# CHARACTERIZING THE OVERWINTERING AND EMERGENCE BEHAVIORS OF THE ADULT SQUASH BUG, *ANASA TRISTIS* (DEGEER)

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## PREFACE

Research was conducted from 2002 to 2004 at the Wes Watkins Agricultural Research and Extension Center (WWAREC) in Lane, Oklahoma to illuminate the specific behaviors of adult overwintering squash bugs during the winter hibernating period and during their spring emergence. These studies were conducted in the field with the adult squash bug, *Anasa tristis* (Degeer), its host plant the yellow crook-necked squash, *Cucurbita pepo* 'lemondrop', and its overwintering habitats consisting of many sheltering objects found in the ecological landscape. The first chapter is introductory and the last two chapters present results as complete manuscripts to be submitted to scientific journals following manuscript guidelines established by the Entomological Society of America.

I would like to acknowledge the following people for valuable advice and assistance throughout my research endeavors at OSU. My sincerest thanks go to my major advisor Dr. Jonathan Edelson. He has given me this wonderful opportunity and the freedom to pursue a project that was both wide in berth and exploratory in nature. The other members of my graduate committee, Dr. Kris Giles, Dr. Thomas Phillips, and Dr. Mark Payton, gave me guidance when I was up against a wall and encouraged me to go a little further. All my committee members have my thanks for reviewing and helping me create a sound scientific study. Thanks also to Dr. Russell Wright, without his support none of this would have been possible.

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# CHAPTER I

# INTRODUCTION AND LITERATURE REVIEW

# INTRODUCTION

Throughout the United States, the squash bug (*Anasa tristis*) has been implicated in the loss of yield of many cucurbit crops. The squash bug feeds on plants in the Cucurbitaceae family, but pumpkins and squash are preferred. Fields of squash or pumpkins have historically been prime habitat for squash bugs, but in southern Oklahoma, watermelons (*Citrullus lanatus*) have been attacked. The emerging overwintered adult squash bugs cause significant damage to fields planted in the early spring. Knowing the specific behaviors and movement of emerging overwintered adults is crucial for controlling squash bug damage.

Squash bugs are somewhat elongate insects in the family Coreidae of the order Hempitera. As hemipterans, squash bugs possess half membranous forewings and piercing-sucking mouthparts. They have been called "stink bugs" because they emit secretions from scent glands, but are not in the stink bug family, Pentatomidae. Squash bugs are a mottled light brown color, with abdominal sclerites being either red or black under the wings and hemelytra. Squash bugs are xylem feeding insects. As the insect feeds, the xylem vessels of the plants are blocked and destroyed. Wilting of the plant occurs as vessels become nonfunctional. The squash bug has been implicated in the transmission of the bacterium *Serratia marcescens*, which is associated with the disease Yellow Vine Decline.

As an important pest of cucurbits, the squash bug (*Anasa tristis*) has been a difficult insect to control. In southern Oklahoma, squash bugs can have as many as 3 generations each year, and this can lead to extremely high populations by the end of the growing season. The adult squash bugs leave the fields to overwinter in protected sites and return to the fields in the late spring. The overwintering sites have been anecdotally described, but these sites have not been systematically sampled. These overwintered adults can rapidly cause stunted growth and death of the host plant seedlings during spring. The timing of the initial influx of bugs is very important for control efforts, yet it is not known why the bugs move when they do, nor is it known if the influx is a one-time event, constant, or sporadic.

