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THE SPOTTED CUCUMBER BEETLE (*DIABROTICA UNDECIMPUNCTATA*
HOWARDI): INTERACTIONS WITH CUCURBITS AND ITS STATUS AS
A SWEETPOTATO PEST IN MISSISSIPPI

By

Daniel Edward Fleming

A Thesis
Submitted to the Faculty of
Mississippi State University
In Partial Fulfillment of the Requirements
for the Degree of Master of Science in
The Department of Entomology and Plant Pathology

Mississippi State, Mississippi

May 2009

THE SPOTTED CUCUMBER BEETLE (*DIABROTICA UNDECIMPUNCTATA*
HOWARDI): INTERACTIONS WITH CUCURBITS AND ITS STATUS AS
A SWEETPOTATO PEST IN MISSISSIPPI

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Pages in Study: 118

Candidate for Degree of Master of Science

The spotted cucumber beetle (*Diabrotica undecimpunctata howardi* Barber) is regarded as a pest of sweetpotatoes in Mississippi; however, its feeding on sweetpotatoes has not previously been documented. They are attracted to cucurbit crops that could be utilized as a trap crop or sentinel plant for management of cucumber beetles in sweetpotatoes. Studies were conducted between 2006 and 2008 to determine if cucurbit plants have the potential to serve as a trap crop or as sentinel plants for the spotted cucumber beetle in sweetpotato fields, and to determine the status of the spotted cucumber beetle as a sweetpotato pest in Mississippi. Cucurbit plants showed some potential to serve as a trap crop or sentinel plant for the spotted cucumber beetle, however, sweetpotato damage assumed to be caused by cucumber beetle larvae did not correlate with the number of adults captured. Spotted cucumber beetle larvae can feed and survive on sweetpotato roots.

ACKNOWLEDGMENTS

The author wishes to thank Dr. Jack Reed for encouraging me to take on the challenge of pursuing this degree and for his constant insight and encouragement throughout the process; Dr. Frank Davis for all his wisdom and insight in insect rearing; Dr. Fred Musser for his assistance with statistical analysis; Dr. David Nagel for germinating the plants needed in my trials; Dr. Randy Little for his help with some economics; Joe MacGown for help making figure 2.1; William Monroe for taking the SEM images; Sandra Woolfolk for identifying the fungi in the colony; Amanda Lawrence for examining specimens from the colony for disease and autoclaving my supplies; and Dr. Gary Lawrence for use of his autoclave. Also, thanks to Amelia Williams, Chris Jackson, Dung Bao, and Matt Holley for all the assistance in the various work that was performed. Also, to my wife Paige, for being patient and encouraging me and praying for me through this process.

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CHAPTER I
INTRODUCTION

Sweetpotatoes

Economics, Ecology, and Storage

Sweetpotatoes (*Ipomoea batatas* (L.)) are a warm season crop primarily grown in tropical and sub-tropical regions of the world, with China accounting for approximately 80% of worldwide production (Horton 1987). In the United States, sweetpotatoes are primarily grown in the Southeast and California. Over 100,000 acres of sweetpotatoes were planted in the United States in 2007. The state of Mississippi accounted for approximately 19,000 of the harvested acres. Most of this acreage is found in the north-central part of the state. Sweetpotato production accounted for \$67 million in the Mississippi economy in 2007 (UDSA-ERS 2008).

Sweetpotatoes are in the morningglory family (Convolvulaceae) and are native to tropical America. They are grown for their tuberous roots and serve as a major carbohydrate and nutritional source for millions of people (Edmond 1971; Swiader and Ware 2002). They can withstand hot temperatures, are fairly drought tolerant (reviews in Bouwkamp 1985), and are nutrient scavengers. Sweetpotatoes have moderately high nutrient demands, but because of an extensive root system, they are usually able to

scavenge much of what they need without additional fertilizer. Their extensive root system, once established, makes them drought-tolerant. Their optimum pH range is 5.5-6.2 (Swiader and Ware 2002).

Sweetpotatoes tolerate the hot summers of the southeastern United States very well. The optimum growing temperature is 24° C, but they do well in the much warmer conditions of the southeastern United States. The hot summers of the Southeast may cause the skin of sweetpotatoes to be tougher which reduces skinning (reviews in Birnbaum 1970) and possibly injury by insects. Sweetpotatoes can grow in a variety of soils, but sandy or silt loams with a clay subsoil tend to grow the best shaped and smoothest skinned roots (Edmond 1971; Bouwkamp 1985). The soil must be well aerated and well drained (Swiader and Ware 2002).

Sweetpotato transplants are grown from storage roots of the previous season's crop. These roots are taken out of storage in early spring, placed in broad furrows, and covered with soil. To protect them from frost, a white plastic covering is placed over them. This covering keeps them warm so they will bud properly in the spring. The sprouts that arise from the buds are called slips. These are cut one or two days before transplanting. In Mississippi, transplanting usually begins in middle to late May and continues until the middle of July with acceptable yields. The slips are transplanted with a vegetable transplanter on raised beds that are approximately 1 m apart and 20 to 40 cm apart in the row. Some fertilizer, herbicides, and insecticides are usually applied pre-plant incorporated (PPI) and some may also be applied later in the season as needed. Once the plants are established they "lay over" and begin to vine. Sweetpotatoes have a

fibrous root system except for the 4-10 roots per plant that swell to form storage roots (Swiader and Ware 2002).

Depending on variety and environmental factors, harvest occurs 80-120 days after planting, and timing of harvest is based on root size since the root will continue to increase in size until the plants are killed by frost. Chilling injury can occur below 10° C, so they must be harvested before the weather cools in the fall. After harvest the roots are cured for about a week at approximately 27.5° C and at 85-90% humidity to help enhance suberization (hardening) for protection from entry by microorganisms (Weimer and Harter 1921; Lauritzen and Harter 1926; Artschwager and Starrett 1931; Birnbaum 1970). This helps injuries on the root to heal by the development of a corky layer. After curing, the roots can be stored for 6 months or more at approximately 14° C and 85-90% humidity (Birnbaum 1970; Swiader and Ware 2002).

Sweetpotato Insect Pests

Sweetpotato roots are attacked by many insect pests, primarily from the Coleoptera. The most common coleopteran pests include: sweetpotato flea beetle larvae (*Chaetocnema confinis* Crotch) (Kantack and Floyd 1956; Cuthbert and Reid 1965; Schalk 1984; Schalk et al. 1991a; Zehnder 1998; Thompson et al. 2002; Jasrotia et al. 2008); red-headed, elongate, and pale-striped flea beetle larvae (*Systema frontalis* (Fabricius), *S. elongata* (Fabricius), and *S. blanda* Melsheimer, respectively) (Cuthbert and Reid 1965; Schalk 1984; Schalk et al. 1991a; Schalk et al. 1991b; Schalk et al. 1993; Zehnder 1998; Thompson et al. 2002; Jasrotia et al. 2008); white grubs (*Phyllophaga*

spp.) (Kantack and Floyd 1956; Rolston and Barlow 1980; Schalk 1984; Schalk et al. 1991a; Schalk et al. 1991b; Zehnder 1998; Thompson et al. 2002); sugarcane beetles (*Euetheola humilis rugiceps* (LeConte)) (Smith 2006); white fringed beetle larvae (*Naupactus leucoloma* (Boheman) and *Naupactus perigrinis* (Buchanan)) (Schalk et al. 1991b; Zehnder 1998; Thompson et al. 2002; Jasrotia et al. 2008); wireworms (click beetle larvae) (*Conoderus* spp., *Heteroderes* spp., and *Melanotus* spp.) (Schalk 1984; Chalfant and Seal 1991; Schalk et al. 1991a; Schalk et al. 1993; Zehnder 1998; Thompson et al. 2002; Jasrotia et al. 2008); sweetpotato weevil adults and larvae (*Cylas formicarius elegantulus* (Summers)) (Floyd 1955; Schalk 1984; Jansson and Raman 1991; Capinera 1998; Zehnder 1998; Horton and Ellis 2005); and banded and spotted cucumber beetle larvae (*Diabrotica balteata* LeConte and *D. undecimpunctata howardi* Barber, respectively) (heretofore BCB and SCB, respectively) (Kantack and Floyd 1956; Schalk 1984; Schalk et al. 1991a; Schalk et al. 1991b; Schalk et al. 1993; Zehnder 1998; Thompson et al. 2002; Smith 2006; Jasrotia et al. 2008). Though many other insects can be found in sweetpotatoes, including foliar feeding insects, root feeding insects are the most important since they feed on the marketable part of the plant.

These root-feeding pests can cause a variety of damage, which can be categorized as holes and gouges. Hole type scars are more common and are caused by a complex of wireworms, flea beetle larvae, and cucumber beetle larvae. Distinguishing between damage of these three insect pests is difficult. However, there are criteria created by researchers in a recent USDA RAMP Southern Sweetpotato IPM project (project no. 640222320003105) that distinguished between types of root scars. Wireworm scars are

considered large, deep holes 2 to 8 mm in diameter sometimes with irregular shaped cavities underneath and usually randomly spaced on the surface of the root (Figure 1.1). *Systema* spp. flea beetle larvae scars are considered pinholes, 1 mm in diameter, possibly with tunneling into the root (Figure 1.2). Larval cucumber beetle scars are considered small, round holes between 1 and 3 mm in diameter, sometimes with irregular shaped cavities under the skin of the root and sometimes clumped on the surface of the root (Figure 1.3). These criteria are helpful. However, confusion exists when considering the size of the damage in relation to the size of the different instars of these insect larvae. For example, a 1st instar cucumber beetle larva could cause damage similar to the pinhole damage associated with *Systema* sp. flea beetle larvae. Likewise, a 3rd instar *Systema* sp. flea beetle larva would probably make a scar similar to small-hole damage associated with cucumber beetle larvae. Also after a sweetpotato root is damaged and continues to grow, the size of the scar will increase and heal making it even more difficult to distinguish which insect caused the damage (Figure 1.4) (Schalk et al. 1991b).

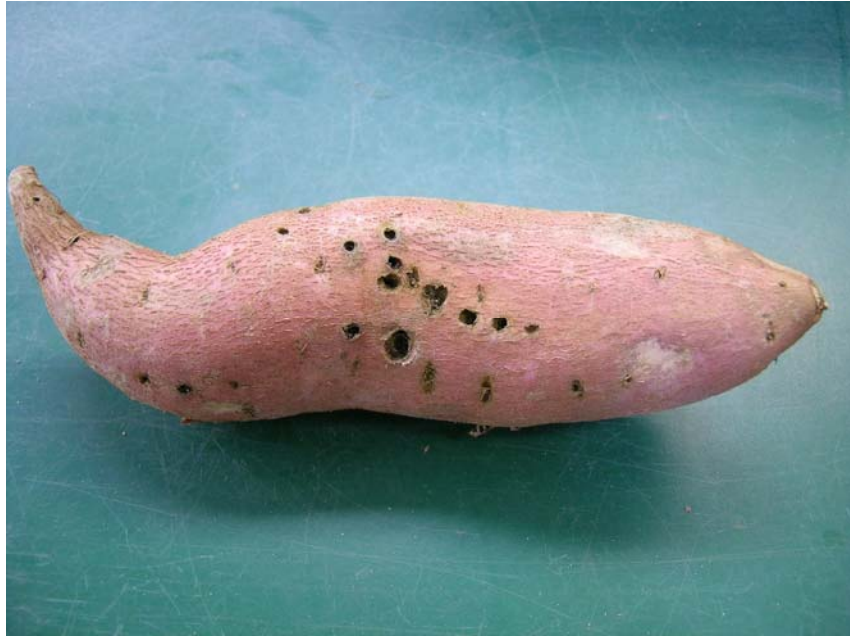


Figure 1.1. Large/deep-hole scars on a sweetpotato root caused by wireworms, according to the criteria of the USDA RAMP Southern Sweetpotato IPM Project. Photo courtesy of Mark Abney, North Carolina State University.

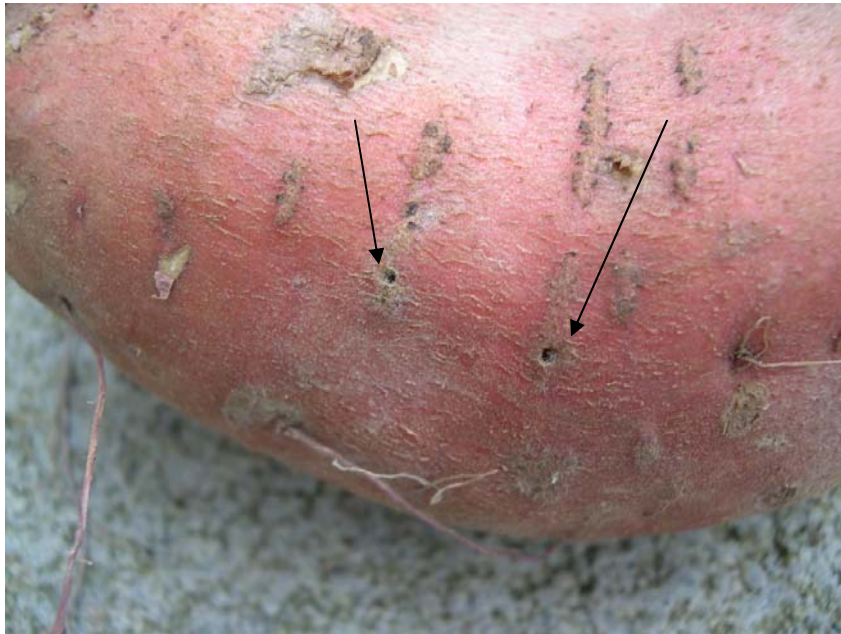


Figure 1.2. Pinhole scars on a sweetpotato root caused by *Systema* spp. larvae, according to the criteria of the USDA RAMP Southern Sweetpotato IPM Project.



Figure 1.3. Small-hole scars on a sweetpotato root caused by either banded or spotted cucumber beetle larvae (*Diabrotica balteata* and *D. undecimpunctata howardi*, respectively), according to the criteria of the USDA RAMP Southern Sweetpotato IPM Project.



Figure 1.4. Old damage that has stretched with the growth of the sweetpotato root making it difficult to identify which insect caused the damage.

Some scientists have simply combined the three hole types of scarring and created a scar type called WDS (wireworm, *Diabrotica*, *Systema*) (Cuthbert and Davis 1971; Schalk et al. 1986b; Schalk et al. 1991a; Schalk et al. 1993). This however is not very practical when considering insect management in the crop. In Mississippi wireworms are less of a problem than the *Diabrotica* and *Systema* species found in the sweetpotato fields. Adult wireworms (click beetles) are rarely captured in sweep-net sampling unlike the cucumber beetle and flea beetle species. In addition, the authors are more confident of correctly identifying the large, deep-hole damage associated with wireworm than correctly distinguishing between small-hole and pinhole damage. Table 1.1 shows the percentage of approximately 40,000 roots damaged, from fields that were part of the Mississippi portion of the Southern Sweetpotato IPM Project, by each of the common root-feeding pests, and Table 1.2 shows the estimated income losses caused by each insect pest (Reed and Fleming, unpublished data). The data in Table 1.2 has been adjusted to take into account that the USDA allows up to 10% of the roots to be damaged without incurring a loss in grading (USDA 1963). These numbers may not be truly representative of insect damage losses. As much as 50% of scars, especially from the WDS complex, could be overlooked (Cuthbert and Jones 1978).

Table 1.1. Percentage of roots damaged in 25 root samples taken between 2004 and 2007 according to each damage type based on results and criteria of the USDA RAMP Southern Sweetpotato IPM Project.

	Mean % of roots damaged	Minimum	Maximum
Small holes (cucumber beetle larvae)	12.7 ± 0.5	0%	100%
Pinholes (<i>Systema flea beetle</i> larvae)	7.0 ± 0.3	0%	80%
Large/deep holes (Wireworms)	6.7 ± 0.3	0%	80%
Shallow/smooth gouges (White grubs)	2.5 ± 0.1	0%	40%
Tracks (Sweetpotato flea beetle larvae)	2.3 ± 0.1	0%	56%
Narrow/winding gouges (White-fringed beetle larvae)	2.1 ± 0.2	0%	60%
Deep/rough gouges (Sugarcane beetles)	1.4 ± 0.1	0%	76%
Total damaged roots	32.2 ± 1.1	0%	100%

Table 1.2. Estimated income losses of USDA grades No. 1 and No. 2 roots from each sweet potato insect pest in Mississippi per acre after adjusting for USDA grading requirements based on data between 2004 and 2007 from the USDA RAMP Southern Sweetpotato IPM Project.

Damage Type	Frequency of more than 10% of roots damaged	*Range of possible loss (in \$/ac)
Small holes (cucumber beetle larvae)	45.6 ± 2.6	0 - total loss
Large/deep holes (wireworms)	24.5 ± 2.3	0 - 2215
Pinholes (<i>Systema</i> flea beetle larvae)	23.6 ± 2.2	0 - 2215
Narrow/winding gouges (white-fringed beetle larvae)	6.3 ± 1.3	0 - 1900
Tracks (sweetpotato flea beetle larvae)	5.7 ± 1.2	0 - 1770
Shallow/smooth gouges (white grubs)	4.9 ± 1.1	0 - 1265
Deep/rough gouges (sugarcane beetles)	2.4 ± 0.8	0 - 2400
All damage	86.8 ± 1.8	0 - total loss

*Loss = X % x Yield x Price; X = Minimum or Maximum of roots damaged from Table 1.1 – 10%.

