*Systena* spp.), is collectively grouped into a complex referred to as the Wireworm- *Diabrotica*- *Systena* complex or WDS complex (Cuthbert 1967).

Cucumber beetles, *Diabrotica* spp., can be serious pests of sweet potato (Chalfant et al. 1990). Larvae of both the banded cucumber beetle, *Diabrotica balteata* LeConte, and the spotted cucumber beetle, *Diabrotica undecimpunctata* Barber, feed on the roots of sweet potato
(Cuthbert 1967). The roots are fed upon throughout their development, resulting in unattractive scarring of the root surface (Schalk et al. 1991). Females lay their eggs in soil where they are feeding and the eggs hatch in ca. two weeks. The larval stage will persist for 8-30 days depending on the availability of food. Pupae are found in cells underground and emerge as adults within one week (Schalk et al. 1991). Larvae of the two species are difficult to distinguish but the adults are easily recognized. The elytra of the banded cucumber beetle are marked with green and yellow bands and those of the spotted cucumber beetle have 11 spots on a yellow-green background (Chalfant et al. 1990). Numerous generations can develop and damage a sweet potato crop within one season (Cuthbert 1967).

Several species of white grubs in the genus *Pyllophaga* are serious pests of sweet potato. White grubs have a generation time of one or two years depending on species (Schalk et al. 1991). The larvae are the damaging stage, chewing wide gouges on the surface of sweet potato roots (Schalk et al. 1991).

Whitefringed beetles (*Naupactus* spp.) feed on numerous plant species including sweet potato. Larvae of these insects have a host range exceeding 380 species and damage is similar to that of other soil insects, namely white grubs (Chalfant et al. 1990). Adults of this beetle are flightless and females are parthenogenetic and can lay in excess of 3000 eggs (Young 1939). The primary means of spread of this insect are by walking and through commercial transport (Chalfant et al. 1990).

**Sweet Potato Insect Control**

Soil insects feeding on the surface of sweet potato roots create holes, scars, and tunnels. This feeding does not usually decrease biomass but quality is compromised and marketable yield is often reduced and the sweet potatoes will not qualify for U.S. No. 1 grade (Chalfant et al.)
Historically, soil insects in sweet potato agroecosystems have been managed with the use of soil incorporated insecticides applied preplant, but alternative methods of control have been sought since the suspension of chlorinated hydrocarbon insecticides (Schalk et al. 1991). Often, efficacy of soil insecticides can be influenced and compromised by formulation, incorporation method and various edaphic properties (Harris 1966, Day 1978, Chalfant et al. 1987). Soil incorporation has been shown to improve activity of chlorpyrifos in the southeastern United States (Day 1978, Chalfant et al. 1987). Foliar adulticide insecticide applications have demonstrated variable results for controlling soil insects (Sutherland 1986, Zehender 1998). Some alternatives to insecticides in sweet potato IPM include insect resistant plants, biological control and the use of sex attractants or pheromones (Schalk et al. 1991).

**Sweet Potato IPM**

Integrated pest management programs for sweet potato insects have been implemented and they have the potential to improve sweet potato insect pest management in the future (Chalfant et al. 1990). A synthetic sex pheromone has been used in managing and monitoring movement of *Cylas formicarius* (Chalfant et al. 1990) and it is also a component of numerous state quarantine programs throughout the southeastern United States (Nilakhe 1991). The sex pheromone for the banded cucumber beetle has been identified and its use in integrated control programs will be helpful in elucidating the biology and ecology of this insect (Schalk et al. 1991). Identification of the attractive agents (host plant volatiles, pheromones) of sugarcane beetle to sweet potato fields could also be a beneficial component of sweet potato integrated pest management.

Breeding programs that select for storage roots with high levels of resistance to insects and good horticultural attributes are underway and mass selection techniques being used may
improve the crop (Schalk et al. 1991). Many studies evaluating sweet potato resistance to sweetpotato weevil have been conducted (Rolston et al. 1979, Mullen et al. 1985, Story et al. 1996), with little progress being made. Recent studies by (Mao et al. 2001) indicate that storage time and production site affect resistance expression. Using cultivars with high resistance to insects would also allow the production of a high quality product and reduce the reliance on insecticides, but the sweet potato industry in the United States is hesitant to change from popular cultivars such as Beauregard (Schalk et al. 1991). Certain IPM methods such as those involving germplasm and biological control methods are not available in developing countries and as a result many of the approaches for managing pests of sweet potato in these areas are remedial and cultural in nature (Jansson and Raman 1991).

Many insecticides currently used in sweet potato production are under review, and may not be available in the future, due to restrictions being instituted by the U. S. Environmental Protection Agency (Schalk et al. 1991, Curtis 2003). Consumer demand for superior quality and an attractive appearance in the United States places some constraints and increases the need for an integrated management approach to manage sweet potato insects (Chalfant et al. 1990). Sweet potato pest management is in its infancy. To improve IPM in sweet potato, more knowledge is needed on the biology of insects, diseases, nematodes, weeds and mites and management programs, as they relate to sweet potato agricultural production systems (Jansson and Raman 1991). Chapter 7 addresses soil insect abundance and damage at various herbicide regimes.

**Sugarcane Beetle Pest Status**

A new significant soil insect pest of sweet potatoes is the sugarcane beetle, *Euetheola humilis* (Burmeister) Scarabaeidae, Coleoptera. The first reported damage of the sugarcane beetle (referred to as the Rough Headed Corn Stalk Borer, *Euetheola rugiceps* LeConte in some
prior literature) in the United States was in Louisiana sugarcane plantations (Comstock 1880, Titus 1905 & Osterberger 1931). Titus (1905) interviewed farmers that remembered sugarcane beetle damage that had occurred 40 –50 years previously. Howard (1888) reported the beetle as a pest of corn in Union County, North Carolina. Douglas and Ingram (1942) described the sugarcane beetle’s pest status in rice in 1942. Sugarcane beetles will feed on a number of host plants. Damage reports vary through the years and it seems the pest status of the sugarcane beetle is somewhat sporadic (Baerg 1942). The sugarcane beetle was first reported as a pest of sweet potato in Louisiana in 2001. In Louisiana, 2002 and 2003, sweet potato producers reported significant economic losses from sugarcane beetles feeding on sweet potato roots. Preliminary field observations of sugarcane beetle damage, suggested that sugarcane beetles aggregate in some fields more than others. Different aspects of the chemical ecology of the sugarcane beetle were evaluated in laboratory experiments. The objective of the research was to determine sugarcane beetle response to sweet potato volatiles, different cultivars and also conspecifics.

Sugarcane beetles have also been deemed a sporadic problem in field corn in recent years in some southern states. *Euetheola humilis* was reported as a pest in field corn in 2003 in Louisiana, with some severe damage and stand losses being noted (Personal observations 2004). Severe sugarcane beetle damage was reported in Tennessee in 2001 and 2002, but infestations were spotty and were not a significant problem in terms of stand or yield losses (Patrick and Thompson 2004). The sugarcane beetle was also reported as a minor pest of corn in Kentucky in 2003 (Johnson 2003).
Population Dynamics of the Sugarcane Beetle

The sugarcane beetle has been reported as a sporadic pest in many southern states (Phillips and Fox 1917). Only the adult beetles are known to cause any crop injury (Baerg 1942). The beetles feed on corn mainly in the seedling stage, but will feed on plants up to 1.2 m tall by burrowing in the soil and attacking the stalk beneath the surface of the soil, chewing a ragged hole in the plant (Baerg 1942).

In sugarcane, the beetles penetrate the soil beside sugarcane rows where they feed on young sugarcane tillers before the apical meristem emerges above ground (Ingram 1935). Titus (1905) thought the dead roots of sugarcane provided a place for the larvae to grow and develop. The adult sugarcane beetle has been reported injuring rice before the first irrigation and before harvesting after the water has been drained (Ingram 1927).

The adult stage of the sugarcane beetle feeds on the roots of sweet potatoes, contrary to all other known soil insect pests of sweet potato with the exception of the sweetpotato weevil, where the larval stage is the damaging stage. The insect burrows in the ground and chews jagged holes in the roots. The beetles can be found burrowed into the sweet potato at harvest. The beetles produce unattractive holes and scars on the roots, which can drastically affect their market value. Larvae of *E. humilis* have not been reported feeding on sweet potato.

Baerg (1942) reported that the adults do little damage to crops in the fall, but Ingram and Bynum (1932) reported newly emerged beetles feeding on old cane and summer plant cane in the fall. It is hypothesized that damage to sweet potato occurs in the fall prior to harvest by the newly emerged generation of beetles. Chapter 6 reports the manipulation of sweet potato planting dates and how these dates relate to soil insect abundance and damage. Planting date studies were designed to investigate sugarcane beetle damage throughout the season. In late
August and September most other host plants the beetle is known to feed on have largely been harvested with the exception of sugarcane. Evaluation of the host plant preference of the sugarcane beetle is another objective that is addressed by this dissertation. The host plant preference of the sugarcane beetle was evaluated in greenhouse choice tests, using several plant species reported as hosts of the beetle.

The pest status of the sugarcane beetle in Louisiana in recent decades is uncertain. It is probable that the beetles were present, but that damage was attributed to other soil dwelling insects, probably those in the genus *Phyllophyga* or *Naupactus*.

**Sugarcane Beetle Biology**

The sugarcane beetle undergoes complete metamorphosis with four distinct life stages; these stages are egg, larva, pupa and adult (Baerg 1942). Mating occurs just above the surface of the soil followed shortly by oviposition (Phillips and Fox 1917). Beetles lay their eggs near to or in contact with the plants they feed upon (Ingram 1927). Douglas and Ingram (1942) reported that adults deposited eggs in sod near cultivated fields. Cornfields have been reported to be unfavorable habitats for reproduction; numerous eggs are laid, but relatively few beetles develop from these eggs, compared to those that are laid in pastures and old sod (Phillips and Fox 1917). Ingram and Bynum (1932) looked at different locations on a sugarcane plantation, and found that no eggs were laid near woodlands compared to 101,640 eggs in a Bermuda grass pasture 3 m from a bayou. Eggs have also been found in unsubmerged rice fields (Douglas and Ingram 1942).

The adult beetle is stout, dull black and ranges in length from 13-16 mm. Teneral beetles are glossy but become dull with age. The pronotum is wider than long and has numerous punctures. The elytra have double rows of coarse punctures and are as wide as they are long.
The beetle has strong fossorial fore legs (Baerg 1942). The egg is oblong, white, smooth and hatches in about two weeks after enlarging to 3x its original weight (Baerg 1942). The eggs are laid primarily in early June and are oviposited singly or in groups beneath the soil where the female beetle is feeding (Phillips and Fox 1917). Baerg (1942) determined that one female might lay up to 100 eggs in her lifetime. The larvae are white grubs with red head shields. Larvae undergo three successive instars for an average overall developmental time of 57 days. At full development the larva is 32 mm long and ca. 6.5 mm in diameter (Baerg 1942). Larvae are known to feed on various rotting and decaying materials, such as cane trash, grass roots and cane plants killed by adult feeding (Ingram and Bynum 1932). Newly formed pupae are white but quickly brown (Phillips & Fox 1917). The pupa is about 15mm in length and has stout, triangular mouth parts (Baerg 1942). The pupal stage of the beetle does not feed and remains under the soil throughout development (Phillips and Fox 1917). Average duration of the pupal stage is 16.5 days (Baerg 1942). Total average time for development is about 85 days (Baerg 1942). The new generation of beetles emerges in mid-September and will feed on available hosts until cold weather arrives. As temperatures decrease, the beetles enter a state of torpor or hibernation (Phillips and Fox 1917, Baerg 1942).

**Sugarcane Beetle Activity Patterns**

Sugarcane beetles are univoltine, with a definite period of torpor or hibernation (Phillips and Fox 1917). The insect remains dormant in the adult stage from late September or early October until late March and early April when increases in temperature draw them out of hibernation to feed (Phillips and Fox 1917, Douglas and Ingram 1942). Holman (1968) found that minimum winter temperatures and average rainfall during larval development have a significant effect on population size from one year to the next, and that colder winters and
decreased rainfall in the spring were advantageous to population growth. White (1990) supported the conclusions of Holman. The number of adult sugarcane beetles he sampled in 1982-83 following a mild winter was significantly less than the number of beetles collected in 1983-84 and 1984-85, years having more severe winters.

Sugarcane beetles are nocturnal and are attracted to light sources. They commonly feed and fly at night (Comstock 1880, Webster 1890, Phillips and Fox 1917, Holman 1968). The beetles, though primarily nocturnal, will occasionally come above ground during the day (Ingram and Bynum 1932). Comstock (1880) conducted studies with trap lanterns and noted the “readiness to which the beetles were attracted to light.” Black light traps operated through 1983-1987 in Louisiana sugarcane fields documented that the beetles were most abundant between March and May (White 1990) but sugarcane beetle flight activity is also detected in August and September with the new generation of beetles (Holman 1968 & White 1990). During the fall, adults spend the majority of time underground, but will come to the surface to feed on a variety of wild grasses on warm days (Phillips and Fox 1917). They also noted that the beetles would fly long distances if an adequate food source was lacking in an area where they emerged.

**Sugarcane Beetle Control**

There are many cultural control practices reported for the sugarcane beetle. Phillips and Fox (1917) recommended the elimination of dormant farmland and pastures, increased cultivation and rotation of crops, and heavy applications of fertilizers to speed growth of plants. Baerg (1942) recommended late planting, which would allow for increased cultivation, and in turn decrease the amount of grasses and sedges present in the field which attract the beetles and promote oviposition. Intense cultivation is also an effective measure of destroying larvae and
pupae that may be present in the soil (Baerg 1942). Eden (1954) found aldrin to be effective for controlling the beetle in corn from time of planting for about 1 month. Aldrin and heptachlor granules and sprays at 1 pound per acre were also found to be effective for controlling beetle damage in corn (Henderson et al. 1958). Howard (1888) recommended submerging infested rice fields. Treating rice seed with kerosene and coal tar was found to be effective under controlled conditions, as well as elimination of sod land, which is a natural breeding habitat for the beetle (Douglas & Ingram 1942). White (1990) recommended cultural control practices that would promote growth, minimize organic material, and promote good drainage. He also recommended using hearty plants and the efficient use of herbicides. There are also several natural predators of the sugarcane beetle such as numerous bird species, skunks, frogs and some dipteran larvae (Douglas and Ingram 1942 & Baerg 1942).

Currently, no insecticides are labeled for control of the sugarcane beetle in sweet potatoes. This research reports results of insecticide bioassays that evaluated the efficacy of selected insecticides for potential control of the sugarcane beetle. Riley (1986) evaluated numerous insecticides for control of the sugarcane beetle in field corn with variable results. Recently, some granular insecticides such as Lorsban 15G (chlorpyrifos) and Counter 15G (terbufos) have been shown to reduce sugarcane beetle infestations in field corn when banded or soil incorporated (Patrick 2004). Tindall et al. (2005) reported a reduction in sugarcane beetle damage to field corn seedlings in field plots treated with bifenthrin, chlorpyrifos, fipronil, clothianidin, cyfluthrin, tebuirimphos, chlorethoxyfos and terbufos compared to untreated control plots. Neonicitinoid field corn seed treatment insecticides including clothianidin, thiomethoxam, and imidacloprid were evaluated in an artificial infestation field experiment in 2004 against the sugarcane beetle and clothianidin (Poncho) applied as a seed treatment at 0.45
milligrams active ingredient per seed was effective in reducing damage to early seedlings by sugarcane beetles (Smith et al. 2005).

**Objectives**

1. To investigate the host plant preference of the sugarcane beetle.

2. To evaluate sugarcane beetle behavior as it relates to sweet potato volatiles, cultivars and response to conspecifics.

3. To determine the susceptibility of the sugarcane beetle to insecticides in laboratory bioassays.

4. To determine the susceptibility of the sweetpotato weevil to insecticides in laboratory bioassays.

5. To investigate the effect of planting date on insect root damage, adult insect abundance and number of storage roots in sweet potato.

6. To determine the effect of herbicide regime on insect root damage, adult *Diabrotica* abundance, and yield in sweet potato.

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CHAPTER 2
HOST PLANT PREFERENCE OF THE SUGARCANE BEETLE IN A GREENHOUSE CHOICE TEST

Introduction

Sugarcane beetles, *Euetheola humilis* Burmeister were first described as pests of sweet potato in Louisiana in 2002 (Hammond 2002). Sweet potatoes are grown in over 100 countries and are second in production among root crops to white potatoes, *Solanum tubersum* L. (Horton 1988). The United States has an average annual of $309 million (USDA 2006). In 2005, sweet potatoes were harvested from 6,800 hectares in Louisiana for a value of $41 million (USDA 2006).

*Euetheola humilis* adults are polyphagous herbivores in the southern states in the United States (Baerg 1942). The adult stage of the beetle feeds beneath the soil surface on sweet potato roots, creating unattractive, jagged scars, but feeding has not been observed on sweet potato stems or leaves (Hammond 2002). The adult is the only life stage of the beetle known to cause damage to crops (Philips and Fox 1917). Historically, *E. humilis*, referred to as *Euetheola rugiceps* (LeConte) in some prior literature, has been reported as a pest of field corn, *Zea mays*, sugarcane, *Saccharum officinarum*, rice, *Oryza sativa*, Johnson grass, *Sorghum halapense*, strawberry, *Fragaria* spp. and some evidence suggests they live and develop on Bermuda grass, *Cynodon dactylon*, and angled sedge, *Scirpus carinatus* (Baerg 1942).

Sugarcane beetles will feed on corn up to 1.2 m tall just below the surface of the soil causing the foliage to wilt and inflicting damage to the apical meristem of plants (Baerg 1942). Damage to rice and sugarcane also occurs on young, tender below ground stems (Ingram 1927, Ingram and Bynum 1932). Damage to strawberry occurs under ground at the base of the plant.
and causes wilting of foliage (Baerg 1942). Although feeding injury by beetles has not been previously described on Bermuda grass, beetles will feed on Bermuda grass roots in the greenhouse (personal observation).

In Louisiana, sweet potato producers reported significant economic losses due to sugarcane beetles feeding on sweet potato roots prior to harvest in 2002 and 2003. Damage reports from *E. humilis* in corn and sugarcane are sporadic, and outbreaks often do not occur in successive years (Philips and Fox 1917). Heavy outbreaks of *E. humilis* in Louisiana sweet potato fields during 2002 and 2003 were followed by two years of minor damage. *Euetheola humilis* demonstrate a propensity to aggregate in sweet potato fields. It is possible that plant-derived allelochemicals, insect pheromones, or a combination of both may be acting as attractive agents in sweet potato fields.

An insect's host plant range is dynamic, and host plant preference can be variable between and within insect populations (Schoonhooven et al. 1998). Factors related to both host plants and insects, including geographical location, seasonality, developmental stage, sex, and temperature, can affect host plant preferences of phytophagous herbivores (Schoonhooven et al. 1998). Singer et al. (1992) defined host plant preference as the chance that an insect will accept a certain host if encountered. Most insects prefer host plants that will optimize their survival and reproduction (Dodge et al. 1990). Generalist feeders are common among insect herbivores, but why they feed on numerous plant species is not yet clear (Tikkanen et al. 2000).