## LITERATURE REVIEW



**Squash Bug Pest Status - Oklahoma**

With the continued expansion of modern farm practices, vast areas of land with monocrops become suitable for invasion by insect pests. These areas of land farmed for one crop create the habitat necessary for high populations of insect pests. Throughout the United States, the squash bug *Anasa tristis* (DeGeer) has been implicated in the loss of crop yield of many cucurbits (Beard 1940, Edelson et al.. 2003, Fargo et al.. 1988). The squash bug feeds on many plants in the Cucurbitaceae family, but pumpkins and squash are preferred (Bonjour and Fargo 1989). Vast fields of squash or pumpkins have historically been prime habitat for squash bugs, but in southern Oklahoma, watermelons *Citrullus lanatus* (Thunb.) Matsun & Nakai have been attacked. This crop has significant economic value in southern Oklahoma where it is planted in the very early season to have mature fruits by the Fourth of July holiday. These early plants are high value transplanted seedlings and are very susceptible to damage by emerging adult squash bugs. The emerging overwintered adult squash bugs can cause significant damage to fields planted in the early spring. Knowing the specific movement of emerging overwintered adults is crucial for managing squash bug damage. If the timing and extent of the emergence was known, sampling and treatment efforts can be more targeted and efficient.

**Squash Bug Natural History**

Squash bugs are somewhat elongate insects in the family Coreidae of the order Hempitera. As hemipterans, the squash bug possesses half membranous forewings and piercing-sucking mouthparts. They have been called "stink bugs" because they emit secretions from scent glands, but are not in the stink bug family, Pentatomidae. Squash bugs are a mottled light brown color, with abdominal sclerites being either red or black under the wings and hemelytra (Hoerner 1938, Beard 1940). Squash bugs possessing red sclerites are more rare than those with black sclerites, occurring in about one third of the general population (Hoerner 1938). The male and female squash bugs look very similar, but can be easily identified by the shield-like appearance of the sternite of abdominal segment nine in the male. The female has an obvious split in the last visible sternite. The males are also generally smaller than the females (Beard 1940).

Squash bug eggs are laid in masses of 15-20. The eggs are yellow to light brown when laid, but they darken quickly to a bronzed, golden brown (Hoerner 1938, Beard 1940, Bonjour and Fargo 1989). After hatching, the squash bug passes through five nymph instar stages before emerging from a molt as the adult. The first instars have a green body and red legs and antennae immediately after hatching. The legs and antennae quickly turn black as sclerotization and melanization of the cuticle occurs. The rest of the instar stages have a light gray body with black legs. The nymphs in the next instar stages look similar, except in the fourth and fifth instars when the bugs have definite wing pads.

The age structure of a squash bug population becomes complex during the summer in southern OK. The overwintered adults can survive into July, thereby resulting in a broad overlap of generations (Fargo et al. 1988). If eggs are laid during the first week of May, the first spring generation of squash bug adults may begin breeding as early as June 15 based on a degree day model of 376.5 degree days from egg to adult with a developmental threshold of 60° F (Fargo and Bonjour 1988). Dependent upon average temperatures, this can lead to the first spring generation of adults by June 15.

Overwintering squash bugs move into fields during the spring, and the size of this initial population influx determines the extent of damage to seedling plants. Knowledge of the timing and behavior of spring squash bug immigration is an important part of squash bug population management strategies (Palumbo 1989).

Adult bugs enter reproductive diapause in the early fall. During this time the bugs stop breeding and begin to convert more food into fat. This causes the fat body to become enlarged and the ovarioles to shrink (Olson et al. 1996, Nechols 1988). Adult females molting from the last instar exhibit shrunken nonfunctional ovarioles. Female bugs that have already laid eggs may also exhibit shrinking ovarioles and go into reproductive diapause (Fielding 1988). The males do not show any physical reproductive tract changes, but do show the behavior change of not initiating mating. Both males and females have a reduction in respiration that is about half that as found in non-diapause bugs (Fielding 1988). During the fall, the bugs feed on any plants remaining in the fields before taking flight to find a suitable overwintering location. During the fall, the bugs have been observed taking flight and flying straight in a randomly selected direction

(Beard 1940). It is assumed that once the bug finds a suitable overwintering site, becomes mostly inactive.