Sweetpotato Integrated Pest Management

Sweetpotatoes, like most crops, have been highly dependent on synthetic insecticides to manage insect pests. Insecticides are applied in sweetpotato fields by three different techniques; pre-plant-incorporated (PPI), lay-by-incorporated, and over-the-top foliar. PPI insecticides are applied to the soil and then incorporated before the slips are transplanted. They provide control of insect larvae that already exist in the soil, such as white grubs and wireworms, and may provide residual control of larvae that

emerge from eggs of foliar feeding adult insects later in the season, such as cucumber beetle larvae and flea beetle larvae. Lay-by-incorporated insecticide applications are applied and incorporated before vines cover the rows (approximately 30 DAP) and may be the same chemical used for the PPI. They provide the crop with potential help managing mid-to-late-season insects by lengthening the residual effects of the insecticides. Over-the-top foliar insecticides are applied to the foliage, often prophylactically, to manage foliage-feeding insects whose larval offspring may feed on roots.

Hybridizing sweetpotatoes for resistance to insect pests, diseases, and nematodes has been an important area of study, especially after the removal of chlorinated hydrocarbons for insect management (Jones et al. 1987). Researchers have developed hybrid sweetpotatoes, to use as an integrated pest management (IPM) tactic, that are resistant to individual or a complex of insect pests or diseases. These insect pests include cucumber beetle larvae, flea beetle larvae, wireworms, white grubs, and sweetpotato weevils (Cockerham and Deen 1947; Cuthbert and Davis 1970; Cuthbert and Davis 1971; Cuthbert and Jones 1972; Jones et al. 1976; Cuthbert and Jones 1978; Waddill and Conover 1978; Rolston et al. 1979; Jones et al. 1980; Mullen et al. 1980; Rolston et al. 1981; Mullen et al. 1982; Jones et al. 1983; Schalk 1984; Hamilton et al. 1985; Jones et al. 1985; Schalk et al. 1986a; Jones et al. 1987; Schalk and Creighton 1989; Schalk et al. 1991a; Mao et al. 2001). Though this has been considered a successful management tactic, cucumber beetle larvae are still considered an important pest of sweetpotatoes. No

other integrated pest management tactic, such as, using predatory insects, sterile insect release, or transgenics, has been recorded for use against SCB as a pest of sweetpotatoes.

An important aspect of integrated pest management (IPM) is the use of thresholds to help determine if a pest density has reached a level that requires a pesticide application. The current threshold for SCB in Mississippi sweetpotato fields is two adults per 100 sweeps with a sweep-net (Catchot 2008). This threshold is based on data for banded cucumber beetle. Feeding of SCB larvae on sweetpotato roots has not been verified.

Trap Cropping Considerations in Sweetpotatoes

What It Is and What Has Been Done

Another IPM tactic that has not been utilized in sweetpotatoes is trap cropping. Trap cropping is a type of IPM cultural control. It has recently gained attention because of the public's concerns about pesticide dangers, insect resistance, and expenses (Shelton and Badenes-Perez 2006). Shelton and Badenes-Perez (2006) defined trap cropping as the use of plants that are deployed to attract, intercept, retain, and/or reduce targeted insects or the pathogens they vector in order to reduce the damage to the main crop. Trap cropping has been used on many agricultural crops with a variety of trap crop species. Trap cropping can be a successful management tactic because insects have preferences for certain plant species or cultivars (Caldwell et al. 2006). Trap crops reduce pest numbers in main crops by attracting them to the trap crop. In addition, they concentrate

the pests in an area in which they can be easily managed. Though trap cropping involves sacrificing acreage for producing a crop that may have no value other than to attract a pest, it prevents damage to the main crop. There are several other advantages to using a trap crop. First, some can be manipulated to attract an insect at a certain time or to a specific area of the field. Second, the trap crop may be able to withstand the pest and require no management to keep the trap crop alive. Third, the trap crop may be an area where beneficial organisms can build a population to naturally control the pest. Finally, the trap crop may reduce the acreage that require insecticide applications by treating only the trap crop (Hokkanen 1991; Caldwell et al. 2006).

There are a few things to consider when implementing a trap crop. Crops are attacked by multiple insect pests, and a trap crop may only be attractive to one pest. The cost of chemical control may be cheaper than setting aside acreage to plant certain trap crops. Trap crops may have different agronomic needs than the main crop. Finally, there are limitations for researchers because there are few companies or organizations willing to give money for trap crop research (Shelton and Badenes-Perez 2006).

Many considerations about the trap crop, insect to be controlled, and main cash crop must be taken into account before choosing to plant a trap crop. The feeding and oviposition sites of the pest, movement patterns of the insect, what insect stage is targeted, how mobile the insect pest is, the insect's host selection behavior, spatial layout of the trap crop, proportion of acreage to be used for the trap crop, and the fate of insects attracted to the trap crop all need to be taken into account (Shelton and Badenes-Perez 2006). The mode of trap crop is also something to consider. Shelton and Badenes-Perez

(2006) defined eight trap cropping modes: 1) Conventional trap cropping simply involves planting a trap crop next to a main crop to attract a pest to the trap crop which serves as a feeding or oviposition site. 2) Dead end trap cropping involves planting a trap crop species that is attractive for oviposition, but that does not support larval survival. 3) Genetically engineered trap cropping involves planting a trap crop that is highly attractive to a pest but has genes in it, such as *Bacillus thuringiensis* (Bt), that kill the pest. 4) Perimeter trap cropping involves planting trap crops entirely around the field early in the season to attract pests as they enter the field from their overwintering sites. 5) Sequential trap cropping involves planting a trap crop at specific time intervals to keep the trap crop as attractive as possible to the pests. 6) Multiple trap cropping involves planting multiple species of trap crops to be attractive to one or more pests. 7) Push-pull trap cropping involves planting a trap crop to attract the pest and planting an additional repellent intercrop to repel the pest. 8) Semiochemical assisted trap cropping involves using chemicals, such as pheromones or kairomones, to enhance the attractiveness of the trap crop.

Trap crops may also have promising economic benefits. They may help diversify a farm and provide an additional source of income if the trap crop is a marketable product. In addition, since trap crops attract the insect pest to a smaller area, insecticide can be applied to the acreage of the trap crop only, thus saving the farmer insecticide costs. An average increase in net profits of 10-30% overall has been shown in trap cropping research. Most of this is from reduced insecticide use and/or reduced pest attack (Hokkanen 1991).

Javaid and Joshi (1995) cite trap crops being used to manage pests in cotton, soybeans, corn, rice, sorghum, and many other crops. Pests such as the boll weevil (*Anthonomus grandis*) in cotton (Mally 1901; Scott et al. 1974), *Helicoverpa* spp. in cotton (Laster and Furr 1972; Pair et al. 1982), Mexican bean beetle (*Epilachna varivestis*) in soybeans (List 1921; Rust 1977; McPherson 1983), European corn borer (*Ostrinia nubilalis*) (Derridj et al. 1988) and corn rootworm (*Diabrotica virgifera virgifera*) (Hill and Mayo 1974) in corn, fall armyworm (*Spodoptera frugiperda*) in sorghum (Castro et al. 1988), and many others have been studied for their potential to be managed using trap crops. Although cucumber beetles are attracted to cucurbits, to the author's knowledge no in-depth work has been done utilizing cucurbit plants as a trap crop in sweetpotatoes.

Though trap crops have been shown to be a successful integrated pest management tactic in some cases, there are also many areas of concern when using trap crops. Economic, agronomic, ecological, and environmental considerations must be taken into account when considering using a trap crop. The cost of using insecticides is often lower than the added cost of implementing a trap crop (Shelton and Badenes-Perez 2006). Trap crops may have different agronomic needs than the main crop, such as different planting dates or fertilizer requirements (Shelton and Badenes-Perez 2006). An ecological danger with trap crops is that they may harbor or act as a breeding ground for some insects that may harm the main crop (Hokkanen 1991; Shelton and Badenes-Perez 2006). Another ecological danger is that the trap crop may attract more insect pests to the field than would have normally existed (Hokkanen 1991). If a trap crop is successful

at aggregating a pest within it and pesticides are regularly used to reduce the pest numbers, this facilitates insecticide resistance selection within a species (Hokkanen 1991). Finally, if natural enemies of the pest being attracted to the trap crop aggregate to their host they may also be harmed by the insecticides (Hokkanen 1991).

Cucurbits and Cucurbitacins

One plant family extensively studied for use as a trap crop is the cucurbit plant family (Cucurbitaceae). This is a moderately large plant family with approximately 900 species in at least 100 genera (Metcalf et al. 1980; Metcalf 1985). These include commonly cultivated plants such as; watermelon (*Citrullus lanatus* (Thunb.)), cucumber (*Cucumis sativus* L.), squash (*Cucurbita pepo* L. and *C. moschata* (Duchesne)), and pumpkin (*Cucurbita maxima* Duchesne). Cucurbits are known to attract *Diabrotica* spp. adults (Chambliss and Jones 1966a; Da Costa and Jones 1971; Howe and Rhodes 1976; Howe et al. 1976; Metcalf 1979; Metcalf et al. 1980; Ferguson et al. 1983; Schroder et al. 2001) and larvae (Deheer and Tallamy 1991).

Cucurbits are attractive to diabroticite beetles because they contain chemicals called cucurbitacins. Cucurbitacins represent more than 20 bitter, toxic, nonvolatile, oxygenated, tetracyclic triterpenes that are biosynthesized in plants and act as powerful arrestants and feeding stimulants for diabroticite beetles (Rehm et al. 1957; Rehm and Wessels 1957; Enslin and Rehm 1958; Chambliss and Jones 1966a; Chambliss and Jones 1966b; Da Costa and Jones 1971; Sharma and Hall 1971; Howe et al. 1976; Metcalf 1979; Metcalf et al. 1980; Rhodes et al. 1980; Ferguson et al. 1983; Metcalf 1986;

Metcalf et al. 1987; Tallamy and Krischik 1989). They occur in the fruit, roots, cotyledons, stems, leaves, and seeds of many cucurbit species (Rehm et al. 1957; Metcalf et al. 1982; Metcalf 1986). The cucurbitacins act as a deterrent to many organisms by being bitter and sometimes toxic (Quin 1928; Rimington 1933; David and Vallance 1955; Nielsen et al. 1977; Gould 1978; Metcalf et al. 1980; Tallamy and Krischik 1989) (reviews in Watt and Breyer-Brandwijk 1962). Diabroticite beetles detect the cucurbitacins in nanogram quantities with sensilla basiconica (Metcalf 1986), located on their maxillary palpi (Metcalf et al. 1980; Metcalf et al. 1987; Halaweish 1993).

It was once hypothesized that cucurbitacins act as a defense mechanism for diabroticite beetles against birds and other predators (Howe et al. 1976). Since then cucurbitacins have been shown to be used by female diabroticites as a defense mechanism in their eggs to deter egg predators (Ferguson et al. 1985) and by the larvae as a defense against ants. Also, Chinese preying mantids have been shown to be less attracted to diabroticite beetles that had eaten cucurbitacin containing foods (Ferguson and Metcalf 1985). It was found that diabroticite adults can sequester cucurbitacins in the hemolymph and can subsequently secrete hemolymph from the tibiofemoral intersegmental areas on the legs and from the buccal area when stimulated (Andersen et al. 1988). Sequestered cucurbitacins are also now believed to be a defense against disease as well (Tallamy et al. 1998). Male SCB pass sequestered cucurbitacins to females through their spermatophore (Tallamy et al. 2000). These unique ecological relationships between diabroticite beetles and cucurbitacins suggest that cucurbits have a great potential for use as a trap crop in sweetpotatoes. The SCB and BCB are two

diabroticite insects for which cucurbit trap crops might be a management possibility in sweetpotatoes.

Plant Volatiles

Since cucurbitacins are not long-range, volatile attractants, they must be accompanied by other compounds found in cucurbit plants to be attractive (Howe et al. 1976; Branson and Guss 1983). According to Metcalf (1987) there are 50,000 to 100,000 secondary plant compounds, many of which may be plant volatiles. These compounds fall into many categories of chemical structures: alkaloids, terpenoids, propanoids, flavanoids, quinones, polyacetylenes, and amino acids, most of which have no known physiological importance to the plant (Metcalf 1987). Metcalf's suggestion is that these compounds provide a rich "menu" to attract insects to the plants to find their food, oviposition sites, or shelter. He also states that the volatiles are released from plants through osmophores of flowers and glandular trichomes.

Howe et al. (1976) were some of the first researchers to hypothesize that diabroticite beetles needed more than cucurbitacins to be attracted to cucurbits. Their hypothesis was that cucurbitacins acted only as feeding stimulants and arrestants, but that unidentified volatile compounds attracted the beetles to the plants. Kairomones are chemicals that act as long-range volatile attractants (Metcalf and Lampman 1989). Orientation and host selection behavior in adult *Diabrotica* spp. seems to be mediated by volatile attractants (Ladd et al. 1983; McAuslane et al. 1986; Andersen and Metcalf 1987; Lampman et al. 1987; Metcalf 1987; Metcalf and Lampman 1989). Eugenol, estragole,

cinnamaldehyde, indole, chavicol, cinnamyl alcohol, phenylacetaldehyde, veratrole, and trans-anethole are some volatile chemicals that have been shown to be attractive to diabroticite beetles (Ladd et al. 1983; Andersen and Metcalf 1986; Lampman and Metcalf 1987; Lampman et al. 1987; Jackson et al. 2005). In Cucurbitaceae, volatiles are released from the flowers (Andersen and Metcalf 1986; Lampman 1986; McAuslane et al. 1986; Andersen and Metcalf 1987; Lampman et al. 1987) and act to attract the beetles from long distances to feed on plants containing cucurbitacins (Andersen and Metcalf 1986; Metcalf and Lampman 1989). The beetles reach the plants and feed on flowers and pollen along with other parts of the plant, and in doing so they become beneficial to the plant, acting as pollinators (Fronk and Slater 1956).

Barbercheck and Warrick (1997) evaluated cucurbit trap crops in peanuts to show their effectiveness in managing SCB larvae (southern corn rootworm). Treatments were peanuts grown with a trap crop and treated either with chlorpyrifos or with nematodes, peanuts without a trap crop and treated with chlorpyrifos or nematodes, and peanuts as an untreated check. The trap crop was 'Blue Hubbard' squash (*Cucurbita maxima*), a commercial variety known to be highly attractive to *Diabrotica* spp. (Fisher et al. 1984). Trap crops were planted on rows 7 and 14 of the peanut plots. Chlorpyrifos was applied at the recommended rate of 2.24 kg/ha, and a parasitic nematode species was applied in some plots. The 1992 results showed a significantly higher yield in peanuts grown with a trap crop than without. The greatest yield difference was between the untreated check (lowest percentage of undamaged peanuts) and trap crop plots treated with chlorpyrifos (highest percentage of undamaged peanuts). In 1993, yields in chlorpyrifos treated plots

tended to be higher. This trend persisted in 1994 with the greatest difference being between the untreated check (lowest percentage of undamaged peanuts) and plots without trap crops treated with chlorpyrifos (highest percentage of undamaged peanuts). Results in 1994 also showed that peanut plants adjacent to the squash trap crop had a lower percentage of undamaged pods than plants 3 rows away. This showed that the squash trap crop tended to concentrate oviposition nearer to the trap crop (Barbercheck and Warrick 1997). This could give farmers the ability to spray only the area near the trap crop, reducing spray costs.

If cucurbit plants are highly attractive to SCB or BCB, they could be considered for use not only as a trap crop but also as a sentinel plant to detect the presence of SCB and BCB in sweetpotatoes. A cucurbit sentinel plant could be planted in small areas (<10 plants per area) in a sweet potato field and could help indicate when a SCB or BCB population reaches a level in the field at which time an insecticide application should be utilized to manage the pest (Reed, personal communication). This would give farmers an indication of when the pest exists in the field rather than applying insecticides preventively if the pest is not even in the field or is occurring in low numbers.

Biology and Ecology of *Diabrotica* spp. and Their Relationship with Sweetpotatoes

The SCB (Figure 1.5) is distributed in most of the United States east of the Rocky Mountains and is very abundant in the southeastern United States (Barbercheck and Warrick 1997). Spotted cucumber beetle larvae are suspected to be the primary cause of

damage to sweetpotatoes in the inland Carolinas (Jackson et al. 2005) where the temperatures are similar to Mississippi. However BCB larvae are considered a primary cause of damage to sweetpotato roots in Louisiana (Pitre and Kantack 1962) where the temperatures are warmer throughout the year. The distribution of the two species is probably determined by temperature (Krysan and Miller 1986) with SCB able to withstand cooler temperatures and BCB (Figure 1.6) not able to withstand extended periods of sub-freezing temperatures. However some evidence indicates the BCB may be acclimatizing to cooler weather (Elsey 1989). Both of these species exist in Mississippi sweetpotato fields with SCB making up approximately 90% of the *Diabrotica* spp. collected in sweetpotato fields from 2004 to 2007 (Reed and Fleming, unpublished data). The SCB is a common insect found in Mississippi and can be collected from most crops anywhere in the state. They overwinter in the adult stage and do not hibernate.



Figure 1.5. Spotted cucumber beetle (*Diabrotica undecimpunctata howardi*) adult.



Figure 1.6. Banded cucumber beetle (*Diabrotica balteata*) adult.