Limited information is available on the ecology and feeding behavior of *E. humilis*, probably because it is a sporadic pest and seldom causes severe damage to crops. The objective of this study was to investigate host plant preference of *E. humilis* among known hosts in a greenhouse choice test. Our hypothesis is that the beetles would prefer to feed on those plant
species that have received the most damage in field situations in recent years, namely sweet potato and corn. Determination of host plant preference will provide insight into the biology and ecology of this sporadic but serious insect pest of several agricultural commodities in the southern United States.

**Materials and Methods**

**Plants**

Choice tests to evaluate host plant preference of the sugarcane beetle were conducted in a greenhouse in 2003 and 2005 located at Louisiana State University, Baton Rouge, LA. The choice tests included seven plant species representative of the beetle's known host range. Plant species used in the choice tests were sweet potato, sugarcane, corn, Bt corn, rice, strawberry and Bermuda grass (Table 2.1). Sweet potatoes, corn, Bt corn, and rice were grown in 18 L polytainer cans (Hummert International, Earth City, MO) in the greenhouse in Jiffy Mix Plus® potting medium. Sugarcane plants were obtained from the Louisiana State University Agricultural Center St. Gabriel Research Station, St. Gabriel, LA and transferred to 18 L polytainer cans with Jiffy Mix Plus® potting medium. Strawberry plants and Bermuda grass were obtained from Louisiana State University Agricultural Center Burden Research Center, Baton Rouge, LA and transferred to 18 L polytainer cans with Jiffy Mix Plus® potting medium before testing. All plants were maintained in the greenhouse at 32 ± 2º C and were watered until the soil was saturated three times weekly.

**Insects**

Sugarcane beetles used in the choice tests were collected using universal black light traps (BioQuip Products, Rancho Dominguez, CA) during April and May of 2003 and 2005. Because we relied on field collections of sugarcane beetles to conduct experiments, we replicated the
choice test over two years to determine if any differences in preference existed between beetles collected in the two years. *Euetheola humilis* were collected at the Louisiana State University Agricultural Center Sweet Potato Research Station, Chase, LA in 2003 and from Zachary, LA in 2005. Beetles collected at Chase, LA, 2003 had possibly fed on sweet potato and/or field corn prior to collection. Beetles collected in Zachary, 2005, most likely emerged from nearby pastures. After collection, beetles were maintained on storage roots 'Beauregard cultivar' and held in plastic containers (5.6 L) with screen covers in a bioclimatic chamber, (Percival Scientific Inc., Perry, Iowa) with a 14:10 light:dark cycle at 28º C and 80 % relative humidity. Beetles were tested within four weeks of collection and all containers were cleaned and beetles were provided fresh roots on a weekly basis.

**Table 2.1.** Plant species used in choice test of host plant preference of *E. humilis*

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Cultivar</th>
<th>Common Name</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ipomoea batatas</em> L.</td>
<td>Beauregard</td>
<td>Sweet Potato</td>
</tr>
<tr>
<td><em>Zea mays</em> L.</td>
<td>DEKALB 69-70</td>
<td>Field corn</td>
</tr>
<tr>
<td><em>Zea mays</em> L.</td>
<td>DEKALB YG (69-70)</td>
<td>Bt field corn</td>
</tr>
<tr>
<td><em>Oryza sativa</em> L.</td>
<td>Cocodrie</td>
<td>Rice</td>
</tr>
<tr>
<td><em>Saccharum</em> spp.</td>
<td>LCP 85-384</td>
<td>Sugarcane</td>
</tr>
<tr>
<td><em>Cynodon dactylon</em> L.</td>
<td></td>
<td>Bermuda grass</td>
</tr>
<tr>
<td><em>Fragaria × ananassa</em> L.</td>
<td>Chandler</td>
<td>Strawberry</td>
</tr>
</tbody>
</table>

**Experimental Design**

All plant species were transferred to grow tubs 0.9 x 0.6 x 0.2 m (Hummert International, Earth City, MO) for the choice test. Grow tubs were divided into seven sections ca. 0.13 m wide arranged longitudinally with plexiglass partitions 0.5 x 0.13 m. Plant species were assigned randomly to one of the sections in the grow tub. Two plants each of sweet potato, sugarcane, and strawberry were placed in a section. Two areas of six seedlings each (12
seedlings/section) of corn, Bt corn, and rice were planted in a section. Corn used in the experiments was V2-V4 stage and rice was in the three-leaf stage. Bermuda grass was transplanted so as to provide 100% coverage of a section. Experiments were conducted immediately following transplant. Five replications (one replicate per tub) of the choice test were conducted each year. New potting soil (84 L) was placed in each grow tub after being moistened with 3.78 L of water before transferring plants. After transplant the soil was leveled across all sections and ca. 0.30 L of water was distributed in each section.

Sugarcane beetles used in experiments were starved 48 h preceding a test. Six beetles were placed in each section of the grow tub. Three beetles were grouped around each plant or clump of plants (six beetles /section). After visually observing that all beetles had burrowed into the soil, hardware cloth was secured across the top of the grow tub to prevent escape. Insects were allowed to feed and move freely for 96 h. Sugarcane beetles could move across plant sections and burrow into different plant sections. However, they could not move underground through sections because of the plexiglass dividers. At the end of 96 h, the hardware cloth was removed, the soil from each section of each grow tub was sifted and number of beetles per section was recorded. This was a conservative procedure for assessing preference because exercising a choice involved rejecting the initial host plant, exiting the soil, moving to a new section, and accepting a new plant. All plants were evaluated for presence of feeding. The amount of plant biomass consumed and feeding scars was not compared across plants because of the wide range of plant species evaluated in the choice test.

Data Analysis

Logistic regression (PROC LOGISTIC, SAS Institute 2004), which assumes a binomial distribution, was used to examine the functional relationship between E. humilis behavioral
responses (choice) and plant species. SAS estimates the coefficients of logistic models with maximum-likelihood estimates on logit values for beetle choice. The analysis compared sweet potato to all other plant species included in the tests. Estimated values from the logistic analysis in the form of odds are automatically back transformed to probabilities with 95 % confidence intervals for each category in the analysis. Comparisons between categories were made with odds ratio tests.

**Results**

Of 420 insects that were used in the choice tests, 97% were recovered and these data are included in the analysis. The response variable, *E. humilis* choice was analyzed with the logistic model in relation to plant species. Because differences were not detected between the two populations of *E. humilis* used in the choice test, data were pooled across years for analysis. In 2003, beetles were recovered primarily from sweet potato and sugarcane (Table 2.2). In 2005, more sugarcane beetles were recovered in sweet potato, corn and Bt corn (Table 2.2).

**Table 2.2.** Mean number of sugarcane beetles recovered from seven plant species in a choice test in 2003 and 2005.

<table>
<thead>
<tr>
<th>Plant species</th>
<th>2003 Mean ± SE SCB recovered</th>
<th>2005 Mean ± SE SCB recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sweet potato</td>
<td>15.00 (4.04)</td>
<td>8.00 (1.67)</td>
</tr>
<tr>
<td>Corn</td>
<td>2.80 (1.24)</td>
<td>13.60 (1.03)</td>
</tr>
<tr>
<td>Sugarcane</td>
<td>12.00 (2.02)</td>
<td>5.00 (2.07)</td>
</tr>
<tr>
<td>Rice</td>
<td>2.40 (0.81)</td>
<td>2.40 (0.68)</td>
</tr>
<tr>
<td>Bermuda grass</td>
<td>4.60 (1.81)</td>
<td>2.20 (0.80)</td>
</tr>
<tr>
<td>Bt Corn</td>
<td>1.60 (0.60)</td>
<td>7.60 (0.40)</td>
</tr>
<tr>
<td>Strawberry</td>
<td>1.60 (1.12)</td>
<td>2.40 (0.40)</td>
</tr>
</tbody>
</table>

The overall effect of plant species in the logistic model was significant ($\chi^2 = 139.297$, DF = 1, 6, P< 0.0001), indicating that beetles exercised a preference for some plants over others.
Sweet potato was preferred over all other hosts included in the test (P< 0.05). Sweet potato was selected ca. 50% more often than corn and sugarcane, the next preferred plant species included in the choice test (Table 2.3, Fig 2.1) The probability of \textit{E. humilis} choosing rice, Bermuda grass, strawberry or Bt corn relative to sweet potato was considerably lower (Table 2.3, Fig 2.1).

\textbf{Table 2.3.} Logistic model for the effects of plant species on \textit{E. humilis} choice$^a$

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Coefficient</th>
<th>SE</th>
<th>Odds ratio</th>
<th>95% C.I.</th>
<th>Wald $\chi^2$</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>-0.445</td>
<td>0.166</td>
<td>1.560</td>
<td>1.129-2.160</td>
<td>7.238</td>
<td>0.0071</td>
</tr>
<tr>
<td>Sugarcane</td>
<td>-0.400</td>
<td>0.164</td>
<td>1.493</td>
<td>1.081-2.058</td>
<td>5.930</td>
<td>0.0149</td>
</tr>
<tr>
<td>Rice</td>
<td>-1.838</td>
<td>0.238</td>
<td>6.289</td>
<td>3.953-10.00</td>
<td>59.911</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Bermuda grass</td>
<td>-1.463</td>
<td>0.210</td>
<td>4.329</td>
<td>2.865-6.535</td>
<td>48.437</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Bt Corn</td>
<td>-1.128</td>
<td>0.191</td>
<td>3.086</td>
<td>2.123-4.505</td>
<td>34.762</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Strawberry</td>
<td>-2.031</td>
<td>0.254</td>
<td>7.634</td>
<td>4.630-12.50</td>
<td>63.743</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Intercept</td>
<td>-0.932</td>
<td>0.110</td>
<td></td>
<td></td>
<td>71.639</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

n = 407.

$^a$The response variable in the model was \textit{E. humilis} choice / plant species

\textbf{Figure 2.1.} Probabilities with 95 % confidence intervals for plant species associated with \textit{E. humilis} abundance levels in a greenhouse choice test
Strawberry and rice were the least preferred plants in the choice test. Sweet potato was selected ca. 7.5 fold more often than strawberry and > 6 fold more often than rice. Sweet potato was also ca. 3 fold more attractive than Bt corn and > 4 fold more preferred than Bermuda grass (Table 2.3, Fig 2.1).

**Discussion**

Insects can be variable in their decisions to accept a food source, initiate feeding or oviposit (Bernays 1995) and insect behavior is influenced by a variety of internal and external factors (Kennedy 1978) such as host plant chemicals in nature (Schultz 1988). Most attractive plant substances are secondary chemicals, assumed to have originated as by-products of plant primary metabolism (Hartmann 1996) and the volatile and non-volatile compounds produced by plants may mediate plant/insect interactions as attractants, repellents, stimulants, or deterrents to feeding/oviposition (Starr et al. 1991).

Increased attraction of sugarcane beetles to sweet potato in the choice test could be due to fluctuations in the chemo-orientation of the beetles, host plant volatile emissions, or a combination of these. Induction of preference is also a possible explanation due to changes in sensitivity of chemoreceptors from previous encounters with host plants (Bernays 1995). It is possible that *E. humilis* used in the choice tests had previously been exposed to one or more of the plants in nature prior to collection. Observations of laboratory held beetles show that initial feeding by one beetle may in turn stimulate feeding by conspecifics.

Volatile attraction has been demonstrated in numerous scarabs such as the Japanese beetle, *Popillia japonica*, (Ahmad 1982, Loughrin et al. 1996, 1998) and *Maladera matrida* Argaman (Harari et al. 1994). Sweet potato plant volatiles from different plant parts have been shown to attract sweetpotato weevils, *Cylas formicarius* (Nottingham et al. 1987, 1989), with
several studies indicating terpenoids as the attractive agents for sweetpotato weevils (Starr et al. 1991, Nottingham et al. 1989). Wang and Kays (2002) found that sweet potatoes release large amounts of terpenes in a volatile form and experiments conducted with a dual choice Y-tube olfactometer have shown that *E. humilis* is differentially attracted to host plant volatiles from injured sweet potato roots as opposed to intact roots.

Sweet potato was the host plant most preferred by *E. humilis* in the greenhouse choice tests conducted in the present study. The strong preferences exhibited by *E. humilis* for sweet potato support the hypothesis that the beetles have been pests of sweet potato for many years and that damage may have been falsely attributed to other soil insect pests of sweet potato.

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http://www.nass.usda.gov:8080/QuickStats/PullData_US

CHAPTER 3

ASSESSMENT OF THE CHEMICAL ECOLOGY OF THE SUGARCANE BEETLE IN LABORATORY EXPERIMENTS

Introduction

Sugarcane beetles, *Euetheola humilis* Burmeister, were first documented as a pest of sweet potato in Louisiana in 2001 (Hammond 2002). In Louisiana, in 2002 and 2003, sweet potato producers reported significant economic losses due to sugarcane beetles feeding on sweet potato roots prior to harvest. Damage reports from *E. humilis* in corn and sugarcane are sporadic, and outbreaks may not occur in successive years (Philips and Fox 1917). The outbreaks of *E. humilis* in Louisiana sweet potato fields, 2002 and 2003, were followed by reduced populations of sugarcane beetle in 2004 and 2005.

Limited information is available on the ecology and feeding behavior of *E. humilis*, probably because it was considered a minor insect pest until recent damage reports warranted concern. *Euetheola humilis* demonstrate a propensity to aggregate in sweet potato fields. In 2003, one 45-hectare field in West Carroll parish had extensive sugarcane beetle damage which resulted in total crop loss at this location. Two weeks prior to harvest the grower inspected the field, taking numerous samples, which showed minimal sugarcane beetle damage, suggesting that the majority of damage occurred within a two week time period (personal communication). One hypothesis for sugarcane beetle aggregative behavior is that there appears to be a cue to which the beetles are responding to over a short period of time, that attracts subsequent beetles to the same location.

Many insect behaviors, such as communication within species and recognition of food sources, are mediated by chemicals (Harris and Foster 1994). The behavior of insects is not controlled by one chemical or cue, but is affected by many innate and external cues (Kennedy
Most attractive plant substances are secondary chemicals, assumed to have originated as by-products of plant primary metabolism (Schoonhoven et al. 1998). The volatile and non-volatile compounds that are produced by plants and are involved in plant/insect interactions can act as attractants, repellents, stimulants, or deterrents to feeding/oviposition (Starr et al. 1991).

Volatiles from different parts of the sweet potato have been shown to be attractive to sweetpotato weevils, *Cylas formicarius* Fab. (Nottingham et al. 1987, 1989), and several studies implicate terpenoids as the attractant volatiles (Starr et al. 1991). Surface chemicals, specifically terpenes, have also been shown to positively mediate oviposition (Nottingham et al. 1989). Sweet potato plant volatiles (sesquiterpenes) have likewise been implicated as possible resistance factors against sweetpotato weevil (Wang and Kays 2002).

Many researchers have investigated the role of host plant volatiles in insects. Japanese beetles are the best known of the polyphagous scarab beetles that respond to and aggregate on particular aromatic host plants (Fleming 1972) and volatile compounds play a key role in host location and acceptance by the Japanese beetle (Ahmad 1982). Induced volatiles from feeding damage account for the majority of aggregative behavior in the Japanese beetle (Loughrin et al. 1995, 1996). The mechanism of aggregative behavior has also been investigated in *Maladera matrida* Argaman (Harari et al. 1994, Yarden and Shani 1994), where it was demonstrated in field and laboratory experiments that males initiate feeding that in turn attracts females to host plant volatiles, eventually forming an equal sex ratio.

Interest in pheromone research is intense. Pheromones allow for more effective control measures in integrated pest management systems (Justsom and Gordon 1989, Leal et al. 1994). Pheromones can mediate many behaviors, and are classified according to function, such as sex pheromones, and aggregation pheromones. Sex pheromones can positively or negatively
influence mating and aggregation pheromones lead to increases in conspecifics in a certain area (Jutsom and Gordon 1989). The female produced sex pheromone of the sweetpotato weevil, *Cylas formicarius* Fab., is currently being used in monitoring and management programs in sweet potato throughout the United States (Jansson et al. 1991).

Smith and Hadley (1926) observed the mating behavior of the Japanese beetle, *Popillia japonica*, in 1926. They recorded distinct movements of males toward plants where females were feeding and also noted males would attempt copulation with the females before they fully emerged from the ground. Female Japanese beetles, *Popillia japonica*, release a volatile sex pheromone that can attract males very quickly and in large numbers (Goonewardene et al. 1970, Ladd 1970). Pheromones have been identified and are used in the monitoring of other scarabs as well (Alm et al. 2004, Nojima et al. 2003, Leal et al. 1994).

Several mechanisms have previously been proposed for pheromone-mediated aggregative responses in insects: 1) aggregation pheromones act alone (Phillips and Burkholder 1981); 2) a synergism exists between aggregation pheromones and host plant volatiles (Bartelt et al. 1990a,b); 3) an aggregation pheromone is produced only when a host plant is encountered (Peng and Weiss 1992); 4) aggregation pheromones are released while feeding (Domeck and Johnson 1988); 5) aggregation pheromones are produced after host plant materials are consumed (Eisner and Meinwald 1987); 6) attractants are produced by an interaction between microorganisms and feeding insects (Domek and Johnson 1990); and 7) attractants are produced by microorganisms developing on a particular host plant (Dolinski and Loschiavo 1973).

Carbohydrates comprise 80-90% of the dry matter of sweet potato. Their numerous functions include structural support, energy reserves, and flavor enhancement (Kays 1992). Carbohydrates are generally separated into three general classes: monosacharides,
oligosaccharides, and polysaccharides. Mono and disaccharides comprise 4-5 % of the dry weight of sweet potatoes, and the three main sugars are sucrose, glucose, and fructose (Kays 1992). Cultivars can differ significantly in total and specific sugar content (Kays 1992).

Beauregard is the predominant sweet potato cultivar grown in Louisiana and throughout the United States (Benedict 2005). Georgia Jet is cultivated on a smaller scale and in preliminary field observations in Louisiana it has been associated with reduced damage from soil insects (personal communication). White Star and Bunch Porto Rico cultivars are in the lineage of Georgia Jet (Harmon 1974). The objectives of these studies were to evaluate sugarcane beetle behavior in relation to sweet potato volatiles, different cultivars, and conspecifics in laboratory tests.