Squash bugs have few specific natural enemies. This is probably due to the fact that the bugs remain under the cover of host plants leaves and stems and their repugnant smell (Beard 1940). Squash bug eggs are parasitized by some hymenopteran parasitoids. These parasites have been shown to be moderately effective as biological control agents. *Gryon pennsulvanicum* (Ashmead) (Hymenoptera: Scelionidae) is an egg parasitoid that has been recorded parasitizing up to 80% of squash bug eggs(Olson et al. 1996). Another well known parasitoid of the squash bug is a dipteran, *Trichopoda pennipes* (Fabricius) in the family Tachinidae. Beard (1940) reported a variety of insects and vertebrates feeding on squash bug adults and nymphs. Those included predaceous pentatomid bugs and other hemipterans, spiders, toads, lizards, and birds. Interestingly, the stomachs of toads and starlings contained 1-3 percent hemipteran parts. The vertebrate predators do not feed on enough squash bugs to seriously consider them as greatly affecting squash bug populations (Beard 1940).

**Host Plants**

Cucurbits are annual and perennial plants belonging to the family Cucurbitaceae. This diverse plant family has a few important characteristics that link the entire group. Cucurbits have three to seven lobed leaves, and they are generally vining, but bush species and cultivars do exist. The plants can be either annual or perennial, and the fruits vary in size from only a few millimeters to a meter or more and hundreds of kilograms (Robinson and Decker-Walters 1996). The flowers are generally monoecious, although there are dioecious species. The flowers must also be insect or hand pollinated.

Domesticated species generally must have adequate pollination or the fruit will be too small for commercial use.

All Cucurbits contain varying levels of a very bitter triterpenoid compound called Cucurbitacin. They are often found in greater quantities in the fruit and roots, and they can be toxic to humans in high doses. Cucurbits are found throughout the world and are used as important sources of food and traditional medicine. These domesticated cucurbits have been selectively bred to reduce bitterness for 10,000 years or more. The seeds were probably the original food sought from cucurbits, as they are not bitter (Robinson and Decker-Walters 1996).

Cucurbits are found throughout the world, but there are many species that have been selectively bred to be edible cultivars. The cucurbits that we call "watermelons" are derived from a line of *Citrullus lanatus* from Africa (Robinson and Decker-Walters 1996, and Whitaker and Davis 1962). Plants in the squash and pumpkin genus, *Cucurbita*, are native to North and South America, as are squash bugs. Only a few species in the genus *Cucurbita* are attractive to squash bugs. Plants native to the squash bug range appear to have developed some defenses that limit feeding and damage by squash bugs. Hosts that are affected by feeding are limited to individual species in the genus *Cucurbita*, namely *C. pepo, C. moschata, and C. maxima* (Bonjour and Fargo 1989).

Squash bugs seem to prefer a few species in the genus *Cucurbita*, with the notable exception of the watermelon, *Citrullus lanatus* (Bonjour and Fargo 1989, Edelson et al.. 2002, and Dogramaci et al.. 2004). Squash bugs prefer feeding in the genus *Cucurbita*, but will readily feed on watermelon if it is the only food source. There are 27 species in the genus *Cucurbita* from the western hemisphere, with many varieties in the *C. pepo* group being selectively bred for human consumption. Other common cucurbits bred to be less bitter are cultivars in the species *Cucurbita maxima* and *Cucurbita moschata* (Robinson and Decker-Walters 1996).

Cucurbits native to Oklahoma are not commonly found in great enough numbers to support abundant populations of squash bugs. That is assuming that the squash bugs can even survive on the native plants. Bonjour and Fargo (1989) showed that squash bug nymphs do not develop past the 2$^{nd}$ instar on Buffalo Gourd *Cucurbita foetidissima* Kunth, a cucurbit native to OK. Cook and Neal (a,b) (1999) found that squash bugs do not develop well on cucumber during their experiments on how squash bugs select acceptable plant hosts. None of the other wild species of cucurbits in Oklahoma are in the genus *Cucurbita* (Hoagland et al.. 2004). There are occasional reports of wild cucurbit cultivars establishing in and around farm fields, but these species generally do not begin growing before fields are planted to domesticated species. Large populations of squash bugs generally only develop on cucurbits grown in gardens or farms.