According to Elsey (1989), SCB uses short photophase (days <13 hours) and cool temperatures to invoke a mild reproductive diapause. Arant (1929) found oviposition to occur as early as January for the overwintered generation of females that had apparently mated in the fall. Females are heartier than males (Sell 1916; Arant 1929) and may comprise the majority of the SCB adults that survive overwintering (Arant 1929). Overwintered SCB adults become most active when temperatures reach 21° C (Metcalf and Metcalf 1993). Peak egg lay occurred in March in Alabama (Arant 1929). Eggs appear white or yellow-orange in color and are covered with hexagonal pits (Garman 1891; Isley 1929; Anonymous 2006) which act as air spaces or “lungs” in flooded environments (Jolivet et al. 1994). After copulating, females feed for an average of six days before ovipositing (Arant 1929). Eggs are deposited in crevices in the soil near the base of plants (Thomas 1912; Sweetman 1926; Arant 1929). Arant (1929) showed that females in a lab could deposit eggs 3 to 8 times with an average of about 45 eggs at each deposition and that the incubation period for the eggs ranged from 8 to 30 days at about 33° C and 16° C, respectively.

Larval development was found to be shorter at higher temperatures. Upon emergence from the egg, the larvae immediately move and feed (Sweetman 1926). Arant (1929) found that development from first instar larvae to pupae ranged from 16 to 29 days. The first instar ranged from 4 to 11 days, the second instar ranged from 5 to 10 days, and the third instar, which includes the pre-pupal period, ranged from 5 to 14 days.

The pupal stage follows the third instar and can require from 3 to 16 days. Pupae are white or yellow and generally occur at the base of plants. The adults emerge from the

pupae and immediately begin to feed. This gives the total life cycle a range from 27 to 87 days. Arant (1929) found that in Alabama three generations per year occurred. The overwintering generation lays eggs from January to April and the larvae emerge as adults in late April or early May. Second generation eggs are laid in May and emerge as adults in June. Third generation eggs are laid in July and adults emerge in August or September to overwinter (Arant 1929). Sweetman (1926), Arant (1929), and Isley (1929) all basically agree on the life cycle of the SCB, with the only differences being on the number of generations due to geographical location.

Limiting Factors of the Spotted Cucumber Beetle as a Sweetpotato Pest

The ecology of the SCB, anatomy of sweetpotato roots, four years of research prior to this research, and uncertainty of distinguishing damage, have led to some questions concerning the amount of damage SCB larvae cause to sweetpotato roots. The SCB ecology includes a polyphagous nature (Quaintance 1900; Webster 1913; Sell 1916; Sweetman 1926; Arant 1929; Isley 1929; Metcalf 1987; Metcalf and Metcalf 1993; Eben and Barbercheck 1997; Eben et al. 1997; reviews in Jolivet et al. 1994), with an attractiveness to blossoms and an apparent need for pollen (Webster 1913; Sell 1916; Arant 1929; Isley 1929; Guss and Krysan 1973; Ludwig and Hill 1975; Fisher et al. 1984; Metcalf 1987; Necibi 1990; Jolivet et al. 1994; Eben et al. 1997; Hesler 1998), a need for moist, smooth-textured soil in the egg and larval stages (Chittenden 1905; Thomas 1912; Webster 1913; Arant 1929; Grayson 1947; Campbell and Emery 1967; Chalfant and Mitchell 1968; Turpin and Peters 1971; Krysan 1976; Lummus et al. 1983; Meinke 1984;

Brust 1989; Brust and House 1990), and a ratio of males to females in late season of about 12:1 (Fleming, personal observation). Common Mississippi crops such as corn (*Zea mays*) and soybeans (*Glycine max*) and common weeds such as pigweed (*Amaranthus* spp.) and morningglory (*Ipomoea* spp.) may be equally or more attractive than sweetpotatoes to SCB (Brust 1989). Sweetpotatoes do not produce many blossoms, which would make them a poor pollen source for SCB. Necibi (1990) found that, in cucurbit fields, cucumber beetles were still highly attracted to cucurbit blossoms even in areas of the field with many weedy host plants. These two findings could indicate SCB adults would leave a sweetpotato field to search for pollen and may lay eggs near their pollen source. The apparent need for moist, smooth textured soil in the egg and larval stages is important because those conditions are not always found in sweetpotato fields. Sweetpotatoes are typically grown on sandy soil (Swiader and Ware 2002), that is frequently dry and coarse textured, and therefore would not be a suitable site for SCB egg hatch or larval survival. After 1 August the number of males to females is 12:1 (Fleming, personal observation). During this time period sweetpotato roots are the most vulnerable to insect damage because most of the PPI insecticides have broken down and damage to roots during this time would not have time to heal before harvest. However, this ratio indicates that most of the cucumber beetles collected would be male and not capable of producing offspring to damage sweetpotato roots. All of these factors, individually or collectively, may have an effect on development and survival of SCB and may make sweetpotatoes an unsuitable or undesirable host for SCB.

The anatomy of the sweetpotato plant includes, as with many other plants, a latex substance that serves as a defense against injury and predation in some cases. This latex is found in special cells called laticifers (Data et al. 1996). The substance is immediately released when plants are cut or injured (Figure 1.7). In sweetpotatoes this latex is comprised of hexadecyl-, octadecyl-, and eicosyl-esters of p-coumaric acid (Data et al. 1996), and has been shown to slightly deter feeding and oviposition on sweetpotato plants by the sweetpotato weevil (*Cylas formicarius* (Fabricius)) (Data et al. 1996). A similar substance has also been shown to “gum-up” the mandibles of the milkweed borer feeding on milkweed (Dussourd and Eisner 1987). The latex substance in sweetpotato could potentially deter cucumber beetle larvae by “gumming up” their mouthparts and making the roots unpalatable. Sweetpotato root anatomy also includes fibrous roots that could be fed upon by SCB larvae (Cuthbert and Jones 1978). These factors could potentially reduce feeding by SCB larvae on the marketable, storage roots of sweetpotatoes.



Figure 1.7. White latex substance that may be a factor in limiting spotted cucumber beetle (*Diabrotica undecimpunctata howardi*) larval ability to feed on sweetpotato roots. Shown exuding from cut sweet potato root.

Another factor that is inconsistent with the important pest status of SCB larvae is the low number of adults present in sweetpotato fields. The mean number of SCB collected between 2004 and 2007 in commercial sweetpotato fields in Mississippi was 0.08 adults per 25 sweeps with a minimum of 0 and a maximum of 1.71 (Reed and Fleming, unpublished data). Other known sweetpotato root feeding pests causing similar damage were also found in Mississippi sweetpotato fields. These included the BCB at 0.01 adults per 25 sweeps, the red-headed flea beetle (*S. frontalis*) at 0.21 adults per 25 sweeps, the elongate flea beetle (*S. elongata*) at 0.02 adults per 25 sweeps and the pale-striped flea beetle (*S. blanda*) at 0.001 adults per 25 sweeps (Reed and Fleming,

unpublished data). The BCB, elongate flea beetle, and pale-striped flea beetle occur in fields in such low numbers that statistical analysis is difficult. However, the red-headed flea beetle occurs in relatively high numbers and the larvae cause damage that could be confused with cucumber beetle larval damage. Analysis of the mean number of SCB collected weekly in a total of 528 field plots over a four year period with scars on a total of 25 roots dug from each of those plots resulted in no correlation (Figure 1.8). However, similar correlation analyses of the number of red-headed flea beetles collected with pinhole scarring associated with flea beetle larval feeding (Figure 1.9) and small-hole scarring associated with cucumber beetle feeding (Figure 1.10) resulted in positive correlation. In addition, a correlation can be seen when combining the number of red-headed flea beetle adults and the number of SCB adults and the combination of pinhole and small-hole scarring (Figure 1.11). These data could indicate that the damage is being misidentified because of similarity in appearance, that red-headed flea beetle larvae are causing both pinhole and small-hole damage, or that the SCB larvae are not causing the small-hole damage and another insect or group of insects is causing the damage. Cuthbert and Reid (1965) show an image of a sweetpotato root with damage similar to small-hole damage, but indicate it was caused by a *Systema* spp. They also indicate that an anthicid beetle, *Notoxus calcaratus* Horn, caused damage similar to small-hole damage.

Mean no. of small hole scars = $0.358 + 0.0632 * \text{Mean no. of spotted cucumber beetle adults collected per 25 sweeps}$
 $r = 0.0071, p = 0.9600$

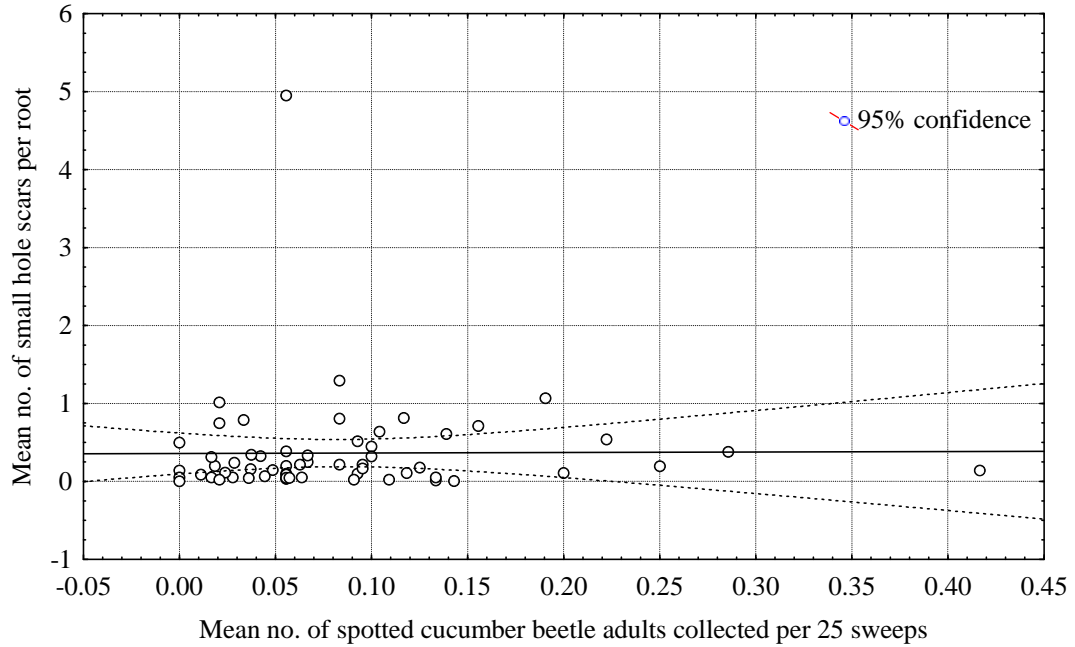


Figure 1.8. Correlation of the mean number of adult spotted cucumber beetles (*Diabrotica undecimpunctata howardi*) collected from 12 to 24 sample sites within each of 65 fields during 2004 to 2007 with the mean number of small-hole scars per root (Data from USDA RAMP Southern Sweetpotato IPM Project).

Mean no. of pinhole scars per root = $0.0815 + 0.3437 * \text{Mean no. of red-headed flea beetle adults collected per 25 sweeps}$
 $r = 0.4400, p = 0.0008$

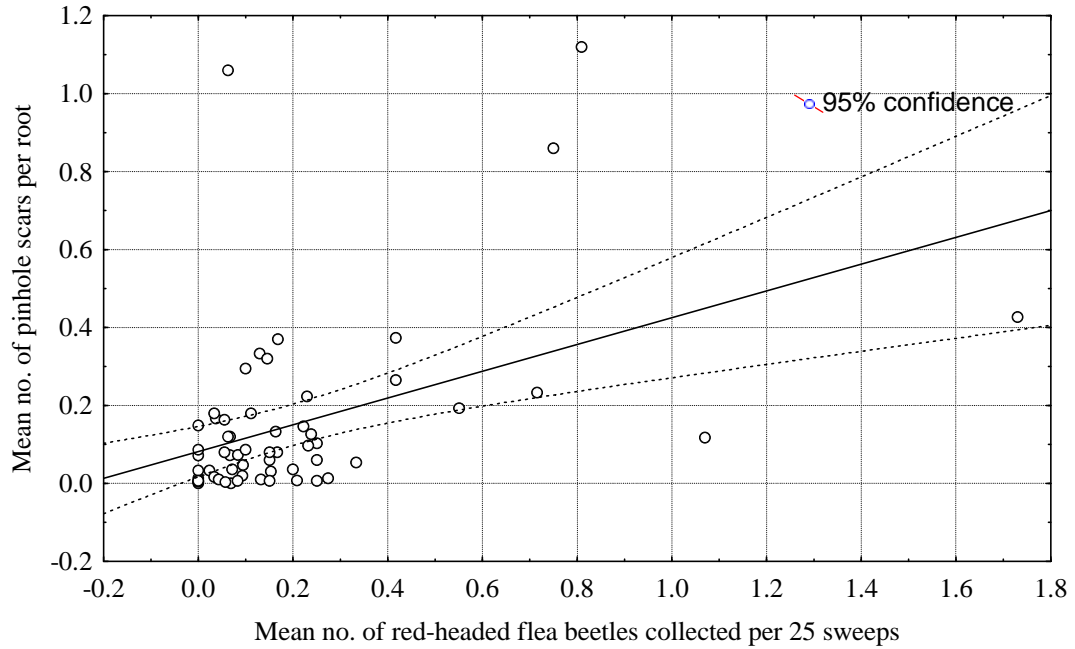


Figure 1.9. Correlation of the mean number of adult red-headed flea beetles (*Systema frontalis*) collected from 12 to 24 sample sites within each of 65 fields during 2004 to 2007 with the mean number of pinhole scars per root (Data from USDA RAMP Southern Sweetpotato IPM Project).

Mean no. of small hole scars per root = $0.19845 + 0.82052 * \text{Mean no. of red-headed flea beetle adults collected per 25 sweeps}$
 $r = 0.3587, p = 0.0050$

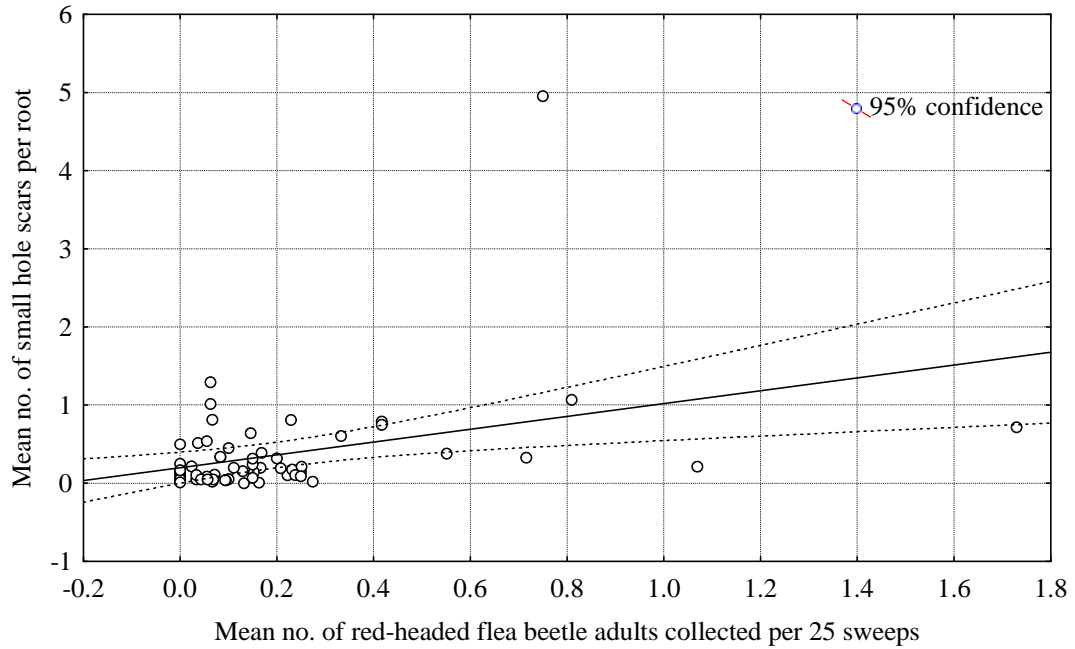


Figure 1.10. Correlation of the mean number of adult red-headed flea beetles (*Systema frontalis*) collected from 12 to 24 sample sites within each of 65 fields during 2004 to 2007 with the mean number of small-hole scars per root (Data from USDA RAMP Southern Sweetpotato IPM Project).

Mean no. of combined small hole and pinhole scars per root = $0.227 + 1.009 * \text{Mean no. of combined spotted cucumber beetle and red-headed flea beetle adults collected per 25 sweeps}$
 $r = 0.3800, p = 0.0024$

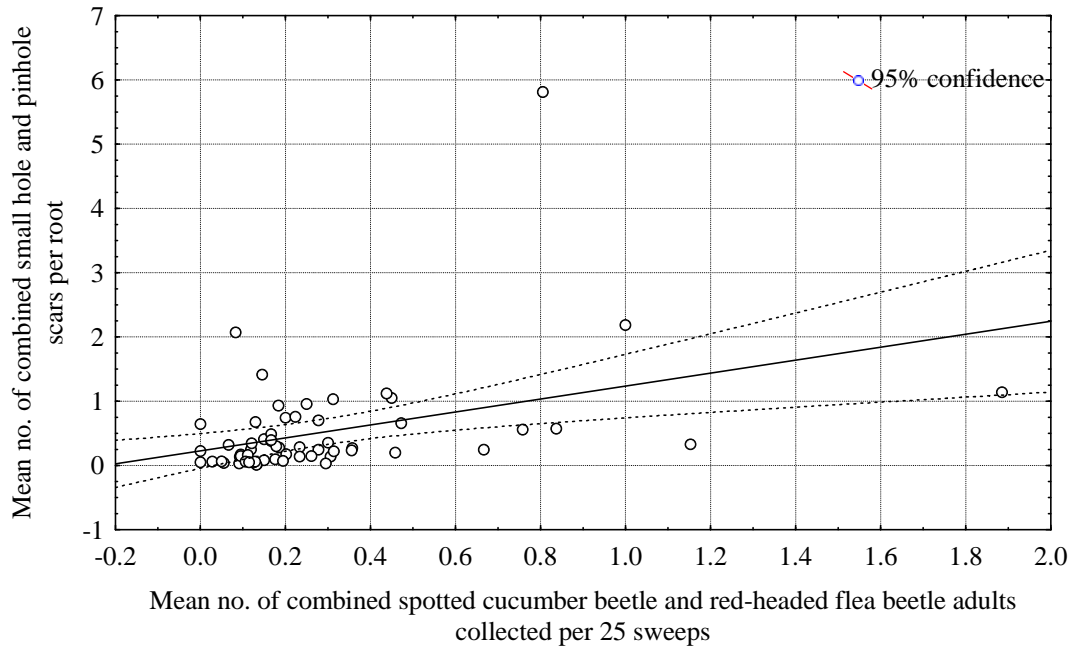


Figure 1.11. Correlation of the mean combined number of adult spotted cucumber beetles (*Diabrotica undecimpunctata howardi*) and adult red-headed flea beetles (*Systema frontalis*) from 12 to 24 sample sites within each of 65 fields during 2004 to 2007 with the mean combined number of small-hole and pinhole scars per root (Data from USDA RAMP Southern Sweetpotato IPM Project).