**Materials and Methods**

**Insects**

Adult sugarcane beetles used in bioassays were collected using universal black light traps (BioQuip Products, Rancho Dominguez, CA) located near Zachary, LA in East Baton Rouge Parish, Louisiana during April and May of 2005. Minimal agriculture production occurs in this area; however, numerous pastures were located in close proximity to the collection site. After collection, beetles were sexed and held in plastic containers (5.6 L) with screen covers in a bioclimatic chamber (Percival Scientific Inc., Perry, Iowa) with a 14:10 light:dark cycle at 28º C and 80 % relative humidity. Sugarcane beetles were maintained on sweet potato roots 'Beauregard' and containers were cleaned and beetles were provided fresh roots on a weekly basis. Beetles used in bioassays were held without sweet potato roots or other food for 48 h prior to testing. Sweet potatoes were used to maintain beetles (Beauregard) and roots used in the olfactometer experiments ('Beauregard' and 'Georgia Jet') were obtained from the Louisiana State
University Agricultural Center Sweet Potato Research Station, Franklin Parish, LA where they were first cured for five days at 30º C and 85 % relative humidity. After curing they were stored in a cinder block building at 60º C and 70-80 % humidity until needed. Sweet potatoes used in container bioassays were obtained from the Burden Center, East Baton Rouge Parish, LA (White Star, Bunch Porto Rico, Beauregard) and the Chase Sweet Potato Station, Chase, LA (Beauregard, Georgia Jet) prior to use. Pairs of sweet potatoes used in experiments were selected for similar shape and size. Unless otherwise specified, sweet potato roots used in experiments were free of any observable insect damage.

**Olfactometer Experiments**

*Euetheola humilis* behavior was investigated by evaluating the anemotactic response of beetles to air streams containing volatiles from sweet potatoes and conspecifics using a Y-tube olfactometer similar in design to that described by Steinberg et al. (1992) and Sullivan et al. (2000). Compressed inlet laboratory air from a single source was split into two streams that were directed through air flow regulators to maintain a flow rate of 100 ml/min through the apparatus. The two air streams then passed through activated charcoal and distilled water to filter and humidify the air. The air stream then passed through either 4 L glass containers or 130 ml Pyrex glass tubes before connecting to the Y-tube with PTFE tubing. The two arms of the Y-tube, separated by a 90º angle, were 10 cm long and had an internal diameter of 1.0 cm. Individual sugarcane beetles were introduced at the end of the Y-tube stem. Bioassays were conducted under ambient laboratory lighting and temperature and relative humidity in the bioassay room were 22 ± 2 º C and 65 ± 5 % respectively. Trials were generally run between the 2-6 h of photophase. Three trials were conducted for each paired test (Table 3.1).
The response of 60 male and 60 female sugarcane beetles were evaluated for four of the odor pairs and 60 beetles (undetermined sexes) were evaluated for the remaining five odor pairs. Insect damaged roots used in olfactometer bioassays were removed from laboratory colonies five days after introduction (sugarcane beetle and sweetpotato weevil). Mechanically damaged roots were injured using a #10 cork borer to a depth of 1.27 cm (4 lesions / root). Washed injured roots were rinsed with distilled water for 5 min prior to testing to remove any regurgitate or frass from the roots.

![Figure 3.1. Y-tube olfactometer with primary components labeled.](image)

Individual beetles were introduced to the system at the Y-tube opening and were initially observed for 2 min. If after 2 min no movement had been detected, the beetle was removed and replaced by an alternate insect. Beetle choice was recorded after a beetle walked 5 cm down one arm of the Y-tube within 8 min of initiating movement. Sugarcane beetles not responding
within 8 min were termed non-responders. Sugarcane beetles were not reused within paired tests and Y-tubes and all connecting tubing were replaced with clean materials between replicate trials. Tubing connecting the sample containers to the Y-tube was manually swapped to opposite ends of the Y-tube between each replicate (beetle) to eliminate any directional bias by the beetles that was unrelated to odor attraction. Between experiments, all sample containers, tubing and Y-tubes were cleaned with soap and water and rinsed with 95 % EtOH followed by oven drying at 60º C for 24 h.

**Olfactometer Data Analysis**

Data for each odor in each test were pooled as a response category and compared to a hypothesized 50:50 ratio to determine significant preferences. Olfactometer data were analyzed with G-tests for goodness of fit with Williams correction for small sample sizes at a significance level of $P = 0.05$ (Sokal and Rohlf 1995).

**Cultivar Preference Experiments**

Preference of sugarcane beetles for different sweet potato cultivars was investigated using 5.6 L plastic containers for arenas. All sweet potato roots used in the study were harvested from the Louisiana State University Agricultural Center Sweet Potato Research Station, Chase, LA (Georgia Jet, Beauregard, Bunch Porto Rico) or the Louisiana State University Burden Research Center, Baton Rouge, LA (Beauregard, Bunch Porto Rico, White Star). Paired tests evaluated included: Georgia Jet vs. Beauregard, Beauregard vs. White Star, and Beauregard vs. Bunch Porto Rico. Coarse vermiculite (80 g) moistened with 50 ml of water was placed in each replicate arena for each paired test. Each arena contained one sweet potato of each cultivar to be tested and ten sugarcane beetles. The Beauregard vs. Georgia Jet cultivar pairing was also conducted using a single beetle per replicate arena. A minimum of five replications was
conducted for each paired test. Sweet potato roots were placed parallel to each other at one end of the arena and sugarcane beetles were introduced at the opposite end of the arena. Arenas were placed in a holding room at 27 °C ± 2 °C and 75 ± 2% relative humidity for 72 h, after which the number of scars per root was recorded. For each paired cultivar test, the number of scars/root was recorded and the data were analyzed using a paired t-test (PROC UNIVARIATE, SAS Institute 2004).

Sugar Analysis

Analysis of total sugar content was determined on the raw roots of the sweet potato cultivars. All analyses were conducted within four weeks following harvest, because changes in sugar content are minimal (Picha 1987). Roots were harvested from their respective locations on the same day. Intact roots were halved longitudinally and uniformly grated over the surface to a depth of 3 mm. The grated tissue from each of the three roots / replication was combined and 10 g was homogenized in 80% ethanol for 1 min at high speed using a Brinkman homogenizer (Brinkman Instruments, Westbury, NY). The resulting slurry was boiled for 15 min, cooled and filtered through Whatman #4 paper. The remaining residue and the original container were washed with 80% ethanol and the filtrate was increased to a final volume of 100 ml. A 20:1 sample was then analyzed using HPLC. Total sugar values were obtained by summing fructose, glucose, and sucrose values for each cultivar. Sugar content was compared between cultivars using ANOVA and means were separated according to Tukey's Test (PROC MIXED, SAS Institute 1999).
Results

Olfactometer Experiments

Female and male sugarcane beetles were significantly more attracted to sugarcane beetle damaged sweet potato roots than to intact sweet potato roots (Table 3.1, Figure 3.2). Beetle injured roots were over six and 3.5 times more attractive, respectively, to male and female beetles (Figure 3.2). Percent response of beetles was also greater for mechanically damaged roots vs. uninjured roots for both male and female sugarcane beetles ($P < 0.05$) (Figure 3.2). Percent response of males was 30 times greater for mechanically damaged roots when compared to intact roots and female beetle response to mechanically damaged roots was 3 times greater than for intact roots (Figure 3.2). Male sugarcane beetle's percent response was significantly greater for beetle damaged roots compared to mechanically damaged roots (Table 3.1, Figure 3.2). No differences were detected in female beetle response to the two injury types (Table 3.1, Fig 3.2). Sugarcane beetles did not respond differentially to washed beetle injured roots vs. unwashed beetle injured roots, suggesting that beetle excretions were not a confounding factor in beetle attraction to wounded sweet potato roots. When given a choice of a sugarcane beetle damaged root or a sweetpotato weevil damaged root, beetle percent response was significantly greater for sweetpotato weevil damaged roots (Table 3.1, Fig 3.2).

Sugarcane beetles previously fed on Beauregard or Georgia Jet roots did not respond differently to these cultivars in olfactometer bioassays (Table 3.1, Fig 3.3), however, percent response was higher in both cases for damaged Beauregard roots when compared to damaged Georgia Jet roots. Beetles similarly did not prefer intact Beauregard roots over intact Georgia Jet roots. Male and female sugarcane beetle's response to conspecifics was also evaluated. In both cases, sugarcane beetle's percent response was significantly greater for the female beetle
sample vs. the male beetle sample, suggesting the presence of a female produced aggregation pheromone (Table 3.1, Fig 3.4).

**Figure 3.2.** Percentage of *Euetheola humilis* adults walking toward one of two paired sweet potato roots in a Y-tube olfactometer. An asterisk (*) indicates a significantly greater response toward one of the two choices using G-tests with William's correction for small samples at a significance level of $P \leq 0.05$. NR = percentage of beetles not responding to either arm within 8 min of introduction.

**Figure 3.3.** Percentage of *Euetheola humilis* adults walking toward one of two paired sweet potato cultivars in a Y-tube olfactometer. An asterisk (*) indicates a significantly greater response toward one of the two choices using G-tests with William's correction for small samples at a significance level of $P \leq 0.05$. NR = percentage of beetles not responding to either arm within 8 min of introduction.
Table 3.1. Sugarcane beetle responses for two-choice odor tests in a Y-tube olfactometer.

<table>
<thead>
<tr>
<th>Beetles Tested</th>
<th>Paired Test</th>
<th>G-statistic (with William's Correction)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>*Beetle injured vs. intact</td>
<td>31.42</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Female</td>
<td>*Beetle injured vs. intact</td>
<td>19.27</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Male</td>
<td>*Mechanically injured vs. intact</td>
<td>59.84</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Female</td>
<td>*Mechanically injured vs. intact</td>
<td>15.95</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Male</td>
<td>*Beetle injured vs. mechanically injured</td>
<td>17.49</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Female</td>
<td>Beetle injured vs. mechanically injured</td>
<td>1.18</td>
<td>0.2763</td>
</tr>
<tr>
<td>Mixed</td>
<td>Washed beetle injured vs. unwashed injured</td>
<td>2.66</td>
<td>0.1025</td>
</tr>
<tr>
<td>Mixed</td>
<td>Intact Bx vs. intact GJ</td>
<td>0.47</td>
<td>0.4922</td>
</tr>
<tr>
<td>Georgia Jet</td>
<td>Beetle injured GJ vs. beetle injured Bx</td>
<td>0.07</td>
<td>0.7855</td>
</tr>
<tr>
<td>Beauregard</td>
<td>Beetle injured GJ vs. beetle injured Bx</td>
<td>3.19</td>
<td>0.0741</td>
</tr>
<tr>
<td>Male</td>
<td>Males SCB vs. female SCB*</td>
<td>5.84</td>
<td>0.0162</td>
</tr>
<tr>
<td>Female</td>
<td>Male SCB vs. female SCB*</td>
<td>6.06</td>
<td>0.0143</td>
</tr>
<tr>
<td>Mixed</td>
<td>SCB injured root vs. SPW injured root*</td>
<td>4.43</td>
<td>0.0357</td>
</tr>
</tbody>
</table>

(*) Indicates a significantly higher response to an odor source at $P < 0.05$. 
**Cultivar Preference Experiments**

Sweet potato cultivars were differentially damaged by sugarcane beetles in paired choice tests. Beauregard was significantly preferred to Georgia Jet in multiple beetle and single beetle experiments (Table 3.2). No differences in feeding preference were detected between Beauregard and White Star; however, Bunch Porto Rico was preferred to Beauregard (Table 3.2).

![Figure 3.4](image)

**Figure 3.4.** Percentage of *Euetheola humilis* adults walking toward male and female conspecifics in a Y-tube olfactometer. An asterisk (*) indicates a significantly greater response toward one of the two choices using G-tests with William's correction for small samples at a significance level of $P \leq 0.05$. Non-responders were < 10% in all trials.

<table>
<thead>
<tr>
<th>Test</th>
<th>Treatment</th>
<th>Mean Difference No. Scars ± (SE)</th>
<th>$t$</th>
<th>P-value</th>
<th>$n$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Beauregard Georgia Jet</td>
<td>4.75 (1.43)</td>
<td>3.31</td>
<td>0.0455</td>
<td>8</td>
</tr>
<tr>
<td>2$^a$</td>
<td>Beauregard Georgia Jet</td>
<td>0.8 (0.29)</td>
<td>2.75</td>
<td>0.0224</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>Bunch Porto Rico Beauregard</td>
<td>10.8 (1.93)</td>
<td>5.58</td>
<td>0.0050</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>Beauregard White Star</td>
<td>1 (1.92)</td>
<td>0.52</td>
<td>0.6306</td>
<td>5</td>
</tr>
</tbody>
</table>

$^a$One beetle evaluated in each replicate container.
Sugar Analysis

Origin of sweet potatoes (location) had no significant effect on sugar content in the cultivars common to the two collection sites. Beauregard in general had the highest sugar content compared to other varieties, with the exception of sucrose in Bunch Porto Rico. Bunch Porto Rico had greater sucrose content than Beauregard at both locations. Significantly more sucrose was also detected in Bunch Porto compared to Beauregard and White Star (Table 3.3).

Table 3.3. Sugar content of four sweet potato cultivars from two locations.

<table>
<thead>
<tr>
<th>Location</th>
<th>Cultivar²</th>
<th>Sugar</th>
<th>Mean g ± (SE)¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burden</td>
<td>Beauregard</td>
<td>Sucrose</td>
<td>0.142 (0.006)  B</td>
</tr>
<tr>
<td>Burden</td>
<td>Bunch Porto Rico</td>
<td>Sucrose</td>
<td>0.255 (0.010)  A</td>
</tr>
<tr>
<td>Burden</td>
<td>White Star</td>
<td>Sucrose</td>
<td>0.144 (0.012)  B</td>
</tr>
<tr>
<td>Burden</td>
<td>Beauregard</td>
<td>Fructose</td>
<td>0.037 (0.007)  A</td>
</tr>
<tr>
<td>Burden</td>
<td>Bunch Porto Rico</td>
<td>Fructose</td>
<td>0.017 (0.006) AB</td>
</tr>
<tr>
<td>Burden</td>
<td>White Star</td>
<td>Fructose</td>
<td>0.009 (0.001)  B</td>
</tr>
<tr>
<td>Burden</td>
<td>Beauregard</td>
<td>Glucose</td>
<td>0.042 (0.008)  A</td>
</tr>
<tr>
<td>Burden</td>
<td>Bunch Porto Rico</td>
<td>Glucose</td>
<td>0.013 (0.007)  B</td>
</tr>
<tr>
<td>Burden</td>
<td>White Star</td>
<td>Glucose</td>
<td>0.023 (0.002) AB</td>
</tr>
<tr>
<td>Chase</td>
<td>Beauregard</td>
<td>Sucrose</td>
<td>0.137 (0.009)  A</td>
</tr>
<tr>
<td>Chase</td>
<td>Bunch Porto Rico</td>
<td>Sucrose</td>
<td>0.202 (0.266) A</td>
</tr>
<tr>
<td>Chase</td>
<td>Georgia Jet</td>
<td>Sucrose</td>
<td>0.190 (0.015)  A</td>
</tr>
<tr>
<td>Chase</td>
<td>Beauregard</td>
<td>Fructose</td>
<td>0.031 (0.003)  A</td>
</tr>
<tr>
<td>Chase</td>
<td>Bunch Porto Rico</td>
<td>Fructose</td>
<td>0.010 (0.002) B</td>
</tr>
<tr>
<td>Chase</td>
<td>Georgia Jet</td>
<td>Fructose</td>
<td>0.011 (0.002)  B</td>
</tr>
<tr>
<td>Chase</td>
<td>Beauregard</td>
<td>Glucose</td>
<td>0.039 (0.004)  A</td>
</tr>
<tr>
<td>Chase</td>
<td>Bunch Porto Rico</td>
<td>Glucose</td>
<td>0.037 (0.005)  A</td>
</tr>
<tr>
<td>Chase</td>
<td>Georgia Jet</td>
<td>Glucose</td>
<td>0.024 (0.007)  A</td>
</tr>
</tbody>
</table>

¹Means followed by the same letter are not significantly different (within sugar and location) Tukey-Kramer, P > 0.05
²Sugar content within cultivar between locations was not significantly different Tukey-Kramer, P > 0.05.
Beauregard had significantly more fructose than White Star and significantly more glucose than Bunch Porto Rico at the Burden location (Table 3.3). Fewer differences were detected between cultivars at Chase. No significant differences in sucrose or glucose content were detected in the three varieties that were evaluated from Chase, LA (Table 3.3). In the case of fructose, however, Beauregard roots had significantly higher levels than either Georgia Jet or Bunch Porto Rico roots.

**Discussion**

Host plant chemistry has the potential to modify not only herbivore host finding, but feeding, oviposition and larval development as well (Wang and Kays 2002). Olfactometer experiments have demonstrated the response of sugarcane beetles to damaged sweet potato roots. Host plant volatiles have also been implicated in other studies as insect attractants (Nottingham et al. 1989, Yarden et al. 1994, Landolt et al. 1999, Ruther and Mayer 2005) and in the aggregation behavior of phytophagous insects (Loughrin et al. 1996, Harari et al. 1994, Heath et al. 2002). Both male and female sugarcane beetle response was greater for beetle injured roots and mechanically injured roots than for the intact sweet potato roots, indicating that beetles are attracted to host plant volatiles released in response to a wounding event. The fact that sugarcane beetles responded to biotic and abiotic damage indicates that there are constitutive odors or volatiles in the sweet potato root that are released when the plant is damaged. Only male beetles responded significantly more toward beetle injured roots vs. mechanically injured roots. It is possible that different volatiles are released after beetle injury vs. mechanical injury, and that male beetles may be responding to induced volatiles after initial feeding.

Plants can respond to a wounding event by releasing an induced response and this may or may not affect herbivores associated with the plant (Karban and Baldwin 1997). Induced
responses are termed induced resistance if they negatively affect the herbivore, or reduce the attractiveness of the plant to the herbivore; however an induced response can also negatively affect the plant and stimulate increased herbivory (Karban and Baldwin 1997).

Insect frass has been shown previously to be attractive to other insects (Sullivan et al. 2000, Hovorka et al. 2005), and in the current research, washing beetle injured roots to remove frass did not affect beetle response to injured sweet potato roots.

Sugarcane beetles evaluated in the olfactometer showed a significantly higher response for sweetpotato weevil injured roots vs. sugarcane beetle injured roots. Nottingham et al. (1989) demonstrated that sweetpotato weevils were attracted to host plant volatiles of sweet potato. Characterization of the volatiles indicated that sesquiterpenes were the major secondary chemicals associated with both the leaves and the storage roots of sweet potato, suggesting that they may be involved in the response of sweetpotato weevil to sweet potatoes in the field. Wang and Kays (2002) found that both biotic and abiotically damaged sweet potato roots release more terpene volatiles than undamaged roots.

The attraction of sugarcane beetles to sweetpotato weevil damaged roots suggests that sesquiterpenes may also be involved in the response of sugarcane beetles to injured sweet potato roots. Sugarcane beetles are polyphagous herbivores. The specific chemicals and quantity of volatile compounds may not be as important for a polyphagous herbivore as they are to monophagous and oligophagous species because the attractive volatiles will likely differ between plant species (Loughrin et al. 1998).