**Squash Bug Damage**

The crop damage in a specific location caused by the squash bug is related to the density of squash bugs that survive the winter, and how that can find the food source in the spring. Because the adult bugs overwinter in debris in and around fields, these bugs can find seedlings that are planted early quite easily. The overwintering habitat sites used by the squash bug have been anecdotally mentioned in literature, but have never been systematically sampled. Attempts at caging squash bugs with various substrates to determine overwintering survival have resulted in extremely high mortality rates (Beard

1940. It is possible that squash bugs do have a very high mortality rate while finding or residing in overwintering sites regardless of the condition of these sites.

Knowledge of where insects overwinter provides valuable data used to predict the damage that a pest species can do during the next growing season. Lam and Pedigo (2000) sampled the overwintering sites of bean leaf beetles, and along with the data for density of late season adults, they were able to estimate winter mortality. If a sampling technique could be established to find squash bugs in overwintering habitats, the likelihood of crop damage by the squash bug the following spring could be predicted. Predicting the survival of insects based on winter habitat and temperature can be invaluable to controlling insect pests. Boll weevil overwintering survivorship knowledge is used to manage that pest (Parajulee et al. 2001). The squash bug has similar habits to these beetles, and the techniques used in these studies could be applied to sampling and control efforts of the squash bug.

Squash bugs damage the plants they feed on by mechanical damage, direct loss of nutrients and water from the plants, and by transmitting pathogens. Their damage to individual plants increases with increasing density (Woodson and Fargo 1991). Squash bugs are primarily xylem feeders, although the stylets do penetrate stem and phloem cells and analysis of gut contents showed that components of these other cell types are ingested (Neal 1993). The typical plant damage caused by the squash bug, called Anasa wilt, is the appearance of burned and dried edges around the leaves. This burnt look precedes general wilting and plant death (Fargo et al. 1988). This is most likely created from the blockage of the xylem vessels of the host plant. Once a significant portion of the vessels in the plant stem has been destroyed, the plants can no longer provide enough water and nutrients to the leaves, thus creating the burnt effect on the leaves. A feeding toxin has not been isolated from squash bug saliva (Beard 1940, Neal 1993).

Cucurbit Yellow Vine Decline, or Yellow Vine Disease is associated with the bacterium *Serratia marcescens* Bizio. Squash bugs have been confirmed as a vector of this pathogen under lab conditions (Pair et al. 2004). Adult bugs were field captured in November, and these were placed on seedling plants. Eleven percent of the bugs transmitted the bacterium, but no plant death occurred on seedlings with 3-5 true leaves. In the field the symptoms occur around the time of fruit set, and involve yellowing of the leaves, and wilting. The disease is preliminarily identified by viewing a cross-section of the stem at ground level. The phloem will appear honey colored in infected plants, instead of a translucent color of healthy plants. *Serratia marcescens* is a common cosmopolitan bacterium, and is the first pathogen implicated with a squash bug vector.

Watermelons have previously been shown to be a poor food source for squash bugs (Bonjour and Fargo 1989). This, however, does not explain the fact that high populations of squash bugs can grow throughout the summer in watermelon fields in southern Oklahoma (Dogramaci et al.. 2004). In the early spring, watermelon growers transplant relatively expensive seedless cultivars to ensure a crop of watermelons in time for the high prices found on the Fourth of July holiday. Transplanting seedless cultivar watermelon seedlings is a high cost production practice for growers. The seeds cost as much as $0.17 each, and that cost escalates when price of transplanting is taken into account (Edelson et al.. 2002).

These early-planted watermelon seedlings must be protected from a variety of insects and environmental conditions. They are planted on raised beds covered in black plastic to ensure heat retention and stop weed growth. The major insect pests of these young plants are cucumber beetles and squash bugs (Pair 1997). Cucumber beetles can cause serious damage to the stems of the young plants by chewing through parts of the stem or by defoliation. This can cause death of the seedling by either direct damage to the xylem and phloem vessels, or by weakening the rigidity of the stem. The compromised structure of the stem is then easily snapped by wind and rain, both of which are very common in southern OK during the spring. Squash bug adults emerging from diapause move into the fields during the middle of April to early May. These large bugs destroy the vascular tissue of the stem (Neal 1993). Two squash bugs can kill a young seedling while feeding (Edelson et al.. 2002).