Objectives

The SCB remains a questionable pest of sweetpotatoes. Though assumed to be a pest, no evidence has been shown that proves the larvae feed on sweetpotato roots. Instead, information indicates it is unlikely that SCB larvae are a major pest of sweetpotato roots. In contrast, BCB larvae are known to feed on sweetpotato roots and are attracted to cucurbit plants. Management strategies for BCB and SCB need to be developed. While cucurbit trap crops appear to be a feasible management tactic, no research has examined this tactic in sweetpotatoes.

One objectives of this research will be to determine the status of SCB as a pest of sweetpotatoes. This will be accomplished by determining if the larvae of SCB will feed on sweetpotato roots, if they can survive on sweetpotato roots, and what factors may influence their ability to feed on sweetpotato roots and survive in a sweetpotato ecosystem in Mississippi. A second objective is to evaluate the possibility of using cucurbit plants as trap crop or sentinel plant to better manage *Diabrotica spp.* in sweetpotatoes.

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CHAPTER II
CUCURBIT PLANTS AS A TRAP CROP OR SENTINEL PLANTS FOR
DIABROTICA SPP. CUCUMBER BEETLES IN MISSISSIPPI
SWEETPOTATOES

Introduction

Cucurbit plants (Cucurbitaceae) and *Diabrotica* spp. cucumber beetles (Coleoptera: Chrysomelidae) share a unique ecological relationship. Cucurbits release kairomonal compounds that attract cucumber beetles. Volatile chemicals from the flowers are naturally released by the plants through osmophores and glandular trichomes (Metcalf 1987) and subsequently attract cucumber beetles (Lampman et al. 1987). Eugenol, estragole, cinnamaldehyde, indole, chavicol, cinnamyl alcohol, phenylacetaldehyde, veratrole, and trans-anethole are some volatile chemicals that have been shown to be attractive to diabroticite beetles (Ladd et al. 1983; Andersen and Metcalf 1986; Lampman and Metcalf 1987; Lampman et al. 1987; Jackson et al. 2005). The volatile chemicals act to attract cucumber beetles, and the cucurbitacins contained in cucurbit plants (Guha and Sen 1975) act as a feeding arrestant and stimulant to keep the beetles on the plant (Metcalf and Lampman 1989).

Cucurbitacins can be a deterrent for many organisms that might feed on cucurbit plants (Quin 1928; Rimington 1933; David and Vallance 1955; Nielsen et al. 1977;

Gould 1978; Metcalf et al. 1980; Tallamy and Krischik 1989). However, diabroticites feed on cucurbits and sequester cucurbitacins for use as a defense against predators (Howe et al. 1976) and disease (Tallamy et al. 1998). The beetles move from plant to plant especially in flowers and act as pollinators for the plants (Fronk and Slater 1956). This relationship has been the focus of several studies utilizing cucurbit plants as trap crops (Barbercheck and Warrick 1997; Boucher and Durgy 2003). Cucurbitacins have been used as toxic baits to manage cucumber beetles (Brust and Foster 1995; Schroder et al. 1998) and in traps to monitor cucumber beetle densities (Shaw et al. 1984). The tactics have been documented for use in various crops, but have never been implemented as a management tactic for sweetpotatoes.

Cucurbits can be broken down into two general categories: bitter and non-bitter. Many commercial cucurbits such as summer squash (*Cucurbita pepo* L.), watermelons (*Citrullus lanatus* (Thunb.)), and cantaloupes (*Cucumis melo* Naudin.) are categorized as non-bitter. Most of the species of wild cucurbits are categorized as bitter and have not been commercialized. However, *Mamordica charantia* Descourt. (bitter melon) and *Lagenaria siceraria* (Molina) (bottle gourd) are two commercial varieties that are categorized as bitter.

The SCB (Figure 2.1) is distributed in most of the United States east of the Rocky Mountains and is very abundant in the southeastern United States (Barbercheck and Warrick 1997). It is suspected to be the primary cause of damage to sweetpotatoes in the inland Carolinas (Jackson et al. 2005) where the temperatures are similar to Mississippi. BCB larvae are considered a primary cause of damage to sweetpotato roots in Louisiana

(Pitre and Kantack 1962) where the temperatures are warmer throughout the year. The distribution of the two species is probably determined by temperature (Krysan and Miller 1986) with SCB able to withstand cooler temperatures and BCB (Figure 2.2) not able to withstand extended periods of sub-freezing temperatures. However there is evidence now that indicates BCB may be acclimatizing to cooler weather (Else 1989). The SCB is a common insect found in Mississippi and can be collected from many crops anywhere in the state.



Figure 2.1. Spotted cucumber beetle (*Diabrotica undecimpunctata howardi*) adult.



Figure 2.2. Banded cucumber beetle (*Diabrotica balteata*) adult.

Cucumber beetle larvae are believed to be important pests of sweetpotatoes in Mississippi. Banded cucumber beetle larvae cause damage that appears as small, round holes 1 to 3 mm in diameter (Pitre and Kantack 1962) (Figure 2.3). It is assumed that SCB larval damage is similar to BCB larval damage, however, no data have been found that indicate SCB larvae feed on sweetpotatoes. Both the BCB and SCB exist in Mississippi sweetpotato fields with SCB making up approximately 90% of the *Diabrotica* spp. collected in sweetpotato fields from 2004 to 2007 in northeastern Mississippi (Reed and Fleming, unpublished data). Scars considered to be from cucumber beetle larval feeding can be found on approximately 13% of sweetpotato roots in Mississippi (Reed and Fleming, unpublished data).



Figure 2.3. Small-hole scars on a sweetpotato root caused by either banded or spotted cucumber beetle larvae (*Diabrotica balteata* and *D. undecimpunctata howardi*, respectively), according to the criteria of the USDA RAMP Southern Sweetpotato IPM Project.

Cucumber beetles in sweetpotato fields are typically managed by the use of insecticides. Growers may apply insecticides prophylactically or when a population of insect pests is found in a field. Cucurbit plants could be used as a trap crop or as sentinel plants to help reduce or eliminate insecticide applications in sweetpotato fields. As a trap crop they may have the potential to aggregate cucumber beetles into a smaller area of the field, which could be sprayed with an insecticide, thus reducing the number of acres requiring insecticide applications. If a cucurbit plant is highly attractive to cucumber beetles it could serve as a sentinel plant to indicate if a population of cucumber beetles

reaches a density that requires an insecticide treatment. Sentinel plots are a concept that was implemented to monitor the spread of soybean rust, a fungal pathogen, in the United States. These plots were planted in various states and monitored to determine if soybean rust had spread to those areas. They served as an early warning system so farmers could apply prophylactic fungicide treatments if soybean rust appeared in the area. Cucurbit plants in sweetpotato fields could be used as a sentinel plant to detect populations of cucumber beetles.

Materials and Methods

Trap Crop Studies

An experiment was conducted in 2006 to determine if cucurbit plants would act as a trap crop for cucumber beetles in a sweetpotato field. A four row strip at the eastern side of a field at the Mississippi State University Plant Science Research Farm, Mississippi State, MS was transplanted with watermelon, squash, and cantaloupe plants on two dates; 11 April and 21 April, with a vegetable trans-planter on hipped rows approximately 2 m apart and spaced 1 m apart on the row. Squash was planted in the northern one-third of the field, followed by cantaloupe, then watermelon in the southern one-third of the field. Sweetpotatoes were transplanted in the adjacent rows on 6 July. Insect counts were taken on 25 plants from both watermelon and cantaloupe on a weekly basis for 16 weeks and on squash for 14 weeks. Cucurbit plants were disked under on 25 July and sweep-net sampling was conducted in the adjacent rows of sweetpotatoes to

observe movement of cucumber beetles into sweetpotatoes. Three sweep-net samples each of 150 sweeps were taken at three sites on each of rows one, ten, and nineteen adjacent to each of the three cucurbit crops on four dates, one pre-disking and three post-disking. Statistical analysis was conducted to separate mean results of experiments using Statistica data analysis software system (Statsoft, Inc. www.statsoft.com) and the general linear model. Homogeneity of variance was determined using the Cochran C test.

Sentinel Plant Studies

An experiment was conducted in 2007 to determine the feasibility of using cucurbit plants as sentinel plants for cucumber beetle adult populations in sweetpotato fields. Five commercial sweetpotato fields were chosen in the north-central Mississippi counties of Chickasaw, Calhoun, and Webster. The cucurbit plants were hand-transplanted in the fields within one week after the sweetpotato slips were transplanted. Sticky cards, 7.6 x 12.7 cm, were immediately placed in the fields for sampling. Cucurbit plants were transplanted into fields 1, 2, and 3 on 29 May, field 4 on 25 June, and field 5 on 16 July. Approximately 2 m of row were cleared of sweetpotato plants for transplanting in each plot. Cucurbit plants were then transplanted approximately 40 cm apart in the cleared part of the row. If field conditions were dry, the transplants were irrigated at planting by applying 1.5 liters of water to the transplant. Two species of bitter cucurbit plants were used in this study, a bottle gourd (*Lagenaria siceraria* (Molina)) and a bitter melon (*Momordica charantia* Descourt.). Bitter cucurbits were chosen to maximize the potential attractiveness to cucumber beetles (Eben et al. 1997),

and if data indicated they were successful at attracting cucumber beetles, then non-bitter, commercially marketable varieties would be chosen for the second year of the study.

These particular species were suggested for use because of the availability of the seeds, the potential of being sold as a food product in the case of bitter melon or as a decorative product in the case of bottle gourd, and their inability to become established as a weed (personal communication Dr. David Nagel). Bottle gourd was planted in field 1 and 4, bitter melon was planted in field 2 and 5, and both species were planted in field 3.

Three treatments were used in each field: edge (EDGE), mid-field (MID) and an untreated check (UTC). In the EDGE treatment the cucurbits were planted near the edge of the field, in the MID treatment the cucurbits were planted near the middle of the field, and in the UTC treatment no cucurbit plants were planted (Figure 2.4). EDGE treatments were planted to see if there was any edge-effect since cucurbit plants on the edge of field might be more discernable to insects entering the field from hosts at the edge of the field. MID treatments were planted to look at effects in two directions from the trap crop. Plots in each treatment were spaced approximately 50 m apart within a row and treatments were placed 75 rows (approximately 75 m) apart from center to center (Figure 2.4). There were three replicates for EDGE and MID treatments and only one replicate of UTC in each field (Figure 2.4). Each plot of EDGE consisted of 5 cucurbit plants (row 0) and sample sites on row 0, two rows from the cucurbit plants (row 2), four rows from the cucurbit plants (row 4), eight rows from the cucurbit plants (row 8), fifteen rows from the cucurbit plants (row 15), and twenty-five rows from the cucurbit plants (row 25) (Figure 2.4). MID was similar, however, sample sites were located bidirectionally (Figure 2.4).

The untreated check was similar to EDGE with the exception of row 0 having been planted only with sweetpotato plants (Figure 2.4). In field 3, separate plots of each species of cucurbit were planted in alternate locations within the field so that there were six plots in each treatment.

Sticky card and sweep-net samples were taken in each field throughout the season. Sticky cards were used as the primary mode of sampling since they could collect insects night and day throughout all environmental conditions. Sticky cards were 7.6 x 12.7 cm with adhesive on both sides and yellow in color. Fields were sampled every one or two weeks. Fields 1, 2, and 3 were sampled on seven dates with sticky cards and on two dates with a sweep-net, Field 4 was sampled on six dates with sticky cards and on two dates with a sweep-net, and Field 5 was sampled on three dates with sticky cards and on one date with a sweep-net. Sampling in Field 5 was reduced due to its late planting date, severe drought, and reduced stand of both sweetpotato and cucurbit plants. Sticky card data for all fields were reported as insects per day by dividing the number of insects by the number of days the cards were left in the field.

Roots were harvested by hand in each field. Field 1 was harvested on 4 September, Field 2 on 21 August, Field 3 on 28 August, Field 4 on 11 September, and Field 5 on 25 September. Twenty-five marketable roots from each sample-site were harvested and evaluated for insect damage. Evaluation of insect damage was based on criteria (Table 2.1) established for the research project called the USDA RAMP Southern Sweetpotato IPM Project (USDA-RAMP agreement #2003-51101-02106). In Field 3, rows 0 and 8 of EDGE had been harvested by the grower before we harvested. Statistical

analysis was conducted to separate mean results of experiments using Statistica data analysis software system (Statsoft, Inc. www.statsoft.com) and the general linear model.

Homogeneity of variance was determined using the Cochran C test.

Table 2.1. Partial criteria for grading sweetpotato roots for insect damage as established for the USDA RAMP Southern Sweetpotato IPM Project, as used in this study.

Insect	Type damage	Damage description
<i>Diabrotica</i> spp. larvae	small-hole	round holes 1-3 mm wide, sometimes clumped on root surface, sometimes with irregular shaped cavities underneath
<i>Systema</i> spp. larvae	pinhole	<1 mm wide
Wireworms	large/deep hole	deep, round holes >3 mm wide, sometimes with enlarged cavities underneath
Sweetpotato flea beetle larvae	Narrow track	narrow, winding channels 1-2 mm wide

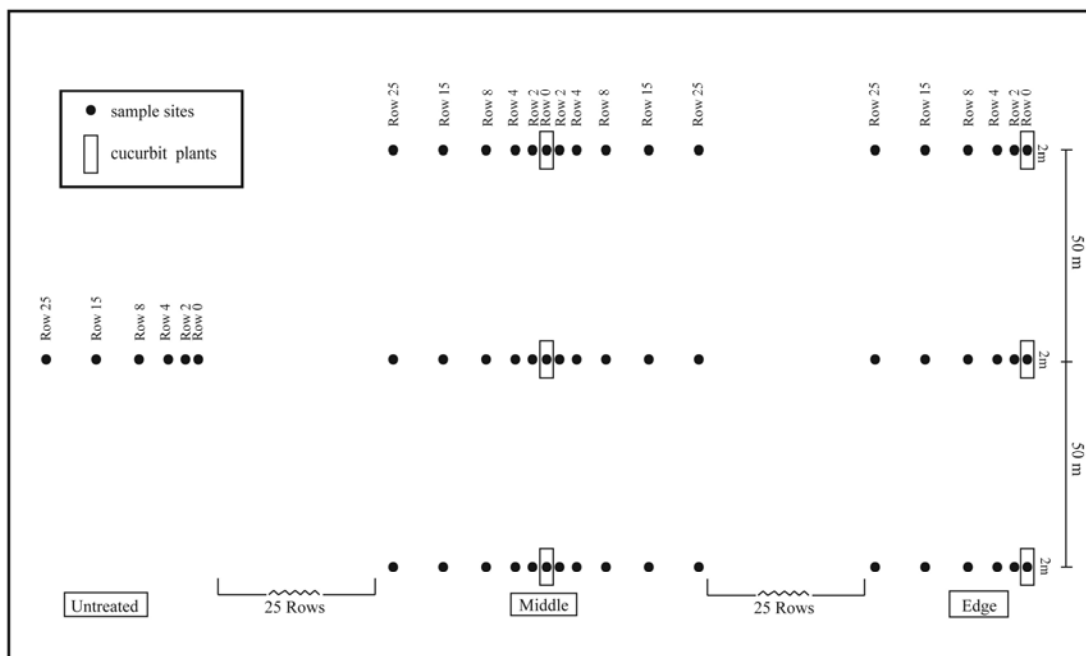


Figure 2.4. Spatial layout of treatments and sample sites in trap crop study.

Results

Trap Crop Studies

Visual sampling of cucurbit plants throughout the season showed watermelon and squash plants to be more attractive than cantaloupe to SCB (Table 2.2). BCB was attracted to watermelon more than cantaloupe, and squash was not significantly different from cantaloupe or watermelon (Table 2.2). Numbers of cucumber beetles in the sweetpotatoes at time of disking were too low for statistical analyses. The number of cucumber beetles found in the sweetpotatoes next to the watermelons in relation to the number of rows from the watermelons and sampling date can be seen in Table 2.3. Only one cucumber beetle was found in the sweetpotatoes prior to disking of the watermelons. Cantaloupe and squash plants had begun dying in early July and by time of disking were host to very few cucumber beetles.

Table 2.2. Season-long mean \pm SE of spotted and banded cucumber beetles (*Diabrotica undecimpunctata howardi* and *D. balteata*, respectively) counted in visual sampling of 25 plants in squash, cantaloupe, and watermelon trap crop bordering sweetpotatoes.