Sugarcane beetle response to intact roots of Beauregard cultivar vs. intact roots of Georgia Jet cultivar was not significantly different. Similarly beetles previously fed on
Beauregard roots and beetles previously fed on Georgia roots did not prefer injured Beauregard roots to injured Georgia Jet roots.

Both male and female beetles were significantly more attracted to female sugarcane beetles vs. male beetles in olfactometer trials. Karlson and Luscher (1959) defined pheromones as "substances which are secreted to the outside by an individual and received by a second individual of the same species in which they release a specific reaction" (Jutsom and Gordon 1989). The fact that females as well as males were attracted to female beetles may indicate the presence of an aggregation pheromone in this insect. Most of the research on the role of semiochemicals in scarab communication has centered on sex pheromones, however, only aggregation pheromones have been reported for the subfamily Dynastinae, of which the sugarcane beetle is a member (Leal 1998). Aggregation pheromones have previously been described for the African rhinoceros beetle (Gries et al. 1994), and the coconut rhinoceros beetle (Hallet et al. 1995) (Leal 1998).

Attraction of sugarcane beetles to sweet potato in the field may be due to a synergistic effect of host plant volatile release and aggregation pheromones. Yarden and Shani (1994) suggested a similar mechanism in the chemical communication of *Maladera matrida*, an exotic scarab species from Israel. They suggested that males initiate feeding on a particular host plant, after which both sexes are attracted to the host plant volatiles and females then emit a sex pheromone. Loughrin et al. (1996) suggested that feeding induced plant odors might be exploited by Japanese beetles and serve as a cue for host location and an indicator that conspecifics are near. Plant volatiles that affect the behavior of insects have the potential to be used in the development of host plant resistance (Wang and Kays 2002). Most economically important insect species use pheromones in communication and they do have the potential to be
used in integrated control programs (Jutsom and Gordon 1989). Monitoring the sweetpotato weevil in sweet potato with a synthetic blend of a female produced sex pheromone is an example (Jansson et al. 1991).

Sugarcane beetles demonstrated a preference in relation to cultivar in the container experiments. When one or 10 beetles were evaluated in the Beauregard / Georgia Jet pairing, significantly more feeding scars were found on Beauregard roots compared to Georgia Jet roots. Georgia Jet is thought to be a less preferred cultivar for insects. It is possible that Beauregard roots are simply more preferred than are Georgia Jet roots. Beauregard is susceptible to soil insects and is preferred over more resistant lines for feeding and oviposition by the sweetpotato weevil (Mao et al. 2001). Beauregard roots in general had more sucrose, glucose and fructose than the other varieties evaluated. Sugar analyses revealed significantly more fructose in Beauregard relative to Georgia Jet. Beauregard also had more sucrose and glucose than Georgia Jet, though the difference was not significant.

Bunch Porto Rico root was preferred to Beauregard in the container bioassays and sugar analyses revealed that Bunch Porto Rico had significantly more (1.8 x) sucrose than Beauregard. However, Beauregard had more glucose and fructose than the Bunch Porto Rico. Sugars are important feeding stimulants for insects and gustation plays a role in food acceptance and rejection by phytophagous insects (Chapman 2003). In the multiple insect tests, a sugarcane beetle may initially sample the roots to determine a more preferred host, possibly detecting a difference in sugar concentration. Once the roots are sampled, volatiles are released and other insects are attracted to the roots.

Additional research investigating the attraction of sugarcane beetles to sweet potato and alternate host plants is warranted. The beetles display an aggregation behavior in the field and
the laboratory. Based on findings thus far, we know that the beetles are attracted to host plant volatiles of injured sweet potato roots, conspecifics and in short range bioassays they may be differentially attracted to different sweet potato cultivars. Our cultivar preference studies and sugar analyses suggest that varieties may vary in susceptibility to sugarcane beetle based on sugar content.

References Cited


Benedict, L. 2005. Louisiana sweet potatoes are the worlds best.


CHAPTER 4

SUSCEPTIBILITY OF SUGARCANE BEETLE TO SELECTED INSECTICIDES IN LABORATORY BIOASSAYS

Introduction

Sugarcane beetles are one of several soil inhabiting insect pests of sweet potatoes, *Ipomoea batatas*. Most soil inhabiting insects feed on the surface of the root of the sweet potato, creating scars, holes, tunnels and other blemishes (Chalfant et al. 1990). Soil insects in sweet potato have traditionally been managed with the use of soil incorporated insecticides applied preplant (Schalk et al. 1991). Their efficacy is often influenced by incorporation method, formulation and edaphic properties (Harris 1966, Day 1978, Chalfant et al. 1987). Root biomass is not usually reduced by soil insects, but quality is compromised and the economic value of the crop is affected (Chalfant et al. 1990). It is possible that the sugarcane beetle has been a pest of sweet potato for many years, and was undetected or damage was attributed to other soil insects. Sugarcane beetle damage to sweet potato roots is similar to other soil insects and their control is necessary.

Sugarcane beetles were first documented as pests of sweet potato in Louisiana in 2002 (Hammond 2002). Farmers reported excessive damage in 2002 and 2003 from sugarcane beetles feeding on roots prior to harvest. Presently there are no insecticides labeled for the sugarcane beetle on sweet potato. Granular organophosphate insecticides such as Lorsban® 15G and Counter® 15G have been shown to reduce sugarcane beetle infestations in field corn when banded or soil incorporated (Patrick 2004). Tindall et al. (2005) reported a reduction in sugarcane beetle damage to field corn seedlings in field plots treated with bifenthrin, chlorpyrifos, fipronil, clothianidin, cyfluthrin, tebupirimphos, chlorethoxyfos and terbufos. Neonicitinoid seed treatments including clothianidin, thiomethoxam, and imidacloprid were also
evaluated in an artificial infestation experiment in field corn, 2004, against the sugarcane beetle. Clothianidin (Poncho®) applied as a seed treatment at 0.45 milligrams active ingredient per seed demonstrated the greatest reduction of damage to early seedlings by sugarcane beetles (Smith et al. 2005).

An adult vial test (AVT) bioassay was used in this study. The bioassay procedure was originally developed by Plapp et al. (1987) for adult tobacco budworm, *Heliothis virescens* (F.), and has since been modified for several other insect species (Snodgrass 1996, Seagraves and McPherson 2003). The objective of this research was to generate baseline dose-mortality responses of *E. humilis* to insecticides using the AVT bioassay technique.

**Materials and Methods**

**Insects**

Sugarcane beetles used in the bioassays were collected using universal black light traps (BioQuip Products, Rancho Dominguez, CA) during April and May of 2003 and 2004 and August-October in 2005. *Euetheola humilis* were collected at the Louisiana State University Sweet Potato Research Station, Chase, LA (Franklin Parish) in 2003 and 2004 and from Zachary, LA (East Baton Rouge Parish) and Deville, LA (Rapides Parish) in 2005. After collection, beetles were maintained on storage roots 'Beauregard cultivar' and held in plastic containers (5.6 L) with screen covers in a bioclimatic chamber (Percival Scientific Inc., Perry, Iowa) with a 14:10 light:dark cycle at 28°C and 80 % relative humidity. Containers were cleaned and beetles were provided fresh roots on a weekly basis.

**Laboratory Bioassays**

Adult vial test procedures were used to evaluate the activity of the organophosphates (chlorpyrifos and phosmet), the pyrethroids (bifenthrin, z-cypermethrin), the neonicitinoids
(clothianidin) and the phenylpyrazole (fipronil) against sugarcane beetle adults during 2003, 2004, and 2005. Stock solutions of chloropyrifos (99.5% w/w, Chem Service, West Chester, PA), phosmet (98% w/w, Chem Service, West Chester, PA), bifenthrin (98% w/w, Chem Service, West Chester, PA), z-cypermethrin (98% w/w, Chem Service, West Chester, PA), clothianidin (98% w/w, Chem Service, West Chester, PA), and fipronil (98% w/w, Chem Service, West Chester, PA), were developed by dissolving technical grade samples in acetone. Dilutions were made from each stock solution to yield the desired number of insecticide concentrations. The number and range of concentrations varied for each insecticide; insecticide, number of concentrations, range of concentrations in µg/vial were: (bifenthrin 2003, 5, 0.5-10), (bifenthrin 2004, 7, 0.35-5), (chlorpyrifos, 5, 0.25-2.5), (z-cypermethrin, 5, 0.05-5), (phosmet, 5, 5-100), (fipronil, 5, 5-100) and (clothianidin, 5, 5-100). Glass scintillation vials (20 mL) were washed with detergent and water, rinsed with acetone, and oven dried at 60°C before treatment. The interior surface of the vials was coated with the appropriate insecticide solution by pipetting 0.5 ml of the insecticide solution into the vials using an Eppendorf micropipetter (Eppendorf North America Inc. New York, New York). Vials used in the control were treated with acetone. Vials were then rotated on a modified hot dog roller (heating element disconnected) until all acetone had evaporated. A residue of insecticide material was left on the interior surface of the vials after evaporation of the acetone. Vials were used the same day of treatment. One adult sugarcane beetle was placed in insecticide treated and untreated vials. A minimum of 20 insects was subjected to each concentration for each insecticide tested. No food was provided to insects during the AVT and all assays were conducted at ambient temperature (ca. 24°C). Mortality was determined 24 h after exposure for all insecticides tested. Beetles were considered dead when they were unable to maintain an upright posture and perform
coordinated movement after dislodgement from the vials. No control mortality occurred in this study. Mortality data were analyzed using probits (PROC PROBIT, SAS Institute 2004). The LC$_{50}$ and LC$_{90}$ values were considered to be statistically different based upon non-overlap of the 95% confidence limits.

**Results**

Bioassays of clothianidin, fipronil and phosmet resulted in 0% mortality at 100µg/vial, the highest dose evaluated. The fit of the probit model was adequate for bifenthrin, z-cypermethrin and chlorpyrifos (Pearson χ$^2$ test; $P > 0.05$).

The LC$_{50}$'s for the pyrethroid insecticides tested ranged from 0.09 to 1.89µg/vial and the LC$_{50}$ and LC$_{90}$ values for chlorpyrifos were 0.44 and 1.16 µg/vial, respectively (Table 4.1, Figure 4.1). Z-cypermethrin, the most toxic chemical tested, was significantly more toxic (4.8, and 17.7-fold) than chlorpyrifos and bifenthrin in 2004 (Table 4.1, Figure 4.1). Chlorpyrifos was significantly more toxic (4.3 and 3.6-fold) than bifenthrin evaluated in 2003 and 2004. No differences were detected in bioassays conducted for bifenthrin in 2003 and 2004.

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>Year</th>
<th>n$^a$</th>
<th>Slope (SE)</th>
<th>LC$_{50}$ (95% CL)$^{b,c}$</th>
<th>LC$_{90}$ (95% CL)$^{b,c}$</th>
<th>χ$^2$ (df)$^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>bifenthrin</td>
<td>2003</td>
<td>150</td>
<td>2.32 (0.51)</td>
<td>1.89 (0.74-4.39)</td>
<td>6.71 (3.26-150.83)</td>
<td>6.52 (3)*</td>
</tr>
<tr>
<td>bifenthrin</td>
<td>2004</td>
<td>240</td>
<td>2.42 (0.37)</td>
<td>1.60 (1.33-2.01)</td>
<td>5.41 (3.67-10.86)</td>
<td>3.28 (5)*</td>
</tr>
<tr>
<td>z-cypermethrin</td>
<td>2005</td>
<td>180</td>
<td>1.37 (0.24)</td>
<td>0.09 (0.05-0.14)</td>
<td>0.78 (0.47-1.91)</td>
<td>1.03 (3)*</td>
</tr>
<tr>
<td>chlorpyrifos</td>
<td>2005</td>
<td>180</td>
<td>3.09 (0.50)</td>
<td>0.44 (0.35-0.53)</td>
<td>1.16 (0.94-1.71)</td>
<td>3.0 (3)*</td>
</tr>
</tbody>
</table>

$^*$Asterisk ( * ) indicates good fit of the data to the probit model ($P > 0.05$).

$^a$Number tested including controls.

$^b$Concentrations reported in µg insecticide per vial.

$^c$LC$_{50}$ and LC$_{90}$ values significantly different if 95% confidence limits did not overlap.
The slopes (Fig 4.1, Table 4.1) of the concentration mortality curves were similar for bifenthrin in 2003 and 2004. The slope of z-cypermethrin was steeper than that of chlorpyrifos and bifenthrin. Chlorpyrifos had the steepest slope of all compounds tested (Table 4.1, Figure 4.1), indicating the dose-response was more sensitive per unit of chlorpyrifos than the other insecticides tested.

![Graph showing the susceptibility of sugarcane beetle to chlorpyrifos, bifenthrin, and z-cypermethrin in an adult vial test.](image)

Figure 4.1. Susceptibility of sugarcane beetle to chlorpyrifos, bifenthrin and z-cypermethrin in an adult vial test

**Discussion**

This study provides baseline dose-mortality data of the sugarcane beetle to bifenthrin, z-cypermethrin, and chlorpyrifos. All of the insecticides evaluated that fit the probit model and exhibited dose-mortality at various concentrations were toxic to sugarcane beetles in the AVT contact bioassay. Two of the insecticides, chlorpyrifos and bifenthrin, are labeled for control of other soil insects on sweet potato in Louisiana. Chlorpyrifos and bifenthrin are recommended
for control of rootworms, wireworms and white grubs in Louisiana sweet potatoes. Chlorpyrifos, (Lorsban® 15%), applied at 6.03 kg / ha and bifenthrin (Capture® 2EC) applied at 0.14 kg ai / ha are recommended for residual control of soil insects for four to six weeks (Hammond 2005). Field studies have shown that the majority of sugarcane beetle damage occurs late season, so the use of preplant residual insecticides to control this insect may not be efficacious.

Clothianidin, fipronil and phosmet were not toxic to sugarcane beetles at 100µg/vial, the highest dose evaluated. These insecticides, namely clothianidin, should be evaluated with an alternative bioassay procedure. Lack of response by sugarcane beetles to this compound is likely due to the inherent toxicological properties of the compound. Smith et al. (2005) reported a reduction in damage to field corn seedlings treated with the neonicitinoid insecticides, but no reduction in mortality, suggesting that the insecticides may be more deterrent than toxic to these insects.

Sugarcane beetle damage in sweet potato has been sporadic and spotty in recent years, but will likely continue to occur. Therefore, the establishment of baseline susceptibility data of insecticides for this insect is a priority and will be useful in selecting an insecticide to control this insect in sweet potato.

References Cited


CHAPTER 5

SUSCEPTIBILITY OF SWEETPOTATO WEEVIL TO SELECTED INSECTICIDES IN LABORATORY BIOASSAYS

Introduction

The sweetpotato weevil, *Cylas formicarius* (F.), is the most damaging insect pest of sweet potato, *Ipomoea batatas* (L.), in both tropical and subtropical areas worldwide (Jansson and Raman 1991). *Cylas formicarius* can attack sweet potatoes in the field and in storage. Larvae feeding on sweet potato roots can produce major economic damage and yield loss (Chalfant et al. 1990). Larval tunneling causes terpene production in the storage roots, which imparts a bitter taste and leaves the sweet potatoes unsuitable for human consumption (Uritani et al. 1975). Reported yield losses worldwide due to sweetpotato weevil damage range from 5-80% (Sutherland 1986).

Sweet potatoes are grown in over 100 countries and are second in production among root crops to white potatoes, *Solanum tuberosum* L. (Horton 1988). The United States has an average annual production valued at $309 million (USDA 2006). In 2005, sweet potatoes were harvested from 6800 hectares in Louisiana for a value of $41 million (USDA 2006).

The distribution of *C. formicarius* is limited to tropical and sub-tropical climates, and is therefore often the object of quarantines that restrict movement of vines and storage roots from infested areas (Nilakhe 1991). *Cylas formicarius* is currently only established in southern regions of Louisiana, and a quarantine program exists to minimize the spread of *C. formicarius* into northern crop areas of the state (LDAF 2004; T. Hardy, personal communication).

The organophosphates phosmet and methyl parathion are recommended for use in a mandatory spray program in south Louisiana. In addition carbaryl, a carbamate, is labeled for use on sweet potato in Louisiana and bifenthrin has received a Section 18 emergency exemption.
for use on sweet potato in Louisiana since 2001 (EPA 2005). The emergency exemption was
granted so that an alternate chemical class (pyrethroids) could be rotated into spray programs to
control C. formicarius and possibly delay organophosphate resistance development. Cyfluthrin
was included in the bioassays as an alternative pyrethroid to bifenthrin. Other insecticides
commonly used to control sweet potato insects in Louisiana, namely carbaryl, phosmet and
methyl parathion, have the same mode of action, and they are scheduled for review by the United
States Environmental Protection Agency (EPA 2005).

Insecticides have traditionally been the primary defense in reduction of root damage by
insects to sweet potato (Schalk et al. 1991). Despite the fact that insecticides are a valuable tool
used in pest management systems, many drawbacks can exist such as the development of insect
resistance (Metcalf 1994). Numerous studies have evaluated chemical control of C. formicarius
in field situations (Pillai et al. 1981, Waddill 1982, Rajamma 1983, Sutherland 1985) with no
resistance to insecticides reported. Mason et al. (1991) using a topical bioassay found
sweetpotato weevils to be more susceptible to chlorpyrifos and parathion than to carbaryl or
endosulfan.

The adult vial test (AVT) used in this study was originally developed by Plapp et al.
(1987) for adult tobacco budworm, Heliothis virescens (F.), and has since been modified for
several other insect species (Snodgrass 1996, Seagraves and McPherson 2003). The objective of
this study was to determine and compare susceptibility of two cohorts of C. formicarius to five
insecticidal compounds and to determine if changes in susceptibility to these compounds were
occurring in the Louisiana cohort.
Materials and Methods

Insects

The Louisiana cohort of *C. formicarius* used in the bioassays was obtained from a laboratory colony established in 2002 from field collected sweet potato roots taken from Avoyelles, Parish, LA, an area with extensive sweet potato production. Sweet potatoes grown at this location received numerous applications of one or more of the insecticides evaluated in this study, indicating that weevils collected from this location experienced multiple insecticide exposures. The Texas cohort was collected using pheromone traps baited with synthetic female *C. formicarius* pheromone placed near wild *Ipomoea* spp. hosts in Frio County, TX, and had limited or no previous exposure to insecticides evaluated in our bioassays. All weevils were maintained on storage roots 'Beauregard cultivar' and held in plastic containers (5.6 L) with screen covers at 28 ± 2°C and 85 ± 10% RH in the laboratory at Louisiana State University. Containers were cleaned and weevils were provided fresh roots on a weekly basis.