**Overwintering Behaviors**

The diapause initiation of the squash bug has been studied to define the environmental control mechanism. The bugs leave the fields during August in northern states, and do not leave the field until September in more southern states. There are three main conditions that could cause the bugs to go into reproductive and behavioral diapause: lack of food source, ambient temperature, and day length. Each of these have been shown to affect the diapause of many other insects (Fielding 1990). The day length was experimentally controlled by Fielding (1988) under laboratory conditions to find if the day length was the prime controller of diapause initiation. Individual squash bugs were reared in a laboratory on squash plants under a long day length period of 17 hours of light and 7 hours of darkness. Individuals from this colony were then held at constant temperatures while the length of light was altered. It was found that the critical time needed to initiate diapause in 100% of the insects was 14 hours of light. With only a half-hour more of light, the diapause initiation rate was about 50%.

Temperature also slightly affects the diapause of squash bugs. A decrease in temperature increases the likelihood of diapause at that critical 14.5 hours of daylight (Fielding 1988). There are 13 hours of daylight in southern Oklahoma during the second week of April, and in order to bring the bugs out of diapause, there was a minimum time of 13 hours of daylight needed in Fielding's study. Increasing temperature and adding a food source aided in bringing the squash bugs out of diapause conditions in this study. The squash bugs were able to reproduce more quickly with the additions of food and higher temperatures.

The developmental rate of squash bugs has been elucidated by research at varying temperatures. Under constant temperatures, the squash bugs developed most rapidly at 26.5° C (Fargo and Bonjour 1988). This information was used to create a degree-day model of the development of squash bugs. Squash bugs can thermoregulate their temperature to optimize development in natural conditions by basking and shading to maintain an optimum temperature. An accurate degree-day model is essential for tracking squash bug growth, but only if there is an accurate measurement of when the bugs moved into the field in the early spring to define an optimum time to begin surveying for eggs laid (Fargo and Bonjour 1988). It has been suggested that by delaying the planting date of many cucurbit crops, the population of squash bugs can be regulated year-round (Fielding and Ruesink 1988). This is probably only partially applicable for southern OK where there can be more than one generation per year. By delaying the planting date of squash, watermelon, and pumpkin crops, the overwintered adults will need to find other suitable host plants for a few weeks. This will cut down on the number of early season eggs, which will, in turn, create lower populations to lessen the damage to crops. Early planted squash will have higher population of squash bugs earlier, because of their larger leaf area (Palumbo 1989). This plan assumes that the squash bugs remain on one host plant after they emerge from overwintering sites.

**Sampling Techniques**

Describing the movement of insects is an important and daunting task for entomologists. Immigration and emigration are extremely important to consider when working with insects that can rapidly migrate into a field and decimate a crop. Understanding how an insect population can quickly reach economically damaging levels often depends on knowledge of insect movement. Squash bugs most likely fly into a field of cucurbit hosts. Dogramaci (2003) found that in watermelon fields and on watermelon plants squash bugs are equally distributed. The have aggregated dispersion, but on a specific plant that is infested there is never a large amount around the periphery of the plant, indicated that the bugs do not walk to a plant and immediately begin feeding. Also, there are no edge effects in watermelon, showing there are just as many suqash bugs in the center of the field. The bugs fly into the field and land, usually settling on a plant where there are already squash bugs. There have been many ways of tracking insect movement examined, from the simple to the extreme. Most tracking of insects is done with sampling of different areas throughout time, without specific insect marking (Pedigo 2002). Techniques range from watching an individual insect move through a field, to internally dyeing thousands of moths and releasing them to see how far they could fly.