Crop	Mean number of insects	
	Spotted cucumber beetles	Banded cucumber beetles*
Squash	0.62 \pm 0.11 b	0.05 \pm 0.03 ab
Cantaloupe	0.23 \pm 0.06 a	0.02 \pm 0.01 a
Watermelon	0.57 \pm 0.10 b	0.06 \pm 0.03 b
Prob F	<0.01	0.04

Means not sharing a common letter differ significantly (Fisher's LSD; $p=0.05$).

*Assumptions for homogeneity of variance were not met according to the Cochran C test.

Table 2.3. Number of cucumber beetles collected in rows parallel to watermelon trap crop.

Date	No. of rows from watermelon trap crop	Number of insects per 150 sweeps	
		Spotted cucumber beetles	Banded cucumber beetles
7/26/2006*	1	0	1
7/26/2006	10	0	0
7/26/2006	19	0	0
7/28/2006	1	0	5
7/28/2006	10	1	2
7/28/2006	19	0	0
8/4/2006	1	1	4
8/4/2006	10	3	1
8/4/2006	19	0	0
8/11/2006	1	1	4
8/11/2006	10	0	4
8/11/2006	19	0	1

* Trap crop destroyed after insect counts on this date

Sentinel Plant Studies

The number of cucumber beetles found in these trials was low, probably as a result of a severe drought throughout the growing season that could have affected egg and larval survival of SCB. Figure 2.5 shows the weekly means of insects collected on sticky cards through the season. The effect of treatment (EDGE or MID) had no effect on the distribution of insects based on sample site (number of rows from the trap crop) for sticky card samples ($p=0.943$) or sweep-net samples ($p=0.475$). Since there was no significant interaction treatment and row sampled, data for EDGE and MID were combined. The distribution of adult insects collected on sticky cards in each field at each sample site can be seen in Figure 2.6 and Figure 2.7. Lady beetles were the most common insects collected on sticky cards. Distance from sentinel plants appeared to have very little impact on the distribution of adult insects. The number of adult insects collected in sweep-net sampling in each field at each sample site can be seen in Figure 2.8 and Figure 2.9. The effect of row in sweep-net sampling was not significant for any insect. There were no trends for the number of adult insects relative to distance from cucurbits in the untreated checks (Table 2.4). These data indicate that the density of lady beetles (LB), twelve spotted cucumber beetles (SCB), *Systema* flea beetles (SFB), and click beetles (CB) varied based on distance from the gourd plants ($p \leq 0.1$) (Table 2.5), whereas only LB and sweet potato flea beetles (SPFB) densities were correlated to distance from the melon species ($p \leq 0.1$) (Table 2.6).

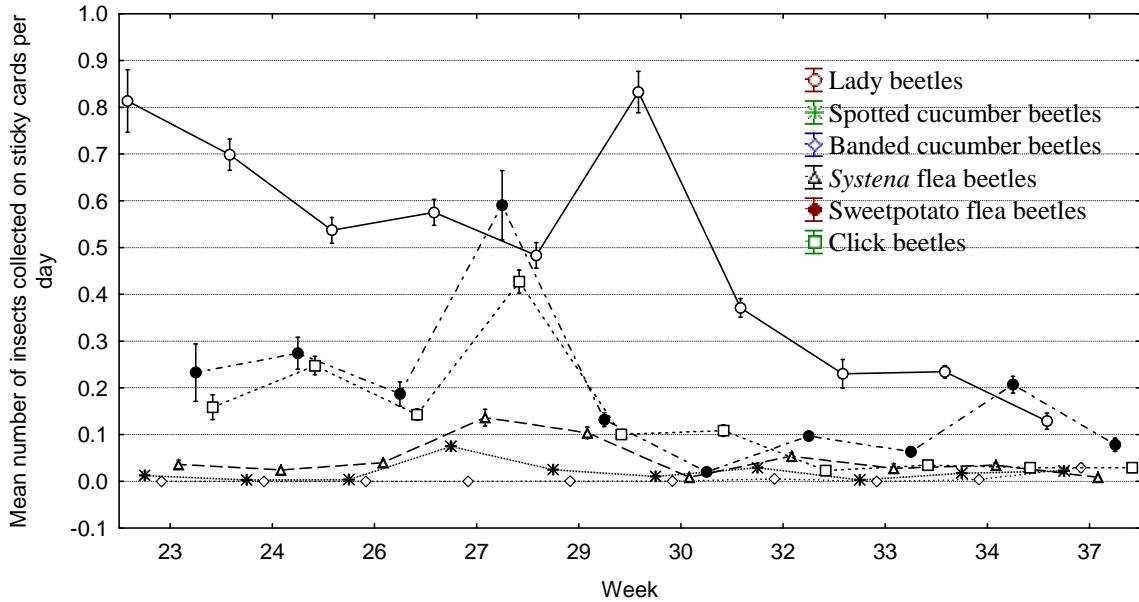


Figure 2.5. Weekly mean density \pm SE of insects in sentinel plots with bitter cucurbits represented as mean insects per sticky card divided by the number of days the card was left in the field (insects per day).

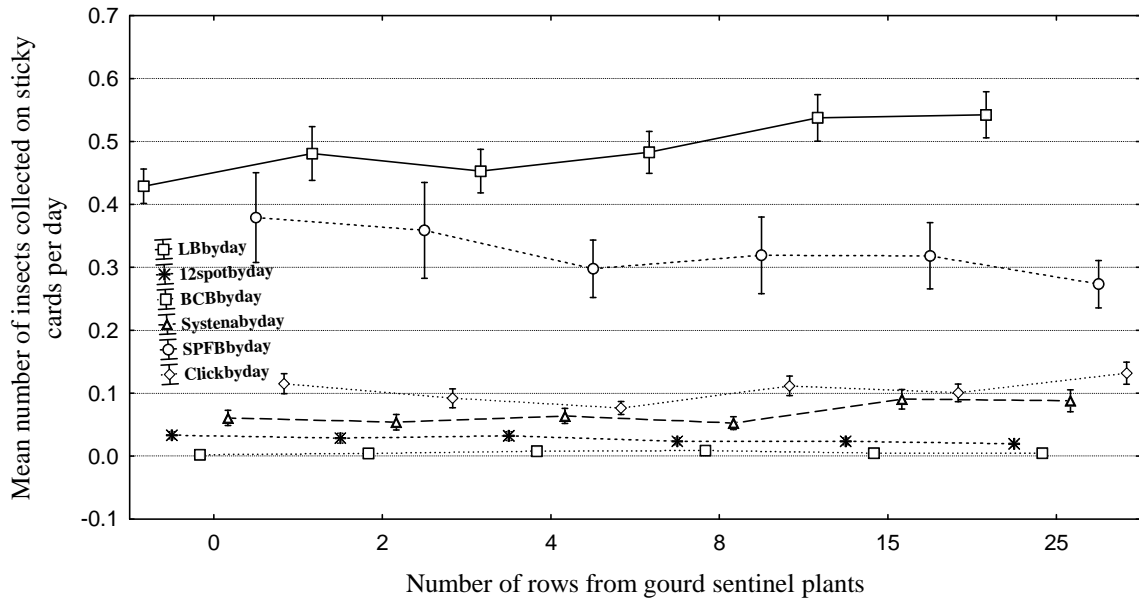


Figure 2.6. Overall mean density \pm SE of insects at various distances from sentinel plots with bottle gourds represented as mean insects per sticky card divided by the number of days the card was left in the field (insects per day).

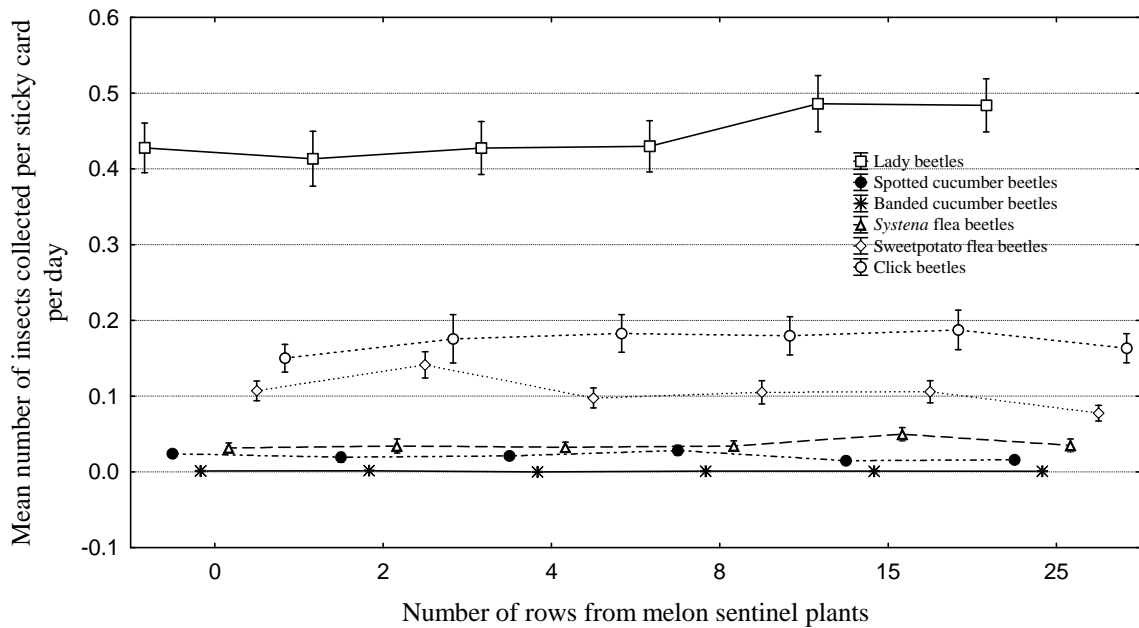


Figure 2.7. Overall mean density \pm SE of insects at various distances from sentinel plots with bitter melons represented as mean insects per sticky card divided by the number of days the card was left in the field (insects per day).

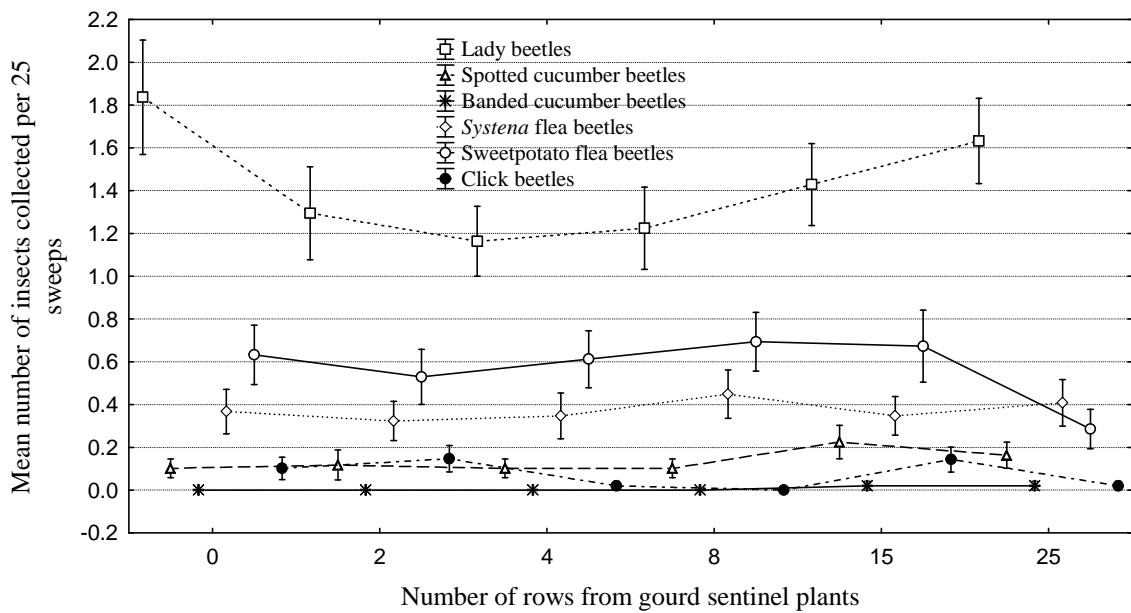


Figure 2.8. Overall mean density \pm SE of insects in sentinel plots with bottle gourds represented as mean insects per 25 sweeps.

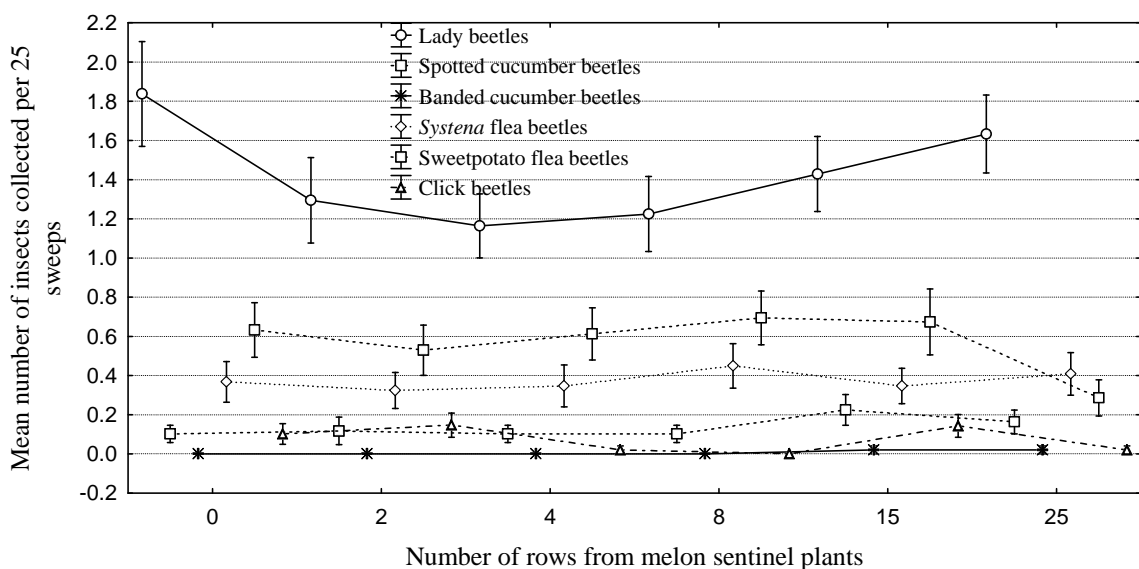


Figure 2.9. Overall mean density \pm SE of insects in sentinel plots with bitter melons represented as mean insects per 25 sweeps.

Table 2.4. Correlation of insects collected on sticky cards with distance (row spacing) from row 0 in the untreated check in all fields containing sentinel plots.

Insect	p	r	slope
Lady beetle	0.62	0.09	0.011
Spotted cucumber beetle	0.63	0.09	0.002
Red-headed flea beetle	0.49	0.13	0.009
Click beetle	0.81	0.05	0.003
Sweetpotato flea beetle	0.84	0.04	0.006

Table 2.5. Correlation of insects, in sentinel fields with gourd plants, collected on sticky cards with distance (row spacing) from sentinel plants.

Insect	p	r	slope
Lady beetle	0.02	0.56	0.004
Spotted cucumber beetle	0.06	-0.46	<-0.001
Red-headed flea beetle	0.01	0.57	0.001
Click beetle	0.04	0.49	0.001
Sweetpotato flea beetle	0.16	-0.35	-0.003

Table 2.6. Correlation of insects, in sentinel fields with melon plants collected on sticky cards with distance (row spacing) from sentinel plants.

Insect	p	r	slope
Lady beetle	0.02	0.53	0.003
Spotted cucumber beetle	0.17	-0.34	<-0.001
Red-headed flea beetle	0.51	0.17	<0.001
Click beetle	0.84	0.05	0.002
Sweetpotato flea beetle	0.09	-0.41	<0.001

The number of LB increased as distance from both species of cucurbit plants increased. The number of SCB decreased as distance from the bottle gourd plants increased, indicating that the bottle gourd plants may have concentrated the SCB adults into the area nearest the cucurbit plants. The number of SFB and CB both increased as distance from the bottle gourd plants increased, indicating a possible allomonal relation between cucurbit plants and SFB and CB. The number of SPFB decreased as distance from the bitter melon plants increased, indicating a possible attraction of SPFB to cucurbit plants. The density of BCB in all fields was too low for analysis.

Small-hole damage (associated with *Diabrotica* species) was the only damage significantly affected by distance from the bottle gourd plants ($p < 0.1$) (Table 2.7). Though the number of adult SCB was higher nearer the bottle gourd plants, the damage showed an opposite trend of being higher farther from the bottle gourd plants. However, small-hole damage showed a trend to decrease as distance from the bitter melon plants increased (Table 2.8). The number of SPFB scars tended to increase as distance from the melon indicator plants increased ($p < 0.1$) (Table 2.8).

Table 2.7. Correlation of the number of scars per root with distance (row spacing) from sentinel plants in sentinel fields with gourd plants.

Damage type	p	r	slope
<i>Diabrotica</i> (small-hole)	0.07	0.44	0.009
<i>Systema</i> flea beetle (pinhole)	0.29	0.26	<0.001
Wireworm (large/deep hole)	0.99	<-0.01	<-0.001
Sweetpotato flea beetle	0.32	-0.24	<-0.001

Table 2.8. Correlation of the number of scars per root with distance (row spacing) from sentinel plants in sentinel fields with melon plants.