Laboratory Bioassays

Adult vial test procedures similar to those described by Plapp et al. (1987) were used to evaluate the activity of bifenthrin, methyl parathion, phosmet, cyfluthrin, and carbaryl against *C. formicarius* adults. Stock solutions of bifenthrin (98% w/w, Chem Service, West Chester, PA), methyl parathion (98.7% w/w, Chem Service, West Chester, PA), phosmet (98% w/w, Chem Service, West Chester, PA), cyfluthrin (98% w/w, Chem Service, West Chester, PA), and carbaryl (99.5% w/w, Chem Service, West Chester, PA) were obtained by dissolving technical grade samples in acetone. Aliquots of these stock solutions were diluted further with acetone to yield the desired concentrations for the assays. The number and range of concentrations varied for each cohort and insecticide tested. Insecticide, number of concentrations, and range of
concentrations in µg/vial for the Louisiana cohort were: (bifenthrin, 7, 0.175-1.25); (methyl parathion, 6, 0.0125-0.125); (phosmet, 6, 0.5-100); (cyfluthrin, 6, 0.5-5); (carbaryl, 7, 0.25-100). Insecticide, number of concentrations, and range of concentrations in µg/vial for the Texas cohort were: (bifenthrin, 4, 0.175-0.75); (methyl parathion, 4, 0.0025-0.125); (phosmet, 5, 0.5-50); (cyfluthrin, 5, 0.5-5); (carbaryl, 5, 5-100). Glass scintillation vials (20 mL) were washed with detergent and water, rinsed with acetone, and oven dried at 60ºC before treatment. The interior surface of each vial was coated with the appropriate insecticide solution by pipetting 0.5 ml into the vials using an Eppendorf micropippeter (Eppendorf North America Inc. New York, New York). Vials used in the control were treated with acetone. Vials were then rotated on a modified hot dog roller (heating element disconnected) until all acetone had evaporated. A residue of insecticide material was left on the interior surface of the vials after evaporation of the acetone. Vials were used immediately following treatment.

One adult sweetpotato weevil was placed in each of the insecticide-treated and untreated vials. Three trials (minimum of 20 insects/concentration) were conducted with the Louisiana cohort for each insecticide tested and two trials (minimum of 10 insects/concentration) was used for each insecticide with the Texas cohort. No food was provided to insects during the AVT and all assays were conducted at ambient temperature (ca. 24ºC). Mortality was determined 24 h after exposure for all insecticides tested. Weevils were considered dead when they were unable to maintain an upright posture and perform coordinated movement after being dislodged from the vials. No control mortality occurred in this study and all data was subjected to probit analysis (PROC PROBIT, SAS Institute 2004). Resistance ratios and their corresponding 95% confidence intervals were calculated using the method of Robertson and Preisler (1992). The susceptible
Texas cohort was assigned a ratio of 1.0. A discriminating concentration was used when the probit model was not appropriate, i.e., when the $\chi^2$ goodness-of-fit test was rejected.

**Results**

The susceptibility of sweetpotato weevil varied by insecticide (Table 1) and the fit of the probit model was adequate in all cases, except carbaryl and phosmet tested against the Louisiana cohort (Pearson $\chi^2$ test; $P > 0.05$).

*Cylas formicarius* adults from both cohorts were most susceptible to methyl parathion, and weevils from the two cohorts were differentially susceptible to this compound (Table 5.1). The Louisiana cohort was over four fold less susceptible to methyl parathion than was the Texas cohort. Both cohorts were also highly susceptible to the pyrethroids bifenthrin and cyfluthrin which were the next most toxic chemicals (Table 5.1, Fig. 5.1). *Cylas formicarius* were less susceptible to phosmet and carbaryl compared to the other insecticides. Differences ($P < 0.05$) in susceptibility of the Louisiana cohort to bifenthrin and cyfluthrin compared to the susceptible Texas cohort were observed at both the LC$_{50}$ and LC$_{90}$ levels. The Texas cohort had 1.5 and 2.3 (RR$_{50}$) decreased sensitivity to cyfluthrin and bifenthrin, respectively compared to the Louisiana cohort. Differences ($P < 0.05$) were detected in susceptibility of weevils to phosmet only at the LC$_{50}$ level with the Texas cohort being over 2.5 fold more susceptible compared to the Louisiana cohort. *Cylas formicarius* from the two cohorts exhibited differences to carbaryl only at the LC$_{90}$ level (Table 5.1).

The chi-square goodness-of-fit tests for both carbaryl and phosmet with the Louisiana cohort were significant, indicating a non-normal response of *C. formicarius* susceptibility to these insecticides. Maximum mortality in the Louisiana cohort for carbaryl and phosmet at 100 $\mu$g/vial, the highest concentration, was 65 and 90%, respectively (Fig. 5.1).
Table 5.1. Susceptibility of two cohorts of *C. formicarius* adults to selected insecticides in an adult vial bioassay

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>Cohort</th>
<th>n</th>
<th>Slope (SE)</th>
<th>LC₅₀ (95% CL)</th>
<th>LC₉₀ (95% CL)</th>
<th>χ² (df)</th>
<th>RR₅₀ (95% CL)</th>
<th>RR₉₀ (95% CL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>bifenthrin</td>
<td>LA</td>
<td>720</td>
<td>1.98 (0.19)</td>
<td>0.51 (0.45-0.58)</td>
<td>2.28 (1.75-3.32)</td>
<td>8.7 (5)*</td>
<td>2.30 (1.72-3.09)</td>
<td>4.08 (2.47-6.72)</td>
</tr>
<tr>
<td></td>
<td>TX</td>
<td>100</td>
<td>3.21 (0.76)</td>
<td>0.22 (0.15-0.28)</td>
<td>0.56 (0.42-1.07)</td>
<td>1.9 (2) *</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>methyl parathion</td>
<td>LA</td>
<td>770</td>
<td>3.17 (0.24)</td>
<td>0.03 (0.03-0.03)</td>
<td>0.08 (0.07-0.09)</td>
<td>6.9 (4) *</td>
<td>4.62 (2.68-7.99)</td>
<td>1.95 (0.92-4.11)</td>
</tr>
<tr>
<td></td>
<td>TX</td>
<td>100</td>
<td>1.64 (0.34)</td>
<td>0.01 (0.00-0.01)</td>
<td>0.04 (0.02-0.12)</td>
<td>0.8 (2) *</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>phosmet</td>
<td>LA</td>
<td>630</td>
<td>1.05 (0.21)</td>
<td>11.59 (3.74-30.95)</td>
<td>&gt;100 (NA)</td>
<td>22.3 (4)</td>
<td>2.63 (1.12-6.21)</td>
<td>3.58 (0.74-17.40)</td>
</tr>
<tr>
<td></td>
<td>TX</td>
<td>120</td>
<td>1.18 (0.23)</td>
<td>4.40 (2.16-7.61)</td>
<td>54.20 (26.09-221.37)</td>
<td>5.0 (3) *</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>carbaryl</td>
<td>LA</td>
<td>570</td>
<td>0.61 (0.17)</td>
<td>58.34 (19.15-2301)</td>
<td>&gt;100 (NA)</td>
<td>17.9 (5)</td>
<td>2.83 (0.91-8.82)</td>
<td>91.94 (2.71-3118)</td>
</tr>
<tr>
<td></td>
<td>TX</td>
<td>120</td>
<td>2.16 (0.34)</td>
<td>20.63 (14.15-28.61)</td>
<td>80.76 (54.46-150.86)</td>
<td>3.9 (3) *</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>cyfluthrin</td>
<td>LA</td>
<td>630</td>
<td>1.70 (0.18)</td>
<td>1.42 (1.22-1.66)</td>
<td>8.01 (5.68-13.33)</td>
<td>2.1 (4) *</td>
<td>1.56 (1.14-2.14)</td>
<td>2.75 (1.48-5.10)</td>
</tr>
<tr>
<td></td>
<td>TX</td>
<td>120</td>
<td>2.53 (0.49)</td>
<td>0.91 (0.66-1.18)</td>
<td>2.91 (2.05-5.76)</td>
<td>0.5 (3) *</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

*a* Asterisk ( *) indicates good fit of the data to the probit model (*P* > 0.05).

*b* Resistance ratio with the 95 % confidence intervals as calculated by the method of Robertson and Preisler (1992) by using the Texas cohort 2005 as the ratio divisor; n is total number of adults tested.

*c* Values exceeded 100µg per vial (highest concentration tested), 100 µg per vial concentration resulted in 65.6% mortality; confidence limits could not be calculated.

*d* Values exceeded 100µg per vial (highest concentration tested), 100 µg per vial concentration resulted in 90% mortality; confidence limits could not be calculated.
At a discriminating concentration of 10µg/vial, phosmet resulted in 34.4% mortality for the Louisiana cohort compared to 65 for the Texas cohort. Carbaryl tested at 50 µg/vial resulted in 45.6 % mortality of weevils from the Louisiana cohort compared to 70 % mortality of weevils from the Texas cohort (Fig. 5.1).

The slopes of the concentration mortality curves were similar for the two pyrethroids tested within cohorts, but the slopes of the Texas cohorts were steeper indicating the dose-response was more sensitive in the Texas cohort. The slopes for phosmet and carbaryl were also steeper for the Texas cohort. Only methyl parathion had an increased dose response in the Louisiana cohort. (Table 5.1, Fig 5.1).

**Figure 5.1.** Susceptibility of adults from two cohorts of *C. formicarius* to selected insecticides in a modified adult vial test.

The slopes (Table 5.1 and Fig. 5.1) of the concentration mortality curves were similar for the two pyrethroids tested within cohorts, but the slopes of the Texas cohorts were steeper indicating the dose-response was more sensitive in the Texas cohort. The slopes for phosmet and
carbaryl were also steeper for the Texas cohort. Only methyl parathion had an increased dose response in the Louisiana cohort. (Table 5.1, Fig. 5.1).

**Discussion**

Our results show lower susceptibility of *C. formicarius* to insecticides in the Louisiana cohort compared to the Texas cohort. Of the chemicals tested that are recommended for use on sweet potato in Louisiana, methyl parathion was the most toxic followed by bifenthrin and phosmet. Mason et al. (1991) found that the organophosphates chlorpyrifos and parathon were more toxic to *C. formicarius* than methomyl, endosulfan and carbaryl in a topical bioassay and carbaryl was found to be the least toxic insecticide of the five chemicals tested in their bioassays.

Sweetpotato weevils from both cohorts were least susceptible to carbaryl and phosmet in the current study, which may indicate lower susceptibility of *C. formicarius* to these insecticides in the field. The Louisiana cohort used in this study was heterogeneous and responses to carbaryl and phosmet were not normally distributed. Homogeneous populations have a linear relationship between log concentration and probit transformed mortality (Hoskins and Craig 1962). The use of a discriminating concentration for these insecticides is an alternative method to evaluate changes in susceptibility between the two cohorts. Resistance to both carbamates and organophosphates has been detected in other insects (Wierenga and Hollingworth 1993, Noronha et al. 2001, Siegfried et al. 2004) and cross-resistance to these insecticides has been reported in the Colorado potato beetle (French et al. 1992).

It is probable that *C. formicarius* adults collected in 2002 for colony establishment were exposed to numerous applications of carbaryl, phosmet and methyl parathion. Phosmet and methyl parathion are both organophosphates and in accordance with the mandatory spray program in south Louisiana, field populations of *C. formicarius* may be exposed to as many as
five applications of phosmet and 12 applications of a microencapsulated formulation of methyl parathion within one season. With the Section 18 emergency exemption, bifenthrin can be rotated into the mandatory spray regimen to replace five applications of either methyl parathion or phosmet.

Significant differences between cohorts were detected for methyl parathion, bifenthrin and cyfluthrin, indicating lower susceptibility in the Louisiana cohort. The AVT bioassays provide baseline toxicological data for five insecticides, four of which are commonly used to control *C. formicarius* in Louisiana. The concentration-mortality response of *C. formicarius* from the Texas cohort to various insecticides can be used as a baseline for future comparisons with *C. formicarius* cohorts collected in sweet potato production areas.

No weevil resistance to insecticides used in this study has been reported thus far in field populations. Adult vial tests (AVT) have been demonstrated to be an effective bioassay technique for the evaluation of insecticides against sweetpotato weevil. Field populations of this insect can be easily subjected to these tests in a timely manner to monitor the susceptibility of sweetpotato weevil to various insecticides.

Surveying insect populations for changes in susceptibility is an integral part of insecticide resistance management and determining the range of initial resistance frequencies among insect populations can allow for early detection of changes in susceptibility to insecticides (ffrench-Constant and Roush 1990). An effective integrated pest management program is multidisciplinary and includes numerous management options. The AVT may become an important tool for establishing baseline dose mortality data and monitoring for resistance development used in conjunction with other components such as pheromone trapping, to improve sweet potato production in Louisiana and other areas of the world.
References Cited


CHAPTER 6

INFLUENCE OF PLANTING DATE ON INSECT DAMAGE, ABUNDANCE AND NUMBER OF STORAGE ROOTS IN SWEET POTATO

Introduction

Sweet potatoes are planted from April through July in Louisiana. Soil insects damage potatoes at various times during the season depending on the availability of roots and the life stage of the insect. A complex of soil insects are associated with sweet potato and can become a major constraint in sweet potato production in the United States (Schalk et al. 1991). Sweet potatoes are cultivated in the summer and fall months, when insect populations are high and developmental time is rapid. Roots can be injured from the onset of root formation, usually in June or July depending on planting date, until harvest in the fall (Cuthbert 1967). Sweet potato producers have a long-standing record of using prophylactic controls. A significant barrier in developing reduced risk management strategies in sweet potato is that most of the pests inflict damage in the larval stage in the soil, thus they are difficult to monitor and control (Curtis 2003).

With most agricultural crops, planting dates or harvest times can be adjusted to avoid some insect losses. In many cases, earlier planting results in fewer losses from insects (Pedigo 1999). Limited research has been conducted concerning soil insect pests of sweet potato in relation to when the roots are damaged.

The banded cucumber beetle, *Diabrotica balteata* Leconte, and the spotted cucumber beetle, *Diabrotica undecimpunctata* Barber, feed on sweet potato. Adults of both species feed on the leaves of sweet potato (Cuthbert 1967) and the larvae of these insects damage sweet potato by chewing small holes on the surface of the root (Schalk et al. 1991). Multiple generations occur in a year and larval developmental time ranges from 20-50 days depending on
temperature; roots are often injured early in the season which can result in numerous blemishes at harvest (Schalk et al. 1991).

Sugarcane beetle, *Euetheola humilis*, damage was first reported in sweet potato in Louisiana, 2001. The adult stage of the beetle feeds beneath the soil surface on sweet potato roots, creating unattractive and jagged scars (Hammond 2002). It is hypothesized that sugarcane beetles damage sweet potatoes in the fall. If this is true, preplant soil insecticides may not be an effective control option for this insect. It may be possible to target this insect later in the season with lay-by insecticide treatments, because the insect comes to the surface of the soil to feed on roots just below the surface and to mate. If it can be determined definitively when sugarcane beetles and other soil insects are damaging sweet potato roots, it may be possible to monitor these insects and control them more directly only when needed. The objective of the study was to evaluate soil insect damage, adult insect abundance, and number of storage roots as they related to time of planting in sweet potato.

**Materials and Methods**

**Study Site and Design**

Studies were conducted at the Louisiana State University Agricultural Center Sweet Potato Research Station near Chase, LA and at the Louisiana State University Agricultural Center Dean Lee Research Station near Alexandria, LA in 2004 and 2005. Three planting dates were evaluated at each location in each year. Sweet potato transplants 'Beauregard cultivar' ca. 30 cm long were cut the day of transplanting and were planted 0.3 m apart. Sweet potatoes were mechanically or manually transplanted on three dates spaced 2-4 weeks apart (Table 6.1).
Table 6.1. Planting dates of 'Beauregard' sweet potato in 2004 and 2005 at two locations in Louisiana.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Location</th>
<th>Year</th>
<th>Planting Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early Planting</td>
<td>Dean Lee</td>
<td>2004</td>
<td>May 25</td>
</tr>
<tr>
<td></td>
<td>Chase</td>
<td>2004</td>
<td>May 27</td>
</tr>
<tr>
<td>Middle Planting</td>
<td>Dean Lee</td>
<td>2004</td>
<td>June 28</td>
</tr>
<tr>
<td></td>
<td>Chase</td>
<td>2004</td>
<td>June 29</td>
</tr>
<tr>
<td>Late Planting</td>
<td>Dean Lee</td>
<td>2004</td>
<td>July 21</td>
</tr>
<tr>
<td></td>
<td>Chase</td>
<td>2004</td>
<td>July 22</td>
</tr>
<tr>
<td>Early Planting</td>
<td>Dean Lee</td>
<td>2005</td>
<td>June 3</td>
</tr>
<tr>
<td></td>
<td>Chase</td>
<td>2005</td>
<td>May 27</td>
</tr>
<tr>
<td>Middle Planting</td>
<td>Dean Lee</td>
<td>2005</td>
<td>June 30</td>
</tr>
<tr>
<td></td>
<td>Chase</td>
<td>2005</td>
<td>June 29</td>
</tr>
<tr>
<td>Late Planting</td>
<td>Dean Lee</td>
<td>2005</td>
<td>July 15</td>
</tr>
<tr>
<td></td>
<td>Chase</td>
<td>2005</td>
<td>July 14</td>
</tr>
</tbody>
</table>

General agronomic and pest management practices recommended by the LSU AgCenter were used to maintain test plots, with the exception of insecticide use. Treatments were arranged in a randomized block design replicated four times. Plot size was four rows spaced 0.96 m, by 30.48 m long at Dean Lee and four rows spaced 1.07 m wide, by 30.48 m long at Chase. Soil type was Norwood silt loam and Gigger silt loam at Dean Lee and Chase, respectively. Treatments were the three planting dates spaced 2-4 weeks apart. In order to evaluate insect populations and any subsequent damage by insects, no soil incorporated or foliar insecticides were applied in either year to the field plots.

**Sampling**

Sampling for insects and storage roots began 40 days after transplant. Plots were sampled for a total of 10 weeks in each year at both locations. Adult insects were sampled by taking 25 sweeps/plot with a standard 0.38 m sweep net. Damage samples were taken by manually digging five whole plants with a pitch fork in each plot. All sweet potato root samples were taken to the laboratory where they were washed to remove any soil and evaluated for insect
damage. The number of storage roots and the number of insect damaged storage roots was recorded. Storage roots were evaluated for soil insect damage including: cucumber beetle, *Diabrotica* spp., sugarcane beetle, *Euetheola humilis*, white grubs, *Phyllophaga* spp., whitefringed beetle, *Naupactus leucoloma*, flea beetles, *Systena* spp. and *Chaetocnema* spp., and wireworms, *Conoderus* spp.

**Data Analysis**

Total insect damaged storage roots, cucumber beetle (*Diabrotica*) damaged storage roots, number of storage roots and adult *Diabrotica* beetle sweep counts were compared among the three planting dates using analysis of variance (PROC GLIMMIX, SAS Institute 2004) assuming a poisson distribution followed by Tukey-Kramer multiple range tests for means separation. Fixed main effects included in the model were treatment (planting date) and sampling time (week). The interaction of main effects was also examined. Year and location were independent of the hypotheses of interests in our study, as a result, location and year were included in the model as random effects and any variability added to the model by location and year is accounted for by placing them as random effects in the model. Sugarcane beetle damage was analyzed separately with logistic regression (PROC LOGISTIC, SAS Institute 2004), assuming a binomial distribution.