The use of mark and recapture techniques have been a staple of population dynamics research and are based on assumptions of estimating populations. There are seven assumptions used when estimating a population using mark and recapture studies. The marking technique must not affect behavior or longevity, the mark is permanent, and the marked insects mix completely with the population. Also, the probability of catching a marked individual is the same as a non-marked individual, sampling must quick, the population is closed, or immigration and emigration is accounted for, and there are no births and deaths during the sample time (Southwood 1978).

Methods to mark insects have been proposed in many forms that can be tailored to the specific need of each situation. Squash bugs have been marked with paint and released in the agricultural fields in a few studies, but these studies have had limited success with recapture (Beard 1940, Fargo et al. 1988). The fact that the insect body form varies so much, makes a standard marking technique impractical for insects. Some insects are too hairy to allow paint to be an effective marker, or the insect may be too small paint. Other marking techniques are necessary, and each must be tailored to the situation. For marking to be effective, the process must be time and labor conscious, the mark should be conspicuous enough to be measured, and must be permanent during the time when samples are taken (Pedigo 2002). Methods of marking insects range from marking individual insects with either paint or by damaging the cuticle to marking populations with rare element tags. Supporting research must be done on any marking technique to ensure that it will not affect the survivorship, behavior, or movement of the insect. If any of these are affected, the data gained about a population will have limited validity.

Some ways that mass marking has been used include the use of radioactive dyes or food, along with marks that include rare trace elements (Akey 1991). These trace elements are fed to the insects in higher quantities than normal surrounding conditions could provide. These techniques can be applied to tag vast numbers of individuals by either catching the insects and feeding them or by lacing their natural food sources with the material. While providing a marking food source, it is most likely impossible to accurately count how many insects have actually been marked. These types of marking are used for presence or absence studies in a given location.

Potato beetles were marked and released in a central location and were then recaptured in trap potato crop fields. The fields were sampled for beetles for 5 to 20 days in each of the four cardinal compass directions extending from the release point. The beetles were shown to move quite rapidly from the field to disperse to uninhabited potato plants. A maximum of 30% of the marked beetles were recaptured, and the number recaptured decreased rapidly based on distance from the release point. It was suggested that potato beetle populations would be limited if potato field rotation areas were at least 0.5 km away from previous infested areas (Follett et al. 1996).

The use of the Global Positioning System (GPS) has been an invaluable tool to accurately record location data for many animal studies. The increased accuracy of modern handheld GPS devices are limited by the signals from the satellites, not by the technology of the unit. With the current technology, these devices are uniformly accurate down to 3 meters. When tracking the movement of insects, the range they travel can be as minute as they are, or long distances. Accurate data, and the technology to back up irrefutably where that data came from could be the deciding factor of whether an experimental study has merit. The spatial structure of insect pests in fields can vastly influence pest management techniques. The movement of overwintered adult Colorado potato beetles was monitored using GPS distance data by Blom and Fleischer (2001). This detailed information was then used to track trends of in-field movement of the pest beetles as the season progressed.

Because of the importance of the squash bug as a pest of early season cucurbits, the movement of these insects must be further studied. The types of overwintering sites in which squash bugs are found have been described by Beard (1940). The types of natural habitats were not systematically investigated. All the natural cues for emergence from overwintering are not known, and it would be helpful to attempt to find predictors for emergence timing. The following chapters will investigate overwintering habitats, as well as track emergence movement in an agricultural setting. For these objectives, there are three major phases of the study. First is finding the location and type of habitats of overwintering squash bugs near a previous infestation point. The next objective is to determine the earliest bug emergence and subsequent field infestation. And finally, immigration and emigration of overwintered adult squash bugs to and from a squash field through the early growing season will be observed.