Damage type	p	r	slope
<i>Diabrotica</i> (small-hole)	0.24	-0.29	-0.001
<i>Systema</i> flea beetle (pinhole)	0.80	-0.06	<-0.001
Wireworm (large/deep hole)	0.27	-0.27	<-0.001
Sweetpotato flea beetle	<0.01	0.65	<0.001

Correlation analysis of the number of adult SCB and SFB on sticky cards with the number of small-hole scars per root was conducted to determine if a relationship between the number of adults present and the damage to roots existed. New, old (damage healed over with new skin), and total insect damage was considered for this analysis. There was no correlation between the number of SCB and the amount of small-hole damage in the bottle gourd study (Table 2.9). The number of SFB did correlate positively with the number of small-hole scars per root in the gourd fields (Table 2.9). In the melon fields there was a negative correlation of the number of SCB adults with new small-hole damage (Table 2.10). The number of SFB adults correlated with the number of new small-hole scars per root in the melon fields (Table 2.10).

Table 2.9. Correlation of spotted cucumber beetle (*Diabrotica undecimpunctata howardi*) and red-headed flea beetle (*Systema frontalis*) adults collected on sticky cards per day with the number of new, old (healed with new skin), and total small-hole scars per root in sentinel fields with gourd plants.

Insect	Small-hole (<i>Diabrotica</i>)	p	r	slope
Spotted cucumber beetle	Old	0.93	0.01	0.23
Spotted cucumber beetle	New	0.29	-0.15	-1.28
Spotted cucumber beetle	Total	0.77	-0.04	-1.05
Red-headed flea beetles	Old	<0.01	0.40	1.59
Red-headed flea beetles	New	<0.01	0.43	0.80
Red-headed flea beetles	Total	<0.01	0.44	2.38

Table 2.10. Correlation of spotted cucumber beetle (*Diabrotica undecimpunctata howardi*) and red-headed flea beetle (*Systema frontalis*) adults collected on sticky cards per day with the number of new, old (healed with new skin), and total small-hole scars per root in sentinel fields with melon plants.

Insect	Small-hole (<i>Diabrotica</i>)	p	r	slope
Spotted cucumber beetle	Old	0.25	0.16	0.39
Spotted cucumber beetle	New	0.01	-0.34	-0.93
Spotted cucumber beetle	Total	0.33	-0.13	-0.53
Red-headed flea beetles	Old	0.66	-0.06	-0.09
Red-headed flea beetles	New	0.05	0.27	0.46
Red-headed flea beetles	Total	0.27	0.15	0.36

Discussion

Trap Crop Studies

The number of cucumber beetles in the 2006 trap crop was too low to determine if cucurbit plants make a suitable trap crop for cucumber beetles in sweetpotatoes.

However, there was a trend for an increased number of cucumber beetles in the

sweetpotato field after the cucurbit plants were disked under. Watermelon seemed to be a better host than either squash or cantaloupe since more cucumber beetles were found in watermelon than the other two crops. Its longer survival time may have been its advantage over the other two crops. Two important considerations for using a cucurbit plant as a trap crop for cucumber beetles in sweetpotatoes would be the attractiveness of the cucurbit crop to cucumber beetles and the timing of the transplanting of both the main crop and the trap crop. An early planted trap crop might be important to attract a pest before the main crop is planted so the pest will establish itself in the trap crop and not the main crop. In the case of cucurbits and sweetpotatoes, the trap crop must survive long enough to continue to attract cucumber beetles throughout the season. Since cucumber beetles are mobile they can leave an unsuitable food source and search for a more suitable one, sweetpotatoes in this case. A second planting later in the growing season would be necessary to extend the longevity of a cucurbit trap crop until the end of the sweetpotato growing season. However, environmental conditions in Mississippi might not be conducive to growing a late planted trap crop.

Sentinel Plant Studies

A severe drought in 2007 could have affected cucumber beetles numbers in this trial. Eggs of SCB are susceptible to drought conditions because there is a 72 hour time-period in the development of a SCB egg that requires 100% relative humidity (Chalfant and Mitchell 1968). Those conditions would not likely be met in a sweetpotato field in Mississippi, especially in a drought year. It has been recorded that SCB are a more

severe pest in areas of a field that stay moist longer or in years with an abundance of rainfall (Chittenden 1905; Thomas 1912; Webster 1913; Arant 1929; Grayson 1947).

Sticky cards were used as the primary method for sampling adult insects because they are in the field through all environmental conditions. Sweep-net samples taken on the rows with cucurbit plants can be considered to be as valid as on rows with sweetpotatoes since the cucurbit have a similar growth habit as sweetpotato plants. Both grow low to the ground and at a similar rate. After approximately six weeks the rows with cucurbits had sweetpotato vines growing over them and the adjacent rows of sweetpotatoes had vines of bitter melon and bottle gourd growing over them.

The higher number of SCB adults in the field near the cucurbit plants was expected. However, since the relation of small-hole damage to distance from the trap crop was opposite that of adult SCB numbers and the correlation of adult SCB with small-hole damage was actually negative for the melon plants, it should not be assumed that this interaction was because of the cucurbit plants affecting the SCB. Since SFB adult numbers correlated positively with small-hole damage, it could be that damage is being mis-identified and that the small-hole damage is caused by SFB larvae rather than SCB larvae.

It is unknown if the responses of the beetles other than the SCB are related to the cucurbit plants. To the author's knowledge, LB, SFB, CB, and SPFB are not known to be affected by cucurbit plants for any reason. The trends these insects showed could be random, although significant correlations were found.

SCB are highly mobile (personal observation) and very polyphagous (Quaintance 1900; Webster 1913; Sell 1916; Sweetman 1926; Arant 1929; Isley 1929; Metcalf 1987; Metcalf and Metcalf 1993; Eben et al. 1997). The author has observed SCB adults able to fly across 20 to 30 rows of sweetpotatoes with relative ease in a matter of two or three seconds. Adult SCB may have been attracted to the cucurbit plants but might have dispersed to lay eggs so as not to overcrowd one area. Weeds in the fields could have been more attractive to SCB females for oviposition sites than the sweetpotatoes or cucurbit plants. The author has often collected SCB on plants such as pigweed and horsenettle, which were common weeds in fields in this trial. It is possible that these plants release volatiles that are attractive to cucumber beetles or these weeds could have served as a source of pollen for SCB which are known to feed on pollen or in blossoms as a regular part of their diet (Webster 1913; Sell 1916; Arant 1929; Isley 1929; Guss and Krysan 1973; Ludwig and Hill 1975; Fisher et al. 1984; Metcalf 1987; Necibi 1990; Jolivet et al. 1994; Eben et al. 1997; Hesler 1998).

A wild cucurbit, Queen Anne's pocket melon (*Cucumis odoratissimus* Moench.) grew in our trap crop study field at the Mississippi State University Plant Science Research Farm. It was found to be highly attractive to cucumber beetles and was considered for use in the sentinel plant study, but was ruled out because there was much potential for it to become a weed pest.

This study shows that cucurbit plants may have potential to act as a trap crop or sentinel plant for SCB or BCB but more work needs to be done to determine their feasibility as an IPM tactic in sweetpotatoes. Larger areas of cucurbit plants might be

necessary to aggregate more of these beetles in an area and retain them there. Small areas of cucurbit plants do not seem to act as sufficiently attractive hosts to serve as good sentinel plants or to cause SCB adults to show less preference for sweetpotato foliage as a host or roots as an oviposition site. Effects of directionality were not considered in these trials. Factors such as wind direction and sunrise and sunset in relation to the direction of sampling in the fields could have affected the results of the sampling. This research was not continued in year two so that more focus could be given to determining the status of SCB as a pest of sweetpotatoes.

Acknowledgments

Thanks to Dr. David Nagel for his assistance in choosing, ordering, and germinating the plants used in these studies.

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CHAPTER III
REARING THE SPOTTED CUCUMBER BEETLE (*DIABROTICA
UNDECIMPUNCTATA HOWARDI*) AND EXPERIMENTS TO
DETERMINE FEEDING PREFERENCES OF SPOTTED
CUCUMBER BEETLE LARVAE ON SWEETPOTATO
(*IPOMOEA BATATAS*) ROOTS

Introduction

The spotted cucumber beetle (*Diabrotica undecimpunctata howardi* Barber) (SCB) (Figure 3.1) is distributed in most of the United States east of the Rocky Mountains and is very abundant in the southeastern United States (Barbercheck and Warrick 1997). It is suspected to be the primary cause of damage to sweetpotatoes in the inland Carolinas (Jackson et al. 2005) where the temperatures are similar to Mississippi. Banded cucumber beetle (*Diabrotica balteata*) (BCB) (Figure 3.2) larvae are considered a primary cause of damage to sweetpotato roots in Louisiana (Pitre and Kantack 1962) where the temperatures are warmer throughout the year. The distribution of the two species is probably determined by temperature (Krysan and Miller 1986) with SCB able to withstand cooler temperatures and BCB not able to withstand extended periods of sub-freezing temperatures. There is however some evidence that indicates the BCB may be acclimatizing to cooler weather (Elsley 1989). Both of these species exist in Mississippi

sweetpotato fields with SCB making up approximately 90% of the *Diabrotica* spp. collected in sweetpotato fields from 2004 to 2007 (Reed and Fleming, unpublished data). The SCB is a common insect found in Mississippi and can be collected from most crops anywhere in the state.



Figure 3.1. Spotted cucumber beetle (*Diabrotica undecimpunctata howardi*) adult.



Figure 3.2. Banded cucumber beetle (*Diabrotica balteata*) adult.

The ecology of the SCB, anatomy of sweetpotato roots, four years of research prior to this research, and the uncertainty of distinguishing damage, have led to some questions concerning the amount of damage SCB larvae cause to sweetpotato roots. The SCB ecology includes a polyphagous nature (Quaintance 1900; Webster 1913; Sell 1916; Sweetman 1926; Arant 1929; Isley 1929; Metcalf 1987; Metcalf and Metcalf 1993; Eben et al. 1997; reviews in Jolivet et al. 1994), with an attraction to blossoms, an apparent need for pollen (Webster 1913; Sell 1916; Arant 1929; Isley 1929; Guss and Krysan 1973; Ludwig and Hill 1975; Fisher et al. 1984; Metcalf 1987; Necibi 1990; Jolivet et al. 1994; Eben et al. 1997; Hesler 1998), and moist, smooth-textured soil in the egg and

larval stages (Chittenden 1905; Thomas 1912; Webster 1913; Arant 1929; Grayson 1947; Campbell and Emery 1967; Chalfant and Mitchell 1968; Turpin and Peters 1971; Krysan 1976; Lummus et al. 1983; Meinke 1984; Brust 1989; Brust and House 1990). Common Mississippi crops such as corn (*Zea mays*) and soybeans (*Glycine max*) and common weeds such as pigweed (*Amaranthus* spp.) and morningglory (*Ipomoea* spp.) may be more attractive to SCB than sweetpotatoes (Brust 1989). Sweetpotatoes do not produce many blossoms, making them a poor pollen source for SCB. Necibi (1990) found that cucumber beetles in cucurbit fields were still highly attracted to cucurbit blossoms even in areas of the field with many weedy host plants. These two findings could mean SCB adults leave sweetpotato fields to search for pollen and may lay eggs near their pollen source. The apparent need for moist, smooth textured soil in the egg and larval stages is important because those conditions are not always found in sweetpotato fields.

Sweetpotatoes are typically grown on sandy soil (Swiader and Ware 2002), that is frequently dry and coarse textured, and therefore would not be a suitable site for SCB egg hatch or larval survival. All of these factors, individually or collectively, may have an effect on SCB development and survival and may make sweetpotatoes an unsuitable or undesirable host for SCB.

The anatomy of the sweetpotato plant includes a latex substance that serves as a defense against injury and predation in some cases. This latex is found in special cells called laticifers (Data et al. 1996). The substance is immediately released when plants are cut or injured (Figure 3.3). In sweetpotatoes this latex is comprised of hexadecyl-, octadecyl-, and eicosyl-esters of p-coumaric acid (Data et al. 1996), and has been shown

to slightly deter feeding and oviposition on sweetpotato plants by the sweetpotato weevil (*Cylas formicarius* (Fabricius)) (Data et al. 1996). A similar substance has also been shown to “gum-up” the mandibles of the milkweed borer feeding on milkweed (Dussourd and Eisner 1987). The latex substance in sweetpotato could potentially deter cucumber beetle larvae by “gumming up” their mouthparts and making the roots unpalatable. Sweetpotato root anatomy also includes fibrous roots that could be fed upon by SCB larvae (Cuthbert and Jones 1978). These factors could potentially reduce feeding by SCB larvae on the marketable, storage roots of sweetpotatoes.



Figure 3.3. White latex substance that may be a factor in limiting spotted cucumber beetle (*Diabrotica undecimpunctata howardi*) larval ability to feed on sweetpotato roots. Shown exuding from cut sweetpotato root.

Another important factor that is inconsistent with the pest status of SCB larvae is the low number of adults present in sweetpotato fields. The mean number of SCB collected between 2004 and 2007 in commercial sweetpotato fields in Mississippi was 0.08 adults per 25 sweeps with a minimum of 0 and a maximum of 1.71 (Reed and Fleming, unpublished data). Other known sweetpotato root feeding pests causing similar damage were also found in Mississippi sweetpotato fields. These included the BCB at 0.01 adults per 25 sweeps, the red-headed flea beetle (*S. frontalis*) at 0.21 adults per 25 sweeps, the elongate flea beetle (*S. elongata*) at 0.02 adults per 25 sweeps and the pale-striped flea beetle (*S. blanda*) at 0.001 adults per 25 sweeps (Reed and Fleming, unpublished data). The BCB, elongate flea beetle, and pale-striped flea beetle occur in fields in such low numbers that statistical analysis is limited. However, the red-headed flea beetle occurs consistently and the larvae cause damage that could be confused with cucumber beetle larval damage.

Analysis of the mean number of SCB collected weekly in a total of 528 field plots over a four year period with scars on a total of 25 roots dug from those plots resulted in no correlation of insect numbers to supposed SCB damage of sweetpotatoes (Reed and Fleming, unpublished data). However, similar correlation analyses of the number of red-headed flea beetles collected with pinhole scarring associated with flea beetle larval feeding and small-hole scarring associated with cucumber beetle feeding resulted in a positive correlation (Reed and Fleming, unpublished data). In addition, a correlation can be seen when combining the number of red-headed flea beetle adults and the number of SCB adults and the combination of pinhole and small-hole scarring (Reed and Fleming,

unpublished data). These data could indicate that the damage is being misidentified because of similarity in appearance, that red-headed flea beetle larvae are causing both pinhole and small-hole damage, or that the SCB larvae are not causing the small-hole damage and another insect or group of insects is causing the damage. Cuthbert and Reid (1965) show an image of a sweetpotato root with damage similar to small-hole damage, but indicate it was caused by a *Systema* spp. They also indicate that an anthicid beetle, *Notoxus calcaratus* Horn, caused damage similar to small-hole damage.

The feeding of spotted cucumber beetle larvae on sweetpotato roots has not been confirmed. It is considered to be a damaging pest of sweetpotatoes (Hammond et al. 2001; Thompson et al. 2002) and adults have been frequently collected in Mississippi sweetpotato fields. However, its status as a damaging pest of sweetpotato roots is unknown. The larvae of the closely related banded cucumber beetle (BCB), *Diabrotica balteata* LeConte, have been observed feeding on sweetpotato roots (Pitre 1962) and are considered a major pest of sweetpotatoes in Louisiana (Pitre and Kantack 1962).

Large numbers of spotted cucumber beetle adults, eggs, and larvae were needed to conduct feeding experiments with sweetpotatoes to determine the status of SCB as a pest of sweetpotatoes. The population of wild spotted cucumber beetle adults was not high enough to provide the number of insects needed for the experiments, so it was concluded that a colony of spotted cucumber beetles would need to be reared to supply necessary number of insects. This insect and the closely related banded cucumber beetle have been reared successfully in the past (Pitre 1962; Chalfant and Mitchell 1968; Cuthbert et al. 1968). The basic rearing methods for these insects have been similar in each case, but

food and environmental factors have varied. Cuthbert et al. (1968) used semi-synthetic diet along with collard leaves and sweetpotato roots to feed the adults, Pitre and Kantack (1962) fed soybean and sweetpotato leaves to the adults, and Chalfant and Mitchell (1968) used sliced squash to feed the adults. Sprouting corn was used to feed the larvae in all three cases, and Pitre and Kantack (1962) also used sweetpotato roots and sprouted soybeans. Temperatures between 21° C and 30° C were used in these studies. Chalfant and Mitchell (1968) state that a relative humidity of 40-80% was used, however they also state that egg hatch only occurred at 100% relative humidity.

Two sets of trials were conducted to help determine SCB larval feeding behavior on sweetpotato roots. The first set involved trials using small sweetpotato plants in cups in a greenhouse that were infested with eggs or larval SCB. This trial was used to help answer the questions: 1) Do SCB larvae feed on sweetpotatoes? 2) How much damage do SCB larvae cause to sweetpotato roots? 3) Do SCB larvae survive on something other than swollen roots? 4) What does the damage to sweetpotato roots look like? The second set of trials involved feeding SCB larvae pieces of sweetpotato or corn seed. This trial was used to help answer the questions: 1) How well do SCB larvae grow when fed sweetpotato roots? 2) Does the sweetpotato root periderm deter feeding of SCB larvae? 3) How much sweetpotato does a SCB larva need to consume in order to complete its larval development?