Correlations between percent damaged storage roots and mean number of cucumber beetles were examined at various seasonal intervals and for the entire season (PROC CORR, SAS Institute 2004). The overall relationship between adult cucumber beetle sweep count numbers and percent cucumber beetle damaged storage roots was also examined (PROC GLM, SAS Institute 2004). Cucumber beetle damage data was averaged over subsamples (plants within replicate plots) for correlation and regression analyses.
Results

The majority of insect species sampled were cucumber beetles, primarily banded cucumber beetles, *Diabrotica balteata*, and the majority of soil insect damage to sweet potato storage roots was attributed to *Diabrotica* larvae. Only three percent of storage roots sampled during the duration of the study from both locations were damaged by insects other than *Diabrotica*. Seasonal abundance of adult cucumber beetles, *Diabrotica*, was variable throughout the sampling period in both years and locations (Figures 6.1-6.4). *Diabrotica* damage steadily increased 40 to 68 days after transplant in most cases (Figures 6.1, 6.2, 6.3). The only exception was seen at Chase in 2005, in which the percentage of storage roots damaged mid season decreased (Figure 6.4).

![Seasonal abundance of cucumber beetles and percent cucumber beetle damaged storage roots at Dean Lee in 2004](image1)

**Figure 6.1.** Seasonal abundance of cucumber beetles and percent cucumber beetle damaged storage roots at Dean Lee in 2004

![Seasonal abundance of cucumber beetles and percent cucumber beetle damaged storage roots at Dean Lee in 2005](image2)

**Figure 6.2.** Seasonal abundance of cucumber beetles and percent cucumber beetle damaged storage roots at Dean Lee in 2005.
Figure 6.3. Seasonal abundance of cucumber beetles and percent cucumber beetle damaged storage roots at Chase in 2004.

Planting date had a significant effect on total insect damage, which included damage from *Diabrotica* and also limited damage from whitefringed beetles, white grubs, and sugarcane beetle (Figure 6.5, Table 6.2). The effects of week (sampling time) and the interaction of planting date * week were also significant (Table 6.2). Initial root damage was greater earlier in the season at late plantings compared to middle or early season sweet potato plantings, and damage throughout the season increased for the late planting date vs. early or middle planting dates (Figure 6.5). Differences in damage were not detected between the early and late plantings but the middle planting dates had significantly more damage than late plantings (Table 6.3).

Cucumber beetle, *Diabrotica*, damage was similar to total insect damage; hence the majority of damage to storage roots in the current study was caused by *Diabrotica* larvae. No differences in *Diabrotica* damage were detected between planting dates in the current study.
Figure 6.5. Mean number of soil insect (all species) damaged storage roots at three planting dates sampled for 10 weeks beginning 40 days after transplant averaged across two locations and two years. Planting date, week and the interaction of planting date * week were significant, $P < 0.05$.

Table 6.2. Effect of planting date on damage, adult *Diabrotica* abundance and damage, and number of storage roots in sweet potato.

<table>
<thead>
<tr>
<th>Variable Measured</th>
<th>Effect</th>
<th>DF</th>
<th>F</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Damaged roots</td>
<td>Planting Date</td>
<td>2, 30</td>
<td>4.33</td>
<td>0.0223</td>
</tr>
<tr>
<td></td>
<td>Week</td>
<td>9, 2326</td>
<td>12.05</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>Planting date * Week</td>
<td>18, 2326</td>
<td>2.17</td>
<td>0.0030</td>
</tr>
<tr>
<td><em>Diabrotica</em> beetle</td>
<td>Planting date</td>
<td>2, 30</td>
<td>2.59</td>
<td>0.0915</td>
</tr>
<tr>
<td>damaged roots</td>
<td>Week</td>
<td>9, 2326</td>
<td>10.71</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>Planting date * Week</td>
<td>18, 2326</td>
<td>2.28</td>
<td>0.0016</td>
</tr>
<tr>
<td>Number storage roots</td>
<td>Planting date</td>
<td>2, 2357</td>
<td>15.65</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>Week</td>
<td>9, 2357</td>
<td>13.64</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>Planting date * Week</td>
<td>18, 2357</td>
<td>2.35</td>
<td>0.0011</td>
</tr>
<tr>
<td><em>Diabrotica</em> beetle</td>
<td>Planting date</td>
<td>2, 435</td>
<td>12.27</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Abundance</td>
<td>Week</td>
<td>9, 435</td>
<td>24.85</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>Planting date * Week</td>
<td>18, 435</td>
<td>11.76</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

Indicates significant effect, ($P < 0.05$)

However, week and the interaction of planting date * week were significant (Table 6.2). The late planting date had more damage earlier in the sampling period than the early and middle planting dates and there was a gradual increase in number of damaged storage roots through the season with all three planting dates (Figure 6.6). The overall number of *Diabrotica* damaged storage roots was greatest in the late planting date, but differences in damage were not detected between the three planting times (Table 6.3).
Table 6.3. Means ± (SE) of total damaged storage roots, *Diabrotica* beetle damaged roots, *Diabrotica* abundance, and number of storage roots at three planting dates.

<table>
<thead>
<tr>
<th>Variable Measured</th>
<th>Planting Date</th>
<th>Mean ± (SE)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total damaged roots</td>
<td>Early</td>
<td>0.77 (0.02) AB</td>
</tr>
<tr>
<td></td>
<td>Middle</td>
<td>0.76 (0.02) B</td>
</tr>
<tr>
<td></td>
<td>Late</td>
<td>1.23 (0.02) A</td>
</tr>
<tr>
<td><em>Diabrotica</em> beetle damaged roots</td>
<td>Early</td>
<td>0.70 (0.04) A</td>
</tr>
<tr>
<td></td>
<td>Middle</td>
<td>0.71 (0.05) A</td>
</tr>
<tr>
<td></td>
<td>Late</td>
<td>1.08 (0.05) A</td>
</tr>
<tr>
<td>Number of roots</td>
<td>Early</td>
<td>4.14 (0.08) A</td>
</tr>
<tr>
<td></td>
<td>Middle</td>
<td>3.59 (0.07) B</td>
</tr>
<tr>
<td></td>
<td>Late</td>
<td>4.00 (0.07) A</td>
</tr>
<tr>
<td><em>Diabrotica</em> adult abundance</td>
<td>Early</td>
<td>3.37 (0.42) B</td>
</tr>
<tr>
<td></td>
<td>Middle</td>
<td>3.64 (0.37) B</td>
</tr>
<tr>
<td></td>
<td>Late</td>
<td>4.46 (0.49) A</td>
</tr>
</tbody>
</table>

*Means followed by the same letter are not significantly different, Tukey-Kramer (P > 0.05).*

![Figure 6.6](image_url)

**Figure 6.6.** Mean number of *Diabrotica* damaged storage roots at three planting dates sampled for 10 weeks beginning 40 days after transplant averaged across two locations and two years. Planting date was not significant, P > 0.05. Week and the interaction of planting date * week were significant, P < 0.05.
The number of adult *Diabrotica* sampled was variable throughout the sampling period (Figure 6.7) and *Diabrotica* abundance was significantly different among the three planting dates (Table 6.2).

**Figure 6.7.** Mean number of adult *Diabrotica* sampled at three planting dates for 10 weeks beginning 40 days after transplant averaged across two locations and two years. Treatment, week and the interaction of planting date * week were significant, *P* < 0.05.

Significantly more adult *Diabrotica* were sampled from the late planting dates vs. early and middle dates (Table 6.3, Figure 6.7). The numbers of adult *Diabrotica* increased ca. three times during the season for each planting date. Weeks one and two, four and five, and eight and nine had an increased number of insects sampled for each planting date respectively compared to other weeks in the sampling period (Figure 6.7).

Planting date had a significant effect on the number of storage roots sampled per plant throughout the season (Table 6.2). Week and the interaction of planting date * week were also significant (Table 6.2). Differences in the total number of storage roots sampled per plant were not detected between the early and late planting date, but both the early and late plantings had
significantly more storage roots than the middle planting date (Table 6.3). The number of storage roots sampled gradually increased until week four, after which it remained fairly uniform for the remainder of the sampling period (Figure 6.8).

**Figure 6.8.** Mean number of storage roots at three planting dates sampled for 10 weeks beginning 40 days after transplant averaged across two locations and two years. Treatment, week and the interaction of planting date * week were significant, $P < 0.05$.

There was a significant relationship between mean number of *Diabrotica* beetles sampled and percent *Diabrotica* larval root damage throughout the season (Figure 6.9). Adult *Diabrotica* beetles sampled throughout the season were positively correlated to damage throughout the season (Table 6.4).

**Figure 6.9.** Relationship between percent damaged roots and mean number of *Diabrotica* beetles sampled at three planting dates for 10 weeks beginning 40 days after transplant, $P < 0.05$. 

$y = 5.0665x$

$R^2 = 0.3663$
Correlations of adult *Diabrotica* abundance with percent damaged roots in relation to sample time were also examined. There was a significant positive relationship between adult beetles sampled early in the sampling period, weeks 1-3, vs. percent root damage middle and late season, weeks 4-6 and 7-9, respectively (Table 6.4). Similarly, beetles sampled mid season, weeks 4-6, were positively correlated with percent root damage in weeks 4-6 and 7-9 (Table 6.4). *Diabrotica* beetles sampled early season were not significantly correlated to percent damaged roots early in the season, and beetles sampled late season, weeks 7-9, were not significantly correlated to percent damaged roots late season (Table 6.4).

**Table 6.4.** Correlations of mean number of *Diabrotica* beetles / 25 sweeps with percent damaged roots sampled in early, middle and late periods.

<table>
<thead>
<tr>
<th><em>Diabrotica</em> adult(^a) sampling period</th>
<th>Root damage(^a) sampling period</th>
<th>(r)</th>
<th>(P^*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early</td>
<td>Early</td>
<td>0.14</td>
<td>0.2509</td>
</tr>
<tr>
<td>Early</td>
<td>Middle</td>
<td>0.48</td>
<td>0.0027</td>
</tr>
<tr>
<td>Early</td>
<td>Late</td>
<td>0.42</td>
<td>0.0098</td>
</tr>
<tr>
<td>Middle</td>
<td>Middle</td>
<td>0.39</td>
<td>0.0174</td>
</tr>
<tr>
<td>Middle</td>
<td>Late</td>
<td>0.35</td>
<td>0.0391</td>
</tr>
<tr>
<td>Late</td>
<td>Late</td>
<td>0.10</td>
<td>0.5480</td>
</tr>
<tr>
<td>Entire Season</td>
<td>Entire Season</td>
<td>0.64</td>
<td>0.0251</td>
</tr>
</tbody>
</table>

\(^*\) Significant relationship, \(P < 0.05\).

\(^a\) Early, middle and late designations represent weeks 1-3, 4-6, and 7-9 of the sampling period respectively.

Sugarcane beetle, *Euetheola humilis*, injury to sweet potato storage roots was negligible in 2004. However, five percent of storage roots sampled in 2005 from Chase were damaged by sugarcane beetle; hence these data were included in an analysis of sugarcane beetle injured roots. Late planted potatoes had 12 and 3.5 times more sugarcane beetle damage than potatoes planted at the early and middle planting dates, respectively (Table 6.5). Planting date had a significant effect on the number of storage roots that were damaged by sugarcane beetle (Table 6.6) and
damage increased throughout the sampling period (Figure 6.10). Probability of damage increased three weeks into the sampling period, ca. 54 days after transplant (Figure 6.10). The late planting date had an overall greater amount of sugarcane beetle storage root damage than the early or middle planting dates (Table 6.5) and the probability of sugarcane beetle storage root damage was highest for the late planting date (Figure 6.10).

<table>
<thead>
<tr>
<th>Table 6.5. Mean sugarcane beetle damaged storage roots at three planting dates, Chase, LA, 2005.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Planting Date</td>
</tr>
<tr>
<td>Early</td>
</tr>
<tr>
<td>Middle</td>
</tr>
<tr>
<td>Late</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 6.6. Effect of planting date on number of sugarcane beetle damaged storage roots at three planting dates, Chase, LA 2005.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effect</td>
</tr>
<tr>
<td>----------------------</td>
</tr>
<tr>
<td>Planting Date</td>
</tr>
<tr>
<td>Week</td>
</tr>
<tr>
<td>Replication</td>
</tr>
</tbody>
</table>
Discussion

Planting date had an effect on total insect damage. Late plantings of sweet potatoes had more total insect damage and more *Diabrotica* damage earlier in the sampling period compared to early and middle plantings. Root damage increased until week five, ca. 68 days after transplant before leveling off for all three plantings, therefore early season scouting and management of *Diabrotica* spp. is important and necessary to reduce root damage later in the growing season. The number of storage roots and the number of damaged roots increased with time until week five or ca. 68 days after transplant for all planting dates, after which the number of roots sampled and damage to those roots remained relatively constant. These data indicate that the majority of storage roots are present 65-70 days after transplant and also suggests that the majority of damage to storage roots occurs early to mid season.

As the average number of *Diabrotica* adults sampled 40-103 days after transplant increased, so did overall percent damage to storage roots. Significant positive correlations suggest that early season control of *Diabrotica* adults may be the most important management
tool available for reducing larval damage throughout the season. Adult beetles sampled early and mid season, weeks 1-3, and 4-6 of the sampling period, respectively were positively correlated with damage mid and late season. This suggests that control of adult *Diabrotica* populations 40-75 days after transplant is important in reducing overall larval damage from these insects at harvest. Presumably, a preplant soil incorporated insecticide would help control early season *Diabrotica* larvae. The recommended threshold for adult cucumber beetles in sweet potato in Louisiana is two beetles / 100 sweeps (Anonymous 1996). Based on our regression analysis, four adult cucumber beetles / 100 sweeps will result in ca. 5% root damage.

The current study was initially designed to determine an approximate window of time that sugarcane beetles, *Eueteola humilis*, were damaging sweet potatoes. Minimal sugarcane beetle damaged roots were sampled in 2004; however in 2005 an adequate number of damaged storage roots were sampled and subjected to analysis.

The original hypothesis was that the new generation of sugarcane beetles that emerge in August and September was causing the majority of damage to sweet potatoes prior to harvest. Results from the current study support this hypothesis. There was an increased probability of sugarcane beetle damage for the late planting date compared to the early and middle planting dates (Figure 6.10). The early planting at Chase in 2005 was harvested on September 8, 2005 and had minimal sugarcane beetle damage, in comparison to the middle and late planted sweet potatoes which were harvested October 13, 2005 and November 1, 2005 respectively. Sugarcane beetle damage was minimal in 2004 at Chase and was not detected at Dean Lee in 2004 or 2005. These data, though based on observations which occurred in only one location in one year, suggests that sugarcane beetle damage to sweet potatoes may be reduced if sweet potatoes are planted early. Initial field observations in 2003 also support our hypothesis. Sweet potatoes
harvested from a 45 ha field on October 10, 2003 in north Louisiana were heavily damaged, presumably by the pre-overwintering generation of sugarcane beetles. Some early season damage from the overwintering generation of the sugarcane beetle may also occur if sweet potato plantings and the initiation of storage root development overlap with the spring flight of beetles. Future studies examining the relationship of planting date with sugarcane beetle injury in sweet potatoes are warranted. Multiple years with heavy sugarcane beetle damage may further support the hypothesis which implicates the fall flight of the sugarcane beetle as the one that damages sweet potatoes prior to harvest.

The effect of planting date on insect biology and damage has been evaluated in other crop systems. Showler et al. (2005) showed that oviposition and feeding damage from boll weevils, *Anthonomus grandis grandis*, was greater in later planted cotton in comparison to earlier planted cotton. Planting earlier resulted in reduced aphid damage and lower incidence of aphid borne diseases in watermelon (Urias-Lopez et al. 2004). Anderson et al. (2003) indicated that later plantings of proso millet were more heavily infested with European corn borer, *Ostrinia nubilalis*, than earlier planted millet.

Studies investigating the effect of planting date on western corn rootworm, *Diabrotica virgifera virgifera*, and northern corn rootworm, *Diabrotica barberi*, have also been conducted. Bergman and Turpin (1986) found fewer larvae and adults of northern and western corn rootworms in later planted corn in comparison to earlier plantings, presumably due to reduced availability of corn plants; while Naranjo and Sawyer (1988) showed that delayed planting resulted in delayed emergence times of northern corn rootworms.

Sweet potatoes are a labor intensive crop, and planting dates are adjusted to ensure that transplants can be planted and roots can be harvested in a timely manner. Current studies have
suggested that later planted sweet potatoes have more total insect damage, *Diabrotica* beetle damage and increased sugarcane beetle damage than do earlier planted sweet potatoes. By planting sweet potatoes as early as possible, a producer may reduce insect damage.

**References Cited**


CHAPTER 7
RELATIONSHIPS BETWEEN HERBICIDE REGIME, ADULT *DIABROTICA* ABUNDANCE, LARVAL ROOT DAMAGE AND YIELD IN SWEET POTATO

**Introduction**


Weeds can affect agroecosystems in many ways, and crop yield reductions due to weeds account for large losses in the production of food, feed and fiber crops (Burnside 1979). Weed competition in vegetable crop production is associated with reductions in yield because weed species can compete with crops for nutrients, light and water (Qasem 1992). Sweet potato producers deal with a complex of weed species. Since the registration of Command® herbicide (clomazone) in sweet potato the populations of morningglories, cocklebur and grasses have been drastically reduced, but other weeds that clomazone does not control continue as pests due to ineffective chemical control measures (Porter 1990, Kelly et al. 2006).

Numerous herbicides have been evaluated for use on sweet potato with variable results on control, residual activity, and yield effects (Harrison et al. 1991, Porter and Canon 1998). Porter (1993) reported increases in yield for all herbicide treatments evaluated compared to untreated weedy plots. Several herbicides have recently been evaluated for use on sweet potato, such as Valor® (flumioxazin) and Sandea® (halosulfuron), with neither having known adverse effects on growth or yield of sweet potatoes when applied as recommended (Kelly et al. 2003, Garrett et al. 2004, Kelly et al. 2004, Shankle et al. 2005).
Historically, the primary problematic weeds species affecting sweet potato production in Louisiana were hophornbeam copperleaf, (*Acalypha ostryifolia*, Riddell), spiny amaranth (*Amaranthus spinosus*, L.), smellmelon, (*Cucumis melo* L.), and yellow and purple nutsedge (*Cyperus rotundus* L.) (Kelly et al. 2006). Carpetweed, *Mollugo verticillata*, is widespread but is not considered to be a problematic weed species (Kelly et al. 2006). Currently, Command® is recommended for control of annual grasses and some broadleaves. Valor® is recommended for control of broadleaf weeds and it also has limited activity against sedges (Anonymous 2006). Sandea® was recommended after approval by the EPA in 2004 and 2005 (Kelly, personal communication).