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# Chapter II

# THE OVERWINTERING HABITATS OF THE SQUASH BUG, *ANASA TRISTIS* (DEGEER)

## INTRODUCTION

The squash bug, *Anasa tristis* (DeGeer), is a pest throughout the US, and has been shown to reduce yield in many cucurbit crops, including pumpkins, squash, and more recently watermelon. The Squash bug feeds on the plant xylem and blocks the vessels when stylets are removed (Neal 1993, Neal 1999). In this way, the bugs not only damage the plant by feeding on the resources necessary for the plant's growth, but they block water movement up the stem. Especially susceptible to this feeding are young seedling plants (Edelson 2002). Mature squash plants can tolerate more damage (Fargo et al. 1988, Fargo and Bonjour 1988). The damage done by bugs in the early growing season is also more devastating because of the low temperatures found during these times (Edelson 2002). The plants are not functioning at their optimum temperatures, and they do not repair as easily.

Adult squash bugs overwinter in protected habitats, but these habitats have never been systematically surveyed in landscapes surrounding cropping habitats. The types of overwintering habitats have been anecdotally described as any litter in a farm situation that provides shelter from the cold, and experimentally created overwintering habitats for squash bugs have repeatedly failed to provide mortality rates less than 90% (Beard 1940). A new investigation into the natural overwintering habitats of the squash bugs is needed to help pest managers control this harmful pest. In the south central U.S. the overwintering adults emerge as early as the last week of April, and these bugs begin searching for host plants (Fargo et al. 1988). At this time of year, the plants suitable for feeding are early planted watermelon and squash. The seedling plants cannot tolerate squash bug feeding because at a density of one bug per plant, growth and yield is reduced (Edelson 2002).

Diapause initiation and termination in the adult squash bug has been found to be mostly daylight mediated (Fielding et al. 1988, Nechols 1988). At about 13.4 hours of daylight, the diapause condition of the squash bug may be terminated. This does not mean that the bugs are incapable of movement before that. The occurrence of reproductive diapause does not effect the muscle activity and feeding capability of squash bugs. The bugs are metabolically slow, but can still feed and move. The natural overwintering habitats of squash bugs have been experimentally manipulated with limited success (Beard 1940). So, in this study we attempted to catalogue the habitats of the adult squash bug during its winter reproductive diapause under field conditions.

## METHODS AND MATERIALS

### Habitat Surveying

One field and the surrounding area in Atoka county was studied during the months of January through June of 2003 and 2004 to examine the overwintering habitats of squash bugs, *A. tristis*. The fields were planted with yellow crook neck squash, *Cucurbita pepo L,* 'Lemondrop' during the previous year, and the area had confirmed large populations of squash bugs (Edelson et al. 2003 and Dogramaci et al.. 2004). The field was plowed and cultivated at the end of the growing season in the fall of 2002 and 2003. There were confirmed squash bug populations in the field both years prior to the winter surveys. A winter wheat cover crop was planted on half of the field during 2002 and the entire field was planted with wheat in 2003.

The area in and surrounding the field was sampled extensively in transects extending beyond the boundaries of the field. All squash bugs encountered were tallied and sex was determined, along with the latitude and longitude of each sample recorded by handheld GPS. Wooded areas to the south that were not adjacent to the field were also surveyed along transect lines. The 21 (covering 570 meters in various habitat types) transect lines were run in three environment types, cultivated field, short-grass pasture, and oak-hickory forest (Appendix 1). All transect line samples were taken every 3 meters. The sample area was 0.25 square meters, and all plant material and litter was searched visually for squash bugs, as well as the soil to a depth of approximately 4 cm. Transect line sampling was initiated in the center of a one hectare field that was naturally infested with squash bugs during the fall of the previous years. The lines were mapped to the four cardinal compass directions (Appendix 1). Thirty samples were taken running from the center of the field in the cardinal directions in 2003 and 2004. These lines extended from the field into the short-grass pasture adjacent to the field. The length of these transect lines were different due to the terrain itself, and time constraints. The north line was bounded by a road, the west by fences and trees, and the east by another crop field. Next, randomly selected lines were chosen to begin at the edge of the wooded area south of the field. These lines began at the pasture edge and headed straight into the woodland. Three or four samples were taken on each line (9-12 meters). Thirty-one samples were taken in this way in 2003, and 