Materials and Methods

Rearing

A colony of SCB was established at the Mississippi State University Insect Rearing Center for use in experiments. Basic rearing procedures of this study were based on the work of Cuthbert et al. (1968). Conditions in this facility were held at $\pm 27^{\circ}$ C and $\pm 55\%$ relative humidity. Adults were placed in a 45 x 45 cm cage made of 50 mesh screen and an aluminum frame (Figure 3.4). A wire tray approximately 3 cm tall was placed in the bottom of the cage to hold food items. Food items were replaced every three days. Food items included: collard leaves (*Brassica oleracea* L.); snap bean leaves (*Phaseolus vulgaris* L.); cucurbit leaves, blossoms, and fruit (*Cucurbita pepo* L., *Cucumis melo* L., and *Mamordica charantia* L.); corn tassels (*Zea mays* L.); and sweetpotato foliage (*Ipomoea batatas* (L.)). Insects were supplied a commercial bee pollen substitute (Betterbee brand pollen substitute) when pollen producing cucurbit blossoms and corn tassels were no longer available.



Figure 3.4. Cage used to house adult spotted cucumber beetles.

Two oviposition dishes were placed in the cage under the food trays. Oviposition dishes consisted of 9 cm Petri dishes filled with sand and covered with a layer of filter paper and four to six layers of cheesecloth dyed with Rit brand dye to make it black. The center of the lids of the Petri dishes were cut out to produce a ring used to hold the cheesecloth on the dish. Oviposition dishes were kept moist by re-saturating with water daily. Eggs were collected every two days. Eggs were left on the cheesecloth and filter paper and placed in a small plastic crisper between layers of moist paper towels and kept there for five days (Incubation crisper) (Figure 3.5).



Figure 3.5. Incubation crisper used to house spotted cucumber beetle eggs for incubation.

After five days the cheesecloth was removed from the crisper and the eggs were gently agitated loose from the cheesecloth and filter paper in a stainless steel container full of tap water. The contents of the container were then poured through muslin to collect the eggs. The eggs and muslin were then soaked in a solution of 0.05% sodium hypochlorite for 5 minutes. The muslin was removed and eggs were rinsed with running tap water for 5 minutes. The muslin and eggs were then placed in a plastic crisper for five days (Hatching crisper). The hatching crisper contained a 2 cm layer of moist sand that was covered with the muslin. Sprouting corn was placed on the muslin as food for the emerging larvae (Figure 3.6).



Figure 3.6. Hatching crisper used to house spotted cucumber beetle larvae emerging from eggs.

After five days the muslin was removed and larvae were washed from the muslin and sprouting corn with tap water through a no. 5 sieve and onto a no. 60 sieve. The no. 5 sieve collected the sprouting corn and the no. 60 sieve collected the larvae. Larvae were then rinsed with distilled water into a larger container, the pupation crisper containing a 2 cm layer of moist sand, to allow maturation and pupation (Figure 3.7). A layer consisting of coarse vermiculite and sprouting corn (2:1 mix) provided a good growing medium for corn and was sufficiently loose to allow larval movement. After approximately ten days the larvae pupated, and at this time the pupation crisper was placed in a cage until the adults emerged. Emergence occurred 5 to 10 days later.



Figure 3.7. Pupation crisper used to house pupating spotted cucumber beetle larvae.

Distilled water was used for keeping the material in the containers moist. Containers and Petri dishes were cleaned with a 10% sodium hypochlorite solution and anti-bacterial soap and rinsed with tap water after each use. The cage containing the adults was cleaned weekly with a 10% sodium hypochlorite solution. Sand, vermiculite, filter paper, muslin, and cheesecloth were autoclaved at 121° C and 15 psi before use. Corn was soaked before use in a 0.25% sodium hypochlorite solution for 15 minutes, rinsed with running tap water, and then soaked in a solution of 50g/L of Captan and rinsed with flowing tap water.

Fungicide Trials

Fungal growth in the hatching and pupation crispers was a problem, so two fungicide efficacy trials were conducted to determine if the standard fungal control practices could be improved. *Aspergillus* spp. were determined to be the major fungal species found in the crispers. The first trial had four different fungicide treatments plus an untreated check, and each treatment had three moisture levels, for a total of 15 scenarios (Table 3.1). The second trial had three treatments including an untreated check, and each treatment had three moisture levels, for a total of 9 scenarios (Table 3.2). This trial was conducted to see if agitation of the corn seed would improve fungicide efficacy. Each trial was set-up as a randomized complete block with four replicates.

The basic substrate of both trials was the same. Four corn seeds were placed on a layer of filter paper which was placed on a 4 mm layer of sand in a standard 9 cm Petri dish. All Petri dishes used in the experiment were sanitized in a 10% sodium hypochlorite solution and rinsed with running tap water, and the filter paper and sand were autoclaved at 121° C and 15 psi. Once sand, filter paper, and treated corn seed were placed in the Petri dish, all dishes received 5 cc of distilled water. Sodium hypochlorite and Captan in all treatments except the unrinsed treatment was rinsed from corn seeds with tap water. Agitation in treatment 3 of trial 2 was done with a VWR Scientific Products orbital shaker at 100 cycles per minute. Agitation was conducted to determine if fungal spores on corn seed were concealed under air bubbles in micro-cracks on the corn surface that standard soaking of seeds could not reach. A 1 cm hole was drilled in the top of each Petri dish to allow access for water to be added.

Table 3.1. Fungicide treatments and moisture levels for fungicide efficacy trial.

Treatment	Treatment details	Moisture level
Untreated	No fungicide	High
	No fungicide	Medium
	No fungicide	Low
Bleach	0.25 % Sodium hypochlorite soaked 1 hour rinsed	High
	0.25 % Sodium hypochlorite soaked 1 hour rinsed	Medium
	0.25 % Sodium hypochlorite soaked 1 hour rinsed	Low
Captan	50g/L Captan soaked 4 hours rinsed	High
	50g/L Captan soaked 4 hours rinsed	Medium
	50g/L Captan soaked 4 hours rinsed	Low
Standard	0.25% Sodium hypochlorite soaked 15 minutes + 50g/L Captan soaked 1 hour rinsed	High
	0.25% Sodium hypochlorite soaked 15 minutes + 50g/L Captan soaked 1 hour rinsed	Medium
	0.25% Sodium hypochlorite soaked 15 minutes + 50g/L Captan soaked 1 hour rinsed	Low
Standard rinsed	0.25% Sodium hypochlorite soaked 15 minutes + 50g/L Captan soaked 1 hour not rinsed	High
	0.25% Sodium hypochlorite soaked 15 minutes + 50g/L Captan soaked 1 hour not rinsed	Medium
	0.25% Sodium hypochlorite soaked 15 minutes + 50g/L Captan soaked 1 hour not rinsed	Low

Table 3.2. Fungicide treatments and moisture levels for agitation trial.

Treatment	Treatment details	Moisture level
Untreated	No fungicide	High
	No fungicide	Medium
	No fungicide	Low
Standard	0.25% Sodium hypochlorite soaked 15 minutes + 50g/L Captan soaked 1 hour rinsed	High
	0.25% Sodium hypochlorite soaked 15 minutes + 50g/L Captan soaked 1 hour rinsed	Medium
	0.25% Sodium hypochlorite soaked 15 minutes + 50g/L Captan soaked 1 hour rinsed	Low
Standard + Agitation	0.25% Sodium hypochlorite soaked 15 minutes + 50g/L Captan soaked 1 hour + agitation rinsed	High
	0.25% Sodium hypochlorite soaked 15 minutes + 50g/L Captan soaked 1 hour + agitation rinsed	Medium
	0.25% Sodium hypochlorite soaked 15 minutes + 50g/L Captan soaked 1 hour + agitation rinsed	Low

Petri dishes were re-moistened with distilled water according to their respective moisture level; High (H) every 24 hours, Medium (M) every 48 hours, and Low (L) every 72 hours. Fungal growth was recorded visually and ranked from 0 to 4 daily: 0, no fungal growth; 1, 0-25% of corn seed covered; 2, 26-50% of corn seed covered; 3, 51-75% of corn seed covered; and 4, 76-100% of corn seed covered. Fungal growth was recorded for seven consecutive days, but only the results from day 7 are presented in this paper. Statistical analysis was conducted to separate mean results of experiments using Statistica data analysis software system (Statsoft, Inc. www.statsoft.com) and the general linear model. Homogeneity of variance was determined using the Cochran C test.

Tests for Disease

A dozen larvae were used in a test to determine if they were infected with a virus, bacteria, or protozoa. Insects were macerated individually in clean cups using sterile distilled water and autoclaved toothpicks. A drop of the homogenate from the cups was smeared on a glass slide and stained with Buffalo Black to detect protozoan spores and/or occluded virus particles. A second portion of the homogenate was streaked onto a microbial agar dish containing TSA (Trypticase soy agar) to monitor microbial growth.

Cup Trials

Eggs and larvae from the rearing colony were used for infestation in two cup trials. Twenty-four ounce, clear plastic cups were used as containers. The cups were washed in a 10% sodium hypochlorite solution prior to use. Small sweetpotato plants from a field at the Mississippi State University Plant Science Research Farm were dug with a shovel and re-planted the same day into the cups. Soil from the same farm was used for replanting. Vines were trimmed and roots too large for the cup were removed from the plants. Two 3 mm holes were drilled into the base of the cup so water could be absorbed into the soil through the base of the cup. Cups were placed into aluminum roasting pans, which acted as a water reservoir to provide moisture for the cups (Figure 3.8). All trials were kept in a greenhouse in which the temperature ranged from 12° C at night to 46° C during the day with a mean of 25° C. Cups were irrigated with distilled water as needed. Cups were immediately infested after re-planting. Cups were infested with eggs or larvae by using a micro-spatula to transfer eggs or larvae from filter paper to

the base of the plants. After infestation, a mosquito netting material was placed over the plants and secured around each cup with a rubber band (Figure 3.8). All cups were spray-painted black after it was realized algae were growing between the soil and inner surface of the cup. Statistical analysis was conducted to separate mean results of experiments using Statistica data analysis software system (Statsoft, Inc. www.statsoft.com) and the general linear model. Homogeneity of variance was determined using the Cochran C test.

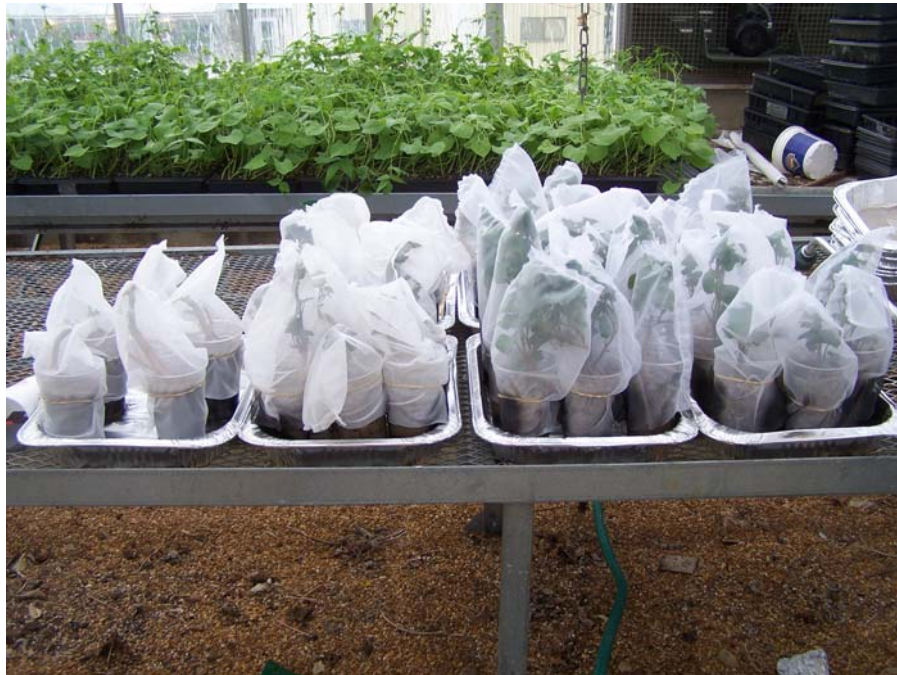


Figure 3.8. Cups used in cup trials in greenhouse, shown sitting in aluminum pan water reservoir. Recently transplanted cups on left and older transplants on right.

Cup trial 1 consisted of five treatments; 8 eggs, 5 one-day-old larvae (1DOL), 3 five-day-old larvae (5DOL), 3 ten-day-old larvae (10DOL), and an uninfested check

(UIC). Uninfested checks were included to verify that roots from the field were free of insects and insect scars prior to planting. They were not used for statistical analysis. Specimens for this trial were reared in a colony in the Mississippi State University Insect Rearing Center. Each treatment except UIC was replicated four times with four cups per replicate. The uninfested check was replicated four times but with only one cup per replicate. Due to an insufficient number of insects in the colony, infestation of all replications of a given treatment could not be conducted on the same day and were infested as follows: egg, 15 August; 1DOL (rep. 1 and 2) 14 August, (rep. 3 and 4) 15 August; 5DOL (rep. 1) 22 August, (rep. 2 and 3) 8 September, (rep. 4) 15 September; 10DOL (rep. 1 and 2) 29 August, (rep. 3) 15 September, (rep. 4) 1 October. Each replicate of each treatment was evaluated when it was believed the specimens had time to reach the pupal stage at which no more feeding would occur. Egg and 1DOL treatments were evaluated 25 days after infestation (DAI), the 5DOL treatment was evaluated 20 DAI and the 10DOL treatment was evaluated 15 DAI. Uninfested checks were evaluated with their respective replicate. Each cup was evaluated by removing all soil from the cup and washing it through a no. 18 sieve to find the number of surviving SCB larvae, pupae, and adults. Roots were washed and evaluated for larval damage.

Cup trial 2 consisted of four treatments; 8 eggs, 16 eggs, 25 eggs, and an uninfested check (UIC). Uninfested checks were included to verify that roots from the field were free of insects and insect scars prior to planting, they were not used for statistical analysis. Specimens for this trial were also from the reared colony. Each treatment except UIC was replicated four times and each replicate had four cups per

replicate. As with cup trial 1, the UIC was replicated four times for each treatment but only had one cup per replicate. Due to an insufficient number of insects in the colony, infestations of all replications of a given treatment could not be conducted on the same day. The 8 egg treatment was infested 15 August; 16 egg treatment (rep. 1, 2, and 3) 19 September, (rep. 4) 22 September; 25 egg treatment was infested 22 September. As with cup trial 1 each treatment replicate was evaluated when it was believed the specimens had time to reach the pupal stage at which time no more feeding would occur.

Vial Trial

Eggs and larvae from the rearing colony were used for infestation of plant material to determine feeding characteristics on corn and sweetpotato roots. Autoclaved, twenty-five ml glass vials were used for this trial. Approximately 1.5 cm of autoclaved sand was placed in the vial and moistened with 2.5 ml of distilled water. Corn seed for the vial trial was sterilized with 0.05% sodium hypochlorite for five minutes and with a 50g/L solution of Captan for one hour. Sweetpotato pieces were washed prior to slicing but not sterilized. An autoclaved cotton ball was used to cap the vials because it allowed some airflow but also held in moisture.

The vial trial was conducted to determine how well sweetpotatoes serve as a food source for SCB larvae. This trial consisted of three treatments: sprouting corn, sweetpotato flesh, and sweetpotato periderm. Each treatment was replicated three times across dates; replicate 1, 22 October, replicate 2, 23 October, and replicate 3, 24 October. Each vial was infested with two larvae. Insects for this trial were reared in the

Mississippi State University Insect Rearing Center. The number of vials per replicate were: replicate 1, six, replicate 2, seven, and replicate 3, ten. Each replicate for each treatment was evaluated six days after infestation with the intention of using half of the surviving larvae to infest again and the other half for measurements, however not enough survived to do both so the experiment was ended. Larval weight and length, percent survival for each treatment, and the number of holes in the sweetpotato treatments was recorded. Statistical analysis was conducted to separate mean results of experiments using Statistica data analysis software system (Statsoft, Inc. www.statsoft.com) and the general linear model. Homogeneity of variance was determined using the Cochran C test.

Results

Rearing, Fungicide Trials, and Tests for Disease

There was no significant interaction between moisture level and fungicide treatment in the first trial. However in both trials all fungicide treatments showed a significant effect on fungal growth but there were no differences among fungicide treatments ($p < 0.1$) (Table 3.3). In the second study agitation was used to see if agitating the seed could help control the fungus better along with the standard treatment. Moisture level had no effect on this trial either. The untreated check had more fungal growth than the standard or the standard plus agitation treatments with no differences between the agitated and standard treatment (Table 3.4).

Table 3.3. Mean \pm SE of fungal growth on corn seed after seven days in fungicide efficacy trial.

Solution	Mean fungal growth rating
Bleach	0.81 \pm 0.13 a
Standard not rinsed	0.79 \pm 0.13 a
Standard	1.08 \pm 0.12 a
Captan	0.88 \pm 0.13 a
Untreated	2.17 \pm 0.13 b
Prob F	<0.01

Means not sharing a common letter differ significantly (Fisher's LSD; $p=0.05$).

Table 3.4. Mean \pm SE of fungal growth on corn seed after seven days in agitation trial.

Treatment	Mean fungal growth rating* ¹
Standard + Agitation	0.48 \pm 0.12 a
Standard	0.31 \pm 0.11 a
Untreated	2.1 \pm 0.23 b
Prob F	<0.01

Means not sharing a common letter differ significantly (Fisher's LSD; $p=0.05$).

*Assumptions for homogeneity of variance were not met according to the Cochran C test.

Larval eclosion from eggs was also a problem. Only 51% of eggs hatched on average, and sometimes none hatched. Adult eclosion rates were also low. Though larvae seemed to feed well on the sprouted corn, only 250 adult beetles emerged in the colony. It is unknown what caused larval and adult eclosion rates to be low. The large amounts of fungi in the environment could have affected the health of the insect and the

sprouting corn. Results from the tests for diseases were negative for viruses, bacteria, and protozoa.