If weeds in monoculture agriculture go uncontrolled, they can decrease yields, interfere with harvest, and reduce the value of the harvested crop (Andow 1988). Insect populations can be influenced by non-host plants or weeds based on the number of host plant species present, the quality and density of the crops, and the relative attraction of the insects for the host plants (Root 1973). Many empirical studies have been conducted on the effects of plant diversity in relation to arthropod populations with two major conclusions: 1) specialized crop pests tend to have reduced populations in diversified systems, and 2) polyphagous crop pests do not show any particular response (Andow 1988). Arthropod-weed-crop interactions are dynamic and it is possible that weeds can serve as a source of a crop pest fauna (Andow 1988). The basic idea is that weeds, by acting as alternate hosts for crop pests, may contribute to crop pest problems (Andow 1988).

Insect damage in sweet potato from a variety of phytophagous pests may reach 60-90% (Chalfant et al. 1990, Jansson and Raman 1991), and all plant parts including roots, stems, and foliage can be affected (Talekar 1992). Soil insects that damage the root are the most harmful
because they can cause significant economic losses even in low numbers (USDA 2001). The banded cucumber beetle, *Diabrotica balteata* Leconte, and the spotted cucumber beetle, *Diabrotica undecimpunctata* Barber, feed on sweet potato. Cucumber beetles are generalist herbivores that will feed on many plant species. Adults of both species feed on the leaves of sweet potato (Cuthbert 1967) and the larvae of these insects damage sweet potato by chewing small holes on the surface of the root (Schalk et al. 1991). Multiple generations occur in a year and larval developmental time ranges from 20-50 days depending on temperature; roots are often injured early season which can result in numerous blemishes at harvest (Schalk et al. 1991). The objective of this study was to examine the effect of different herbicide regimes on insect abundance, insect damage to sweet potatoes and sweet potato yield.

**Materials and Methods**

**Study Site and Design**

Studies were conducted at Thornhill Farms, located near Gilbert, LA in 2004 and 2005. Sweet potato transplants 'Beauregard cultivar' ca. 30 cm long were cut the day of transplanting and were planted 0.3 m apart. Sweet potato plants were mechanically transplanted June 8, 2004 and June 24, 2005. General agronomic and pest management practices recommended by the LSU AgCenter were used in the test plots.

Treatments were arranged in a randomized block design with four replicates. Plot size was four rows spaced 1.01 m wide, by 15.24 m long. Treatments included Valor (flumioxazin 0.063 lb ai/A + Sandea (halosulfuron at 0.032 lb ai/A), Command (clomazone 1 lb ai/A) + Sandea (halosulfuron at 0.032 lb ai/A), Valor (flumioxazin 0.063 lb ai/A) + Command (clomazone 1 lb ai/A) + Sandea (halosulfuron at 0.032 lb ai/A), Command (clomazone 1 lb ai/A) + Valor (flumioxazin 0.063 lb ai/A) and an untreated control. All treatments were applied
immediately prior to or following transplanting to weed free beds using a CO₂-powered sprayer calibrated to deliver 190 L/ha. Clomazone was applied post transplant. Flumioxazin was applied immediately prior to transplanting and halosulfuron was applied 28 days after transplant (DATr). In order to evaluate numbers of adult insects and any subsequent larval damage, no soil incorporated or foliar insecticides were applied in either year to the field plots.

The flumioxazin + halosulfuron combination targeted broadleaf weeds and annual sedges, whereas, the clomazone + halosulfuron combination targeted annual grasses and sedges. The combination of flumioxazin + halosulfuron + clomazone targeted broadleaf weeds, grasses and sedges. The goal of the treatment regime which included all three herbicides was to establish weed-free plots with little competition.

**Sampling**

Sampling for insects and storage roots began 40 days after transplant. Plots were sampled a total of six times in each year. Adult insects were sampled by taking 25 sweeps/plot with a standard 0.38 m sweep net. Root samples were collected by manually digging three whole plants in each plot with a pitch fork. All roots samples were taken back to the laboratory where they were washed to remove any soil and evaluated. The number of storage roots / plant and the number of roots damaged by insects were recorded. Roots were evaluated for soil insect damage including: cucumber beetle, *Diabrotica* spp., sugarcane beetle, *Euetheola humilis*, white grubs, *Phyllophaga* spp., whitefringed beetle, *Naupactus leucoloma*, flea beetles, *Systena* spp. and *Chaetocnema* spp., and wireworms, *Conoderus* spp.

Weed counts were taken only in 2005 to determine the primary weed species present and the efficacy of the various herbicide regimes in controlling those weeds. Three random weed
counts were taken in each plot 70 days after transplant using a 30.48 square cm quadrant. Weed count data was averaged within replicate plots for analysis.

At 115 DATr, one row of each plot was harvested using a PTO-powered chain digger. Sweet potato roots were graded according to USDA standards (Anonymous 1997) and separated into three grade classes: U.S. No. 1 and 2, Canners, and Jumbos. U.S. No. 1 and 2 grades were combined, because they constitute the most profitable portion of sweet potato yield.

**Data Analysis**

Damage to sweet potato roots was compared among five herbicide treatments using analysis of variance, (PROC GLIMMIX, SAS Institute 2004), assuming a binomial distribution followed by Tukey-Kramer multiple range tests for means separation. Adult *Diabrotica* abundance was analyzed with analysis of variance, (PROC GLIMMIX, SAS Institute 2004), assuming a poisson distribution followed by Tukey-Kramer multiple range tests for means separations. Within each herbicide regime, the main effect of year and the interaction of year * herbicide regime were also examined. Sampling time was included in the analyses as a random effect. Correlations between percent *Diabrotica* damaged roots and mean number of adult *Diabrotica* were also examined (PROC CORR, SAS Institute 2004). Yield and weed count data were analyzed with analysis of variance, (PROC MIXED, SAS Institute 2004) and means were separated according to LSD for yield data and Tukey-Kramer for weed count data.

**Results**

Soil insect damage to sweet potato roots in 2004 and 2005 was minimal and damage data for all insect species was combined for purposes of analysis (Figure 7.1). No damage was recorded for the first sampling time in either year. The majority of insect damaged roots, approximately 1% of all roots sampled during the study were damaged by *Diabrotica* larvae.
Data were pooled across years because the interaction of herbicide regime * year was not significant, \( F = 0.99, \text{df} = 4, 495, P = 0.4141 \). Differences in insect damage between herbicide regimes were not detected \( F = 1.82, \text{df} = 4, 76, P = 0.1342 \); however, the effect of year on insect damage was significant \( F = 4.19, \text{df} = 1,495, P = 0.0412 \).

![Figure 7.1. Means ± SE of damaged sweet potato roots at five herbicide regimes in 2004 and 2005. Means were not significantly different between treatment regimes (\( P > 0.05, \text{Tukey-Kramer} \)). Number designations for treatments are: 1) flumioxazin / halosulfuron, 2) clomazone/halosulfuron, 3) flumioxazin / clomazone, 4) flumioxazin / clomazone / halosulfuron, 5) untreated control.](image)

### Table 7.1. Diabrotica abundance and mean Diabrotica damaged roots sampled from five herbicide regimes in 2004 and 2005.

<table>
<thead>
<tr>
<th>Year</th>
<th>Treatment</th>
<th>Mean ± SE Diabrotica Damaged Roots</th>
<th>Mean ± SE Diabrotica Adults</th>
</tr>
</thead>
<tbody>
<tr>
<td>2004</td>
<td>1</td>
<td>0 (0)</td>
<td>0.54 (0.15)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.14 (0.04)</td>
<td>0.58 (0.16)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.29 (0.09)</td>
<td>0.71 (0.24)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.13 (0.06)</td>
<td>0.38 (0.16)</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.01 (0.01)</td>
<td>1.08 (0.33)</td>
</tr>
<tr>
<td>2005</td>
<td>1</td>
<td>0.03 (0.02)</td>
<td>1.42 (0.31)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.03 (0.02)</td>
<td>1.04 (0.27)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.14 (0.04)</td>
<td>0.92 (0.22)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.01 (0.01)</td>
<td>0.96 (0.26)</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.03 (0.02)</td>
<td>1.21 (0.37)</td>
</tr>
</tbody>
</table>

\(^a\) Cucumber beetle samples 25 sweeps / plot were averaged over replicate plots.

\(^b\) Number designations for treatments are: 1) flumioxazin / halosulfuron, 2) clomazone/halosulfuron, 3) flumioxazin / clomazone, 4) flumioxazin / clomazone / halosulfuron, 5) untreated control.
Treatment three (flumioxazin / clomazone) had the greatest number of damaged roots, but differences in damage were not detected between treatment regimes (Figure 7.1). The mean number of damaged roots was similar for treatment one (flumioxazin / halosulfuron) and the untreated control, while treatments two (clomazone/ halosulfuron) and four (flumioxazin / clomazone / halosulfuron) received similar amounts of damage (Fig. 7.1).

The majority of adult insects sampled were cucumber beetles, *Diabrotica* spp. Adult *Diabrotica* beetle numbers were also pooled across years for analysis because the interaction of herbicide regime * year was not significant (F=1.51, df = 4, 115, P =0.2043). Differences in *Diabrotica* abundance were not detected between treatment regimes (F = 0.94, df = 4,92, P =0.4469). Mean number of *Diabrotica* adults sampled in 2004 and 2005 are presented in (Table 7.1). The highest numbers of *Diabrotica* beetles were sampled from untreated control plots but differences in mean number of *Diabrotica* beetles were not detected between treatments (Figure 7.2). No significant correlations were found between mean number of *Diabrotica* beetles and percent damaged roots within each treatment (P >0.05) (Table 7.2).

![Figure 7.2. Mean number ± SE of adult Diabrotica beetles sampled at five different herbicide regimes. Means were not significantly different between treatment regimes (P > 0.05, Tukey-Kramer). Number designations for treatments are: 1) flumioxazin / halosulfuron, 2) clomazone/halosulfuron, 3) flumioxazin / clomazone, 4) flumioxazin / clomazone / halosulfuron, 5) untreated control.](image-url)
Table 7.2. Correlations of mean number of *Diabrotica* beetles / 25 sweeps vs. percent damaged roots at five herbicide regimes.

<table>
<thead>
<tr>
<th>Treatment</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>flumioxazin/</td>
<td>0.56</td>
<td>0.24</td>
</tr>
<tr>
<td>halosulfuron</td>
<td></td>
<td></td>
</tr>
<tr>
<td>clomazone/</td>
<td>-0.31</td>
<td>0.55</td>
</tr>
<tr>
<td>halosulfuron</td>
<td></td>
<td></td>
</tr>
<tr>
<td>flumioxazin/</td>
<td>-0.26</td>
<td>0.62</td>
</tr>
<tr>
<td>clomazone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>flumioxazin/clomazone</td>
<td>0.77</td>
<td>0.08</td>
</tr>
<tr>
<td>halosulfuron</td>
<td></td>
<td></td>
</tr>
<tr>
<td>untreated control</td>
<td>0.66</td>
<td>0.16</td>
</tr>
</tbody>
</table>

* Significant relationship, P < 0.05

Data were averaged for 2004 and 2005

Yield data for 2004 and 2005 is reported (Table 7.3). The interaction of herbicide regime * year was not significant for U.S. No. 1 and 2, canners, jumbos or total yield grades (Table 7.4), therefore, yield data in 2004 and 2005 were combined (Table 7.5). A significant effect of herbicide regime on yield was not detected (Table 7.4). Year was significant in all yield analyses (Table 7.4). U.S. No. 1 and 2 yield was significantly higher in three of the herbicide regimes compared to the untreated check (Table 7.5).

Table 7.3. Sweet potato yields with five herbicide regimes in 2004 and 2005.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Year</th>
<th>Mean ± SE a</th>
<th>Mean ± SE a</th>
<th>Mean ± SE a</th>
<th>Mean ± SE a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>U.S. No. 1 and 2</td>
<td>Canner</td>
<td>Jumbo</td>
<td>Total</td>
</tr>
<tr>
<td>1</td>
<td>2004</td>
<td>51.43 (15.96)</td>
<td>34.65 (7.37)</td>
<td>1.65 (1.65)</td>
<td>87.73 (24.24)</td>
</tr>
<tr>
<td>2</td>
<td>2004</td>
<td>74.80 (7.46)</td>
<td>27.50 (4.79)</td>
<td>6.05 (3.74)</td>
<td>108.35 (10.09)</td>
</tr>
<tr>
<td>3</td>
<td>2004</td>
<td>63.25 (5.49)</td>
<td>33.55 (7.64)</td>
<td>5.23 (3.65)</td>
<td>102.03 (7.74)</td>
</tr>
<tr>
<td>4</td>
<td>2004</td>
<td>63.80 (11.74)</td>
<td>30.80 (3.70)</td>
<td>3.57 (1.51)</td>
<td>98.18 (15.84)</td>
</tr>
<tr>
<td>5</td>
<td>2004</td>
<td>44.55 (14.69)</td>
<td>27.22 (7.92)</td>
<td>4.95 (3.16)</td>
<td>76.73 (24.50)</td>
</tr>
<tr>
<td>1</td>
<td>2005</td>
<td>54.73 (15.68)</td>
<td>17.87 (1.70)</td>
<td>0.28 (0.28)</td>
<td>76.60 (15.37)</td>
</tr>
<tr>
<td>2</td>
<td>2005</td>
<td>51.92 (8.96)</td>
<td>20.02 (2.27)</td>
<td>3.52 (2.47)</td>
<td>71.94 (10.75)</td>
</tr>
<tr>
<td>3</td>
<td>2005</td>
<td>71.78 (14.80)</td>
<td>19.53 (1.87)</td>
<td>3.85 (3.16)</td>
<td>91.30 (16.52)</td>
</tr>
<tr>
<td>4</td>
<td>2005</td>
<td>57.75 (12.49)</td>
<td>25.85 (1.82)</td>
<td>7.15 (4.34)</td>
<td>83.60 (12.26)</td>
</tr>
<tr>
<td>5</td>
<td>2005</td>
<td>5.50 (2.77)</td>
<td>14.67 (3.88)</td>
<td>0 (0)</td>
<td>20.17 (6.61)</td>
</tr>
</tbody>
</table>

* All yield data is reported in kg sweet potato / 15 row meters.

Number designations for treatments are: 1) flumioxazin / halosulfuron, 2) clomazone/ halosulfuron, 3) flumioxazin / clomazone, 4) flumioxazin / clomazone / halosulfuron, 5) untreated control.
Table 7.4. Effect of different herbicide regimes evaluated in 2004 and 2005 on yield in sweet potato.

<table>
<thead>
<tr>
<th>Yield Grade</th>
<th>Effect</th>
<th>DF</th>
<th>F</th>
<th>P&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>U.S. No. 1 and 2</td>
<td>Treatment</td>
<td>4, 16</td>
<td>2.30</td>
<td>0.1041</td>
</tr>
<tr>
<td>Year</td>
<td></td>
<td>1, 14</td>
<td>32.13</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Trt * Year</td>
<td></td>
<td>4, 14</td>
<td>0.48</td>
<td>0.7532</td>
</tr>
<tr>
<td>Canner</td>
<td>Treatment</td>
<td>4, 16</td>
<td>0.32</td>
<td>0.8633</td>
</tr>
<tr>
<td>Year</td>
<td></td>
<td>1, 14</td>
<td>54.03</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Trt * Year</td>
<td></td>
<td>4, 14</td>
<td>0.36</td>
<td>0.8317</td>
</tr>
<tr>
<td>Jumbo</td>
<td>Treatment</td>
<td>4, 16</td>
<td>0.62</td>
<td>0.6580</td>
</tr>
<tr>
<td>Year</td>
<td></td>
<td>1, 14</td>
<td>4.74</td>
<td>0.0472</td>
</tr>
<tr>
<td>Trt * Year</td>
<td></td>
<td>4, 14</td>
<td>0.56</td>
<td>0.6987</td>
</tr>
<tr>
<td>Total Marketable</td>
<td>Treatment</td>
<td>4, 16</td>
<td>1.45</td>
<td>0.2644</td>
</tr>
<tr>
<td>Year</td>
<td></td>
<td>1, 14</td>
<td>43.84</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Trt * Year</td>
<td></td>
<td>4, 14</td>
<td>0.16</td>
<td>0.9559</td>
</tr>
</tbody>
</table>

Table 7.5. Effect of herbicides on sweet potato yield 115 days after transplant

<table>
<thead>
<tr>
<th>Treatment</th>
<th>U.S. No. 1 and 2</th>
<th>Canner</th>
<th>Jumbo</th>
<th>Total Marketable</th>
</tr>
</thead>
<tbody>
<tr>
<td>flumioxazin/halosulfuron</td>
<td>10641 AB</td>
<td>5884 A</td>
<td>241 A</td>
<td>16766 A</td>
</tr>
<tr>
<td>clomazone/halosulfuron</td>
<td>13737 A</td>
<td>5143 A</td>
<td>890 A</td>
<td>19771 A</td>
</tr>
<tr>
<td>flumioxazin/clomazone</td>
<td>13390 A</td>
<td>5847 A</td>
<td>965 A</td>
<td>20202 A</td>
</tr>
<tr>
<td>flumioxazin/clomazone/halosulfuron</td>
<td>12514 A</td>
<td>5904 A</td>
<td>966 A</td>
<td>19385 A</td>
</tr>
<tr>
<td>untreated check</td>
<td>7033 B</td>
<td>4677 A</td>
<td>891 A</td>
<td>12601 A</td>
</tr>
</tbody>
</table>

1 Means followed by the same letter are not significantly different (P > 0.05, LSD).

a Yield data is reported in kg/ha and was combined in 2004 and 2005.
Yield in the (clomazone/halosulfuron), (flumioxazin/clomazone), and (flumioxazin/clomazone/halosulfuron) treatment regimes was > 1.5 times that of untreated control plots (Table 7.5). No differences in yield were detected for canners, jumbos or total marketable yield between treatments \((P > 0.05)\) (Table 7.5).

Weed counts were taken in 2005 to examine the efficacy of different herbicide regimes in controlling different weed species. Spiny amaranth, *Amaranthus spinosus*, was significantly reduced in all treatment regimes compared to the untreated check (Table 7.6). Treated plots had 4-18 times improved control of *A. spinosus*, compared to untreated plots (Table 7.6).

Carpetweed, *Mollugo verticillata*, was significantly reduced in three of the herbicide regimes compared to the untreated check (Table 7.6). Treatments of (flumioxazin/halosulfuron), (flumioxazin/clomazone), and (flumioxazin/clomazone/halosulfuron), had significantly fewer *M. verticillata* compared to untreated plots and plots treated with clomazone/halosulfuron only (Table 7.6).

**Table 7.6. Effect of herbicide regime on weed control 70 Days after transplant, Gilbert, LA, 2005**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean ± SE Spiny amaranth</th>
<th>Mean ± SE Carpetweed</th>
<th>Mean ± SE Yellow nutsedge</th>
<th>Mean ± SE Copperleaf</th>
<th>Mean ± SE Grasses</th>
</tr>
</thead>
<tbody>
<tr>
<td>flumioxazin/halosulfuron</td>
<td>2.15 (0.57) B</td>
<td>0.25 (0.17) B</td>
<td>1.07 (0.55) A</td>
<td>0.43 (0.25) A</td>
<td>0.75 (0.75) A</td>
</tr>
<tr>
<td>clomazone/halosulfuron</td>
<td>0.58 (0.38) B</td>
<td>2.18 (0.64) A</td>
<td>1.43 (0.88) A</td>
<td>0.40 (0.21) A</td>
<td>0 (0) A</td>
</tr>
<tr>
<td>flumioxazin/clomazone</td>
<td>0.43 (0.17) B</td>
<td>0 (0) B</td>
<td>1.51 (1.08) A</td>
<td>0 A</td>
<td>0 (0) A</td>
</tr>
<tr>
<td>flumioxazin/clomazone/halosulfuron</td>
<td>0.50 (0.22) B</td>
<td>0.18 (0.18) B</td>
<td>1.18 (0.29) A</td>
<td>0.43 (0.25) A</td>
<td>0 (0) A</td>
</tr>
<tr>
<td>untreated check</td>
<td>9.33 (1.36) A</td>
<td>2.03 (0.62) A</td>
<td>2.18 (0.77) A</td>
<td>0 A</td>
<td>0 (0) A</td>
</tr>
</tbody>
</table>

\(^1\)Means ± SE. Means followed by the same letter are not significantly different, \((P > 0.05, Tukey-Kramer)\).
**Discussion**

Differences in soil insect damage were not detected between herbicide treatment regimes in the current study. Insect abundance and subsequent damage from all species evaluated was minimal in both years. It was hypothesized that untreated plots with natural weeds would have larger insect populations and increased damage; however this difference was not detected in the current study. Lack of significant differences between treatments was likely due to low insect numbers in both 2004 and 2005.

*Diabrotica* numbers reached economically damaging levels. The recommended threshold for adult *Diabrotica* is two beetles / 100 sweeps. More *Diabrotica* were sampled from untreated plots, but differences in *Diabrotica* abundance were not detected between herbicide treatments. Buckelew et al. (2000) did not find significant differences in the number of bean leaf beetles sampled from different weed management systems in soybean, *Glycine max*, but other studies relating insect numbers to weed control in soybean have produced variable results (House and Stiner 1983, Troxclair and Boethel 1984).

The various herbicide regimes evaluated did affect sweet potato yield. The significant effect of year in our study was most likely due to environmental fluctuations. Yields were higher in 2004 compared to 2005. A possible explanation for the reduction in yield in 2005 is lack of precipitation and reduced activation of herbicides due to dry conditions. More than 46 cm of rain was recorded during the duration of the test in 2004, compared to 28 cm in 2005.

The increase in yield of U.S. No. 1 and 2 in three of the four herbicide treatment regimes compared to the untreated check suggests the importance of practicing integrated weed management in sweet potato. Differences in U.S. No. 1 and 2 yield were not detected between the flumioxazin/halosulfuron herbicide regime and the untreated check. Kelly et al. (2006) also
found increases in U.S. No. 1 and 2 and total yields with different herbicide treatments; plots treated with flumioxazin had significantly greater yields compared to plots treated with clomazone only (Kelly et al. 2006). Similar results in yield have been demonstrated in other crops. Young et al. (1994) found that increased weed management resulted in increased yield in winter wheat.

The majority of weed species present in the current study were broadleaf weeds, such as spiny amaranth, *Amaranthus spinosus*, and Carpetweed, *Mollugo verticillata*. However some grasses were present. All herbicide regimes reduced the number of spiny amaranth and all regimes with the exception of clomazone/ halosulfuron, the only treatment regime without flumioxazin, reduced the number of carpetweeds in 2005. Kelly et al. (2006) also noted good control of these weeds, at least 86%, with flumioxazin applications.

Cultivation can also be an important aspect of integrated weed management. The expense of cultivation adds to the overall production costs, so it is important for growers to be efficient in weed control (Seem et al. 2003). A critical weed free period in agricultural crop production is the period of time when competition of weed species with the crop should be kept to a minimum to prevent yield and quality reductions (Weaver et al. 1992). The critical weed free period is different depending on the crop and the weed species (Seem et al. 2003). Beauregard, the predominant sweet potato variety grown in Louisiana and the United States, is a high yielding sweet potato cultivar, (Rolston et al. 1987) and is not as tolerant to weed interference as are lower-yielding varieties (Labonte et al. 1999). Seem et al. (2003) suggested that the majority of yield loss in Beauregard brought about by weed interference throughout the season was due to weed interference 2-8 weeks after transplant.
These data indicate that greater yields may be achieved in sweet potato if herbicides are integrated into an overall pest management program. Treatment regimes that included flumioxazin and clomazone, herbicides currently recommended for use on sweet potato in Louisiana, provided good control of spiny amaranth and carpetweed, two predominate weed species in Louisiana sweet potato production. More research is needed to define the relationship between adult *Diabrotica* abundance and larval damage of these insects in relation to various weed management options (herbicide combinations). Insect populations were low throughout the course of this study, as a result minimal damage to sweet potato roots occurred. Planting sweet potato in close proximity to selected trap crops may improve opportunities to investigate the relationship of sweet potato insects and weed control tactics in future experiments.

References Cited


CHAPTER 8
SUMMARY AND CONCLUSIONS

The United States is a major contributor to sweet potato production worldwide and sweet potato production plays a vital role in the agroecosystem of the southern United States. Sweet potatoes are the most important vegetable crop grown in Louisiana with respect to acreage and economic impact. At least 19 insect species affect sweet potato in the United States and insect damage from a variety of phytophagous insects may reach 60-90%. Soil insects that damage the roots are the most harmful because they can cause significant economic losses even in low numbers. Species that are problematic in Louisiana include the: sweetpotato weevil, *Cylas formicarius* Fab., cucumber beetles, *Diabrotica* spp., white grubs, *Phyllophaga* spp., whitefringed beetle, *Naupactus leucoloma*, and the sugarcane beetle, *Eutheola humilis*, Burmeister.

A recently significant soil insect pest of sweet potato is the sugarcane beetle, *Eutheola humilis* (Burmeister) Scarabaeidae, Coleoptera. The sugarcane beetle is a polyphagous herbivore that has traditionally been a sporadic pest of field corn and sugarcane. No insecticides are currently labeled for control of the sugarcane beetle in sweet potato, but numerous insecticides have been shown to reduce sugarcane beetle damage to field corn. Limited information is available on the ecology and feeding behavior of *E. humilis*, probably because it has been a sporadic pest in recent years. *Eutheola humilis* demonstrate a propensity to aggregate in sweet potato fields. It is hypothesized that there is a cue to which the beetles are responding over a short period of time, which attracts subsequent beetles to a particular location.

The host plant preference of the sugarcane beetle was evaluated in greenhouse choice tests. Seven plant species including: sweet potato, *Ipomoea batatas*, corn and Bt corn, *Zea mays,*
sugarcane, *Saccharum* spp., rice, *Oryza sativa*, Bermuda grass, *Cynodon dactylon*, and strawberry, *Fragaria* spp. were evaluated. Sweet potato was preferred over all other hosts included in the test (P< 0.05). Sweet potato was selected ca. 50% more often than corn and sugarcane, the next preferred plant species included in the choice test. In addition, sweet potato was preferred ca. 7.5 times to strawberry and > was 6 times preferred over rice. Sweet potato was also ca. 3 times more attractive than Bt corn and > 4 times preferred to Bermuda grass. These results suggest that when given a choice of known host plants, sugarcane beetles prefer to feed on sweet potato. In field situations, host plants are not available at the same time during the year. Sugarcane and corn were also popular choices for the beetles in the current study. Corn and sugarcane are damaged in the spring of the year, and it is hypothesized that damage to sweet potato occurs in the fall after most alternative host plants are unavailable.

The chemical ecology of the sugarcane beetle was evaluated in olfactometer and cultivar preference tests. Numerous paired odor tests were conducted using a classical Y-tube olfactometer. Beetle injured and mechanically injured sweet potato roots were evaluated against uninjured sweet potato roots. Washed beetle injured roots were compared with unwashed beetle injured roots. Two cultivars were evaluated in the olfactometer and sugarcane beetle damaged roots were tested against sweetpotato weevil injured roots. Male and female sugarcane beetles were also evaluated for their response toward conspecific beetles. Sugarcane beetle preference for four cultivars including: Beauregard, Georgia Jet, White Star and Bunch Porto Rico were evaluated in small container paired choice tests. In addition, sugar analyses were conducted on the four cultivars. In olfactometer studies, female and male sugarcane beetles were significantly more attracted to sugarcane beetle damaged sweet potato roots compared to intact sweet potato roots. Beetle injured roots were over six and three times more attractive respectively to male and
female beetles. Percent response of beetles was also greater for mechanically damaged roots for both male and female sugarcane beetles. Percent response for males was 30 times greater for mechanically damaged roots when compared to intact roots and female beetle response to mechanically injured roots was 3 times greater than for intact roots. When comparing beetle injured roots to mechanically injured roots, male beetle's percent response was significantly greater for beetle injured roots compared to mechanically injured roots and female beetle response to the two injury types was not significantly different. Sugarcane beetles did not respond differentially to washed beetle injured roots vs. unwashed beetle injured roots, indicating that beetle frass and / or regurgitate was not a confounding factor in beetle attraction to wounded sweet potato roots. When given a choice of a sugarcane beetle injured root or a sweetpotato weevil injured root, beetle percent response was greater for sweetpotato weevil injured roots ($P < 0.05$). Sweet potato cultivars were differentially damaged by sugarcane beetles in paired choice tests. In small container tests, Beauregard was preferred to Georgia Jet in multiple beetle and single beetle evaluations, $P < 0.05$. No differences in feeding preference were detected between Beauregard and White Star; however, Bunch Porto Rico was preferred to Beauregard. Beauregard, in general, had the highest sugar content compared to other cultivars, with the exception of sucrose in Bunch Porto Rico. Results from chemical ecology studies provide insight into the behavior of the sugarcane beetle. We now know that the beetles respond to host plant volatiles of injured sweet potato roots and to conspecifics. These data suggest that aggregations in the field may be due to host plant volatiles released after a wounding event, aggregation / sex pheromones or a combination of these.

Sugarcane beetles were first documented as pests of sweet potato in Louisiana in 2002 when farmers reported excessive losses from sugarcane beetles feeding on roots prior to harvest.
There are currently no insecticides labeled for sugarcane beetle on sweet potato. The activity of the organophosphates (chlorpyrifos and phosmet), the pyrethroids (bifenthrin, z-cypermethrin), the neonicitinoid (clothianidin) and the phenylpyrazole (fipronil) was evaluated against sugarcane beetle adults during 2003, 2004, and 2005 in laboratory adult vial bioassays. Z-cypermethrin, the most toxic chemical tested, was significantly more toxic (4.8, and 17.7-fold) than chlorpyrifos and bifenthrin in 2004. Chlorpyrifos was significantly more toxic (4.3 and 3.6-fold) than bifenthrin evaluated in 2003 and 2004. Bioassays of clothianidin, fipronil and phosmet resulted in 0% mortality at 100µg/vial, the highest dose evaluated. Bioassays have yielded valuable baseline toxicological data for three insecticides for potential control of the sugarcane beetle. Information provided here will be useful in selecting an insecticide to control this insect in sweet potato.

Adult vial tests were used to evaluate two cohorts of sweetpotato weevil, *Cylas formicarius* Fab. The sweetpotato weevil is currently confined to the southern regions of Louisiana. The weevil is closely monitored with the use of a synthetic sex pheromone and a mandatory spray program is in place to slow the movement of the weevil into northern regions of the state. The cohorts tested were a laboratory maintained population of sweetpotato weevils which were collected from a sweet potato production field in Avoyelles Parish, Louisiana and had prior exposure to insecticides in the field before collection, and a susceptible Texas cohort collected from wild *Ipomoea* spp. which had no known previous exposure to insecticides evaluated in bioassays.

*Cylas formicarius* adults from both cohorts were most susceptible to methyl parathion, and weevils from the two cohorts were differentially susceptible to this compound. Both cohorts were also highly susceptible to the pyrethroids bifenthrin and cyfluthrin which were the next
most toxic chemicals. *Cylas formicarius* were less susceptible to phosmet and carbaryl compared to the other insecticides. Differences (*P* < 0.05) in susceptibility of the Louisiana cohort to bifenthrin and cyfluthrin compared to the susceptible Texas cohort were observed at both the LC$_{50}$ and LC$_{90}$ levels (ug/vial). The Texas cohort had 1.5 and 2.3 (RR$_{50}$) decreased sensitivity to cyfluthrin and bifenthrin respectively compared to the Louisiana cohort. Differences (*P* < 0.05) were detected in susceptibility of weevils to phosmet only at the LC$_{50}$ level with the Texas cohort being over 2.5 fold more susceptible compared to the Louisiana cohort and weevils from the two cohorts exhibited differences to carbaryl only at the LC$_{90}$ level. At a discriminating concentration of 10µg/vial, phosmet resulted in 34.4% mortality for the Louisiana cohort compared to 65 for the Texas cohort. Carbaryl tested at 50 µg/vial resulted in 45.6 % mortality of weevils from the Louisiana cohort compared to 70 % mortality of weevils from the Texas cohort.

The influence of planting date on insect abundance, damage to roots and number of storage roots was evaluated in a two year study. The majority of adult insects sampled were cucumber beetles, *Diabrotica* spp., and the majority of root damage was caused by larval *Diabrotica* in 2004 and 2005 and sugarcane beetle in 2005. Seasonal distribution of adult cucumber beetles, *Diabrotica*, was variable throughout the sampling period in both years and locations, which is indicative of the life cycle of the insect. *Diabrotica* damage steadily increased 40 to 68 days after transplant in most cases. Planting date did have a significant effect on total insect damage, which included damage from *Diabrotica* and also limited damage from whitefringed beetles, white grubs, and sugarcane beetle.

Differences in *Diabrotica* damage were not detected between planting dates, but sampling time (week) and the interaction of planting date * week did affect total insect and
Diabrotica only damage. Significantly more adult *Diabrotica* were sampled from the late planting dates vs. early and middle dates and *Diabrotica* numbers increased ca. three times during the season for each planting date. There was a significant relationship between mean number of *Diabrotica* beetles sampled throughout the season and percent root damage from these insects. Adult *Diabrotica* beetles sampled throughout the season were also positively correlated to larval damage throughout the season and at various seasonal intervals.

Planting date also had a significant effect on the number of storage roots sampled throughout the season. Differences in total number of storage roots per plant were not detected between the early and late planting dates, but both the early and late plantings had significantly more storage roots than the middle planting date. The number of storage roots sampled gradually increased until week four, staying fairly uniform for the remainder of the sampling period.

Planting date also had a significant effect on the number of roots that were damaged by sugarcane beetle and sugarcane beetle damage did increase throughout the sampling period. Late planted potatoes had 12 and 3.5 times more damage than sweet potatoes planted at the early and middle planting dates, respectively and the probability of sugarcane beetle damage increased three weeks into the sampling period, ca. 54 days after transplant.

These studies suggest the importance of using a structured multidisciplinary integrated pest management program in sweet potato. Early season control of adult insects is important for reducing damage later in the season and it may be possible to minimize sugarcane beetle damage by planting sweet potatoes as early as possible.

Numerous weed species can be problematic in sweet potato production and weeds have been known to influence insect population dynamics. Insect damage, abundance, number of
storage roots, yield and weed density were compared at five herbicide regimes. Herbicide regimes evaluated included: Valor (flumioxazin 0.063 lb ai/A + Sandea (halosulfuron at 0.032 lb ai/A), Command (clomazone 1 lb ai/A) + Sandea (halosulfuron at 0.032 lb ai/A), Command (clomazone 1 lb ai/A) + Valor (flumioxazin 0.063 lb ai/A ), Valor(flumioxazin 0.063 lb ai/A) + Command (clomazone 1 lb ai/A) +Sandea (halosulfuron at 0.032 lb ai/A), and an untreated control.

No differences in *Diabrotica* beetle abundance or insect damage were detected between herbicide treatments in the current study. Insect abundance and subsequent damage from all species evaluated was minimal in both years. It was hypothesized that untreated plots with natural weed populations would have larger insect populations and increased damage; however this was not seen in the current study. Herbicide regime affected yield and weed density. U.S. No. 1 and 2 yield was significantly higher in all herbicide regimes compared to the untreated check with the exception of the flumioxazin / halosulfuron treatment regime. Weed counts were taken in 2005 and spiny amaranth, *Amaranthus spinosus*, was significantly reduced in all treatment regimes compared to the untreated check, while carpetweed, *Mollugo verticillata*, was significantly reduced in all herbicide regimes except the clomazone / halosulfuron regime compared to the untreated check.

Integrated pest management programs for sweet potato insects are being implemented and they have the potential to improve sweet potato insect pest management in the future. Current studies have yielded valuable information on the host plant preference and chemical ecology of the sugarcane beetle. We now know that sugarcane beetles will respond to host plant volatiles and conspecifics, and different cultivars may be differentially damaged by the sugarcane beetle. Further identification of the attractive agents (host plant volatiles, pheromones)
responsible for the aggregative behavior of sugarcane beetle to sweet potato fields is the next logical step in elucidating the behavior of this insect. Many of the insecticides currently being used in sweet potato production are being reviewed by the United States Environmental Protection Agency. Currently no insecticides are labeled for control of sugarcane beetle in sweet potato. Adult vial bioassays have provided baseline toxicological data for this insect. The overall demand for a superior quality and attractive product in the United States places some constraints but also increases the need for an integrated management approach to manage sweet potato insects. This is especially true in managing the sweetpotato weevil with a mandatory spray program. Susceptibility of the weevil to labeled insecticides must be carefully monitored to slow the development of tolerance and resistance to insecticide chemistries in this insect. Cultural control tactics are an important part of integrated pest management. By manipulating planting and harvesting dates, we can reduce damage by some insects such as the sugarcane beetle. These studies have shown that insect damage is reduced in earlier planting dates and has specifically stressed the importance of early season management of adult *Diabrotica* to reduce larval damage throughout the season. Judicious use of various control options is necessary to achieve optimum crop production. Results of these studies will be useful in improving and refining the integrated pest management of insects affecting sweet potato.
VITA

Tara Parker Smith, the daughter of Robert Parker and Delilah Crooks, was born in Alexandria, Louisiana, on June 28, 1978. She graduated from Holy Savior Menard High School in Alexandria, Louisiana, in 1996. In 2000, she earned a bachelor of science degree in biology from University of Louisiana at Monroe. She completed her master of science in biology from Louisiana Tech University in 2001. Tara married Joey Smith in 2001, and she began studies for the degree of Doctor of Philosophy in entomology in 2002 at Louisiana State University and Agricultural and Mechanical College under the supervision of Dr. Abner M. Hammond. Currently, she is a doctoral candidate in the Department of Entomology.