During August it was noticed egg production quickly declined in the cage housing adults collected from the field. A survey of adults from the field showed a male to female ratio of 12:1. Sexing the beetles was done by observing the beetles for a pad on tarsomere 1. Absence of the pad indicates a female, presence of the pad a male (Hammack and French 2007).

Cup Trials

Larvae in the cup trials survived when given only sweetpotato roots as food. Data from cup trial 1 suggest that all stages of SCB larvae are capable of feeding on sweetpotato roots (Table 3.5). The mean number of scars resulting from surviving SCB larvae in cup trial 1 can be seen in Table 3.5. The data also indicate that SCB larvae cause only a small amount of damage per larva and that only a small percentage survive when fed sweetpotatoes (Tables 3.5 and 3.6). Pictures of damaged roots from these trials show large variability in scar type. Some roots had very small pinhole scars (<1mm) which are usually considered to be damage of *Systema* flea beetle larvae (Figures 3.9 and 3.10). Other roots had larger scars typical of small-hole damage associated with cucumber beetle injury (Figures 3.11 and 3.12). One root was found to have a probable entry hole (~0.5mm) from a neonate larva, a large excavation under the skin and a larger probable exit hole (~1.5mm) (Figure 3.13). This particular larva may have fed enough in

this root to complete its larval development, however this is a unique case, as most scars were superficial.

Table 3.5. Mean \pm SE number of scars per survivor in each cup and mean \pm SE percent survival in each cup from Cup Trial 1.

Treatment	Mean no. of scars per survivor*	Mean % survival* ¹	N
egg	1.25 \pm 0.35 b	20.00 \pm 2.04 b	10
1 DOL	2.40 \pm 0.31 b	28.00 \pm 3.27 b	10
5 DOL	1.81 \pm 0.60 b	45.83 \pm 6.10 bc	8
10 DOL	1.18 \pm 0.28 b	69.23 \pm 9.59 c	13
Prob F	0.08	<0.01	

Means not sharing a common letter differ significantly (Unequal N HSD; p=0.05).

*Least square means used for the mean no. of scars per survivor because of uneven N due to loss of some cups from fungus and lack of survivors in some cups.

¹Assumptions for homogeneity of variance were not met according to the Cochran C test.

Table 3.6. Mean \pm SE number of scars per survivor in each cup and mean \pm SE percent survival in each cup from Cup Trial 2.

Treatment	Mean no. of scars per survivor*	Mean % survival	N
8 eggs	1.25 \pm 0.35	20.00 \pm 2.04	10
16 eggs	1.21 \pm 0.21	11.98 \pm 2.23	12
25 eggs	1.32 \pm 0.26	8.29 \pm 1.49	14
Prob F	0.92	0.15	

Means not sharing a common letter differ significantly (Unequal N HSD; p=0.05).

*Least square means used for the mean no. of scars per survivor because of uneven N due to loss of some cups from fungus and lack of survivors in some cups.

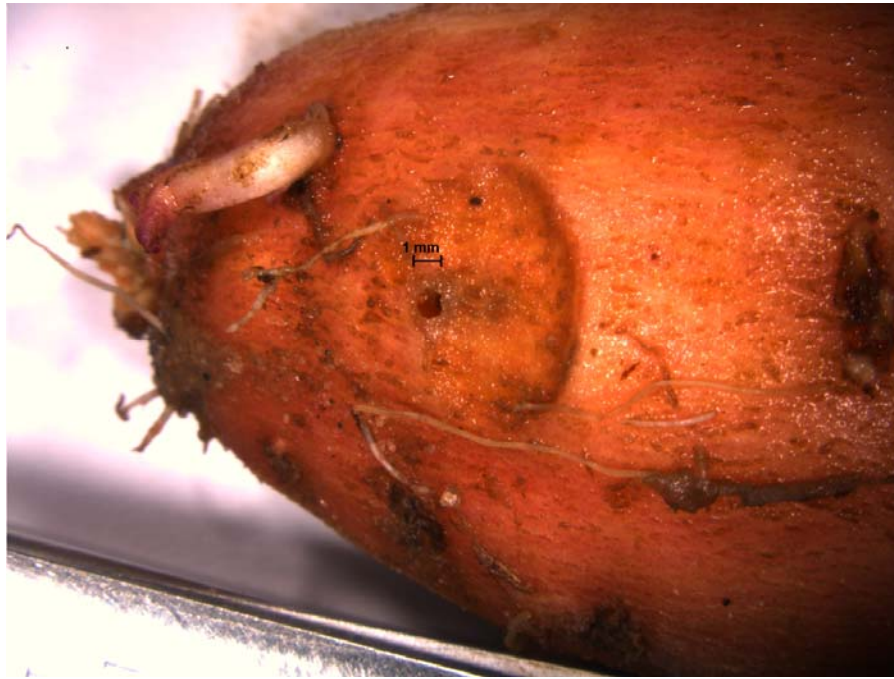


Figure 3.9. Damage on root from cup trial showing pinhole injury.



Figure 3.10. Damage on root from cup trial showing two pinhole scars.



Figure 3.11. Damage on root from cup trial showing small-hole injury.



Figure 3.12. Cross-section of damage on root from cup trial.



Figure 3.13. Damaged sweetpotato root from cup trial with an apparent entry and exit hole.

In the second cup trial the rate of egg infestation was evaluated to determine the rate damage may increase with an increasing number of larvae. There was an increase in the number of scars between treatment 1 of 8 eggs and treatment 3 of 25 eggs, however this increase was not significant (Table 3.7). The amount of damage significantly increased as the number of survivors increased ($p < 0.01$, $r = 0.59$). However, damage did not significantly increase with the increased number of eggs ($p = 0.8$, $r = 0.26$).

Table 3.7. Mean \pm SE of scars per cup in Cup Trial 2.

Treatment	Mean no. of scars per cup*	N
8 eggs	1.25 \pm 0.49 a	15
16 eggs	2.01 \pm 0.49 a	15
25 eggs	2.44 \pm 0.48 a	16
Prob F	0.23	

Means not sharing a common letter differ significantly (Unequal N HSD; $p=0.05$).

*Least square means used for the mean no. of scars per survivor because of uneven N due to loss of some cups from fungus and lack of survivors in some cups.

Vial Trial

In the vial trial, weights and lengths of SCB larvae were taken to compare growth of SCB larvae when fed corn, sweetpotato flesh, or sweetpotato periderm for a period of six days. Larval mortality was too high to continue the trial beyond 6 days because there were not enough surviving larvae for statistical analysis. After 6 days of feeding, SCB larvae weighed more when feeding on sprouting corn than on either sweetpotato flesh or periderm (Table 3.8). Body length was also affected by food type. Corn-fed larvae were significantly longer than larvae developing on sweetpotato flesh or periderm (Table 3.9). Larvae developing on sweetpotato flesh were significantly longer than larvae developing on sweetpotato periderm (Table 3.9). Survival of larvae fed the three different food types did not significantly differ between treatments (Table 3.10).

Table 3.8. Mean \pm SE of the weight of larvae fed the three different food types (corn, sweet potato flesh, and sweet potato periderm) in the vial trial.

Treatment	Mean weight per larvae (in milligrams)* ¹	N
Corn	1.10 \pm 0.13 b	17
Periderm	0.24 \pm 0.16 a	11
Flesh	0.42 \pm 0.11 a	23
Prob F	<0.01	

Means not sharing a common letter differ significantly (Unequal N HSD; p=0.05).

*Least square means and unequal N highest significant difference used because of uneven N due to uneven number of surviving specimens and fungi.

¹Assumptions for homogeneity of variance were not met according to the Cochran C test.

Table 3.9. Mean \pm SE of the body length of larvae fed the three different food types (corn, sweet potato flesh, and sweet potato periderm) in the vial trial.

Treatment	Mean body length of larvae (in mm)* ¹	N
Corn	5.49 \pm 0.21 c	17
Periderm	2.67 \pm 0.25 a	12
Flesh	3.41 \pm 0.17 b	23
Prob F	<0.01	

Means not sharing a common letter differ significantly (Unequal N HSD; p=0.05).

*Least square means and unequal N highest significant difference used because of uneven N due to uneven number of surviving specimens and fungi.

¹Assumptions for homogeneity of variance were not met according to the Cochran C test.

Table 3.10. Mean \pm SE of percent survival in each treatment (corn, sweet potato flesh, and sweet potato periderm) in the vial trial.

Treatment	Mean % survival	N
Corn	45.02 \pm 8.92 a	19
Periderm	30.59 \pm 8.05 a	23
Flesh	50.15 \pm 8.05 a	23
Prob F	0.21	

Means not sharing a common letter differ significantly (Unequal N HSD; $p=0.05$).

*Least square means and unequal N highest significant difference used because of uneven N due to loss of some vials from fungi.

The number of scars in the sweetpotato flesh and periderm from the vial trial was evaluated to help determine the feeding preference of SCB larvae on sweetpotatoes.

Larvae fed more aggressively on the sweetpotato flesh than on the periderm (Table 3.11).

This suggests that the periderm may be less suitable to SCB larvae than the flesh.

Table 3.11. Mean \pm SE of the number of holes in the sweetpotato food types (sweet potato flesh and sweet potato periderm) in the vial trial.

Treatment	Mean no. of holes per piece of food
Flesh	16.27 \pm 1.71 a
Periderm	7.55 \pm 1.40 b
Prob F	<0.01

Means not sharing a common letter differ significantly (Fisher's LSD; $p=0.05$).

Discussion

Spotted cucumber beetle larvae exhibit the ability to feed on sweetpotato roots, though they may not prefer sweetpotato roots compared to other hosts such as corn. The amount of damage SCB larvae can cause when given only sweetpotato roots was not as high as expected. Based on the evidence of little scarring occurring per larva and scars generally not appearing to be large enough to sustain a larva through its life cycle, it can be assumed that the larvae fed on something other than swollen roots, likely fibrous roots, to survive.

Limited attempts were made to observe larvae feeding on fibrous sweetpotato roots, however no larvae were seen feeding on fibrous roots. The data of these trials indicate that the population of SCB in sweetpotato fields are not generally high enough to cause the amount of damage normally attributed to SCB larvae. Based on these results, SCB larvae may not damage sweetpotatoes in Mississippi as much as has been previously assumed, unlike BCB which is known to be a major pest of sweetpotatoes in Louisiana (reviews in Pitre 1962).

Spotted and banded cucumber beetles occur in low number in Mississippi fields (0.8 and <0.1 per 25 sweeps, respectively) (Reed et al. In Press). Though the adults occur in low numbers, small-hole damage is found on 13% of roots (Reed and Fleming, unpublished data), and is considered to be caused from cucumber beetle larvae. *Systema* flea beetles are more common than SCB in Mississippi sweetpotato fields (0.23 per 25 sweeps) (Reed and Fleming, unpublished data) and may cause injury similar to small-hole damage (Cuthbert and Reid 1965). Also, there is a positive correlation between the

number of *Systema* flea beetles collected in Mississippi sweetpotato fields and the small-hole damage found in corresponding plots. Though SCB and BCB occur in Mississippi sweetpotato fields, and damage known to be comparable to cucumber beetle larval damage is found in sweetpotato fields, SCB and BCB may not be the primary cause of the small-hole damage. *Systema* flea beetle larvae may be causing a considerable amount of the small-hole damage found in Mississippi sweetpotato fields. Banded cucumber beetles occur in such low numbers and in few of the fields in the sweetpotato producing area of Mississippi that they are probably only contributing a small amount of the small-hole damage found in sweetpotato fields. This complex of insects and their impact on sweetpotatoes needs to be fully investigated to determine the causes of small-hole damage to sweetpotato roots in Mississippi.

Acknowledgments

Thanks to Amanda Lawrence for performing the disease tests on the larvae.

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CHAPTER IV

CONCLUSION

Limiting Factors of the Spotted Cucumber Beetle as a Pest of Sweetpotatoes

The spotted cucumber beetle (*Diabrotica undecimpunctata howardi*) has a limited ability to be a major sweetpotato pest. According to Reed et al. (In press) spotted cucumber beetles do not occur in Mississippi sweetpotato fields in high numbers, but more roots are thought to be damaged by cucumber beetle larvae than any other insect pest. No correlation between the numbers of spotted cucumber beetle adults and the amount of small-hole damage that is assumed to be caused by cucumber beetle larvae has been demonstrated. There is, however, a correlation between *Systema* flea beetles and the small-hole damage. Soil moisture and relative humidity are some factors limiting the ability of SCB to be a sweetpotato pest (Chittenden 1905; Thomas 1912; Webster 1913; Arant 1929; Grayson 1947; Campbell and Emery 1967; Chalfant and Mitchell 1968; Turpin and Peters 1971; Krysan 1976; Lummus et al. 1983; Meinke 1984; Brust 1989; Brust and House 1990). Spotted cucumber beetle eggs require a relative humidity of nearly 100% to hatch (Chalfant and Mitchell 1968). This condition is rarely met in a sweetpotato field (Fleming, personal observation). Another factor limiting their pest ability is their need to feed in blossoms for pollen to lay viable eggs (Webster 1913; Sell 1916; Arant 1929; Isley 1929; Guss and Krysan 1973; Ludwig and Hill 1975; Fisher et al.

1984; Necibi 1990; Jolivet et al. 1994; Eben et al. 1997; Hesler 1998). Pollen is not readily available in a weed-free sweetpotato field since sweetpotatoes do not produce a large amount of blossoms, nor do the blossoms produce much pollen. Weeds in or around sweetpotato fields may produce substantial amounts of pollen and could draw SCB from the sweetpotatoes to the weeds for oviposition. Finally the male to female ratio in a sweetpotato field during the most critical time for sweetpotatoes to be pest free (post August 1) is not supportive for them to be a major sweetpotato pest (Fleming, personal observation). The fact that there were many more males than females late in the season coupled with low field populations suggest that the population of larval SCB in a sweetpotato field after August 1 would not be high enough to cause a substantial amount of damage to sweetpotatoes.

Trap Crop and Sentinel Plant Studies

No data was found concerning the use of cucurbit plants as a trap crop or sentinel plants for cucumber beetles in sweetpotatoes, however they have been used as a trap crop in other main crops (Barbercheck and Warrick 1997). The population of spotted cucumber beetles in our 2006 trap crop study was not high enough to determine if cucurbit plants were a good trap crop. However, spotted cucumber beetles were found to be more attracted to watermelon and squash than cantaloupe. Spotted cucumber beetle adults did not seem to be highly attracted to the cucurbit plants we used in our sentinel plant study, nor was damage by spotted cucumber beetle larvae concentrated near the cucurbit plants. It was discovered however, that the number of *Systema* flea beetles

collected on sticky cards correlated with the number of small-hole scars on the sweetpotato roots, which is similar to results of the USDA RAMP Southern Sweetpotato IPM Project (Reed and Fleming, unpublished data).

The number of plants we used in each plot may not have been sufficient to produce enough floral volatiles to attract cucumber beetles from a large area to the cucurbit plants. Larger plantings of cucurbit plants could be more effective as a trap crop for spotted or banded cucumber beetles in sweetpotatoes. Also, a cucurbit plant that releases large amounts of floral volatiles might be more effective in attracting cucumber beetles to cucurbit plants and away from sweetpotato plants. Our decision to use bitter melon and bottle gourd was based on those species being bitter, the documented relationship between diabroticites and cucurbits, the potential marketability of these species and their inability to become established as a weed. The quantity of floral volatiles rather than the quantity of cucurbitacin in a cucurbit species may be more significant in attracting cucumber beetles over a large area to serve as a trap crop. In research plots at the Mississippi State University Plant Science Research Farm I have observed up to a dozen cucumber beetles in individual blossoms of crookneck squash, a non-bitter cucurbit. In the same field I observed a wild cucurbit, Queen Anne's pocket melon (*Cucumis odoratissimus* Moench.), to be highly attractive to spotted cucumber beetles. More research could determine the feasibility of using cucurbit plants as trap crops for cucumber beetles in sweetpotatoes where cucumber beetles are major pests.

Rearing and Fungicide Trials

Problems in the rearing colony can most likely be attributed to the *Aspergillus* spp. of fungi that were growing in the pupation containers. The fungal problems were not curable with the standard fungicide treatments deemed safe for use in a rearing colony (sodium hypochlorite and Captan) and agitation with fungicides did not improve fungicidal efficacy. However, the use of fungicide control reduced the fungi compared to no fungicide.

Evaluations to Determine the Feeding Behavior of Spotted Cucumber Beetle Larvae

Spotted cucumber beetle larvae will feed on sweetpotato roots. The extent of feeding and their relatively low level of survival on sweetpotato roots does not indicate that sweetpotatoes would be a preferred host, thus they are not likely to be a major pest of sweetpotatoes. Feeding scars were found to be usually 1 to 2 mm wide and sometimes up to 3 mm deep into the root, but typical scars were superficial. Larvae only caused between one to two scars per larva. The periderm of the sweetpotato root did not seem to be preferred by the larvae when compared to sweetpotato flesh or sprouting corn, and a latex substance in the sweetpotato root could be a deterrent to spotted cucumber beetle larval feeding.

Future Considerations

Farmers that make insecticide applications specifically for spotted cucumber beetle could be applying insecticides unnecessarily. However those insecticide

applications could be managing other insects such as *Systema* flea beetles that are known pests of sweetpotatoes and can cause damage similar to SCB larvae (Cuthbert and Reid 1965). The current threshold of two SCB adults per 100 sweeps (Catchot 2008) seems too low in light of the results of this study. Further research that considers soil moisture, the male/female ratio, and pollen sources in fields may contribute to development of accurate management thresholds for SCB.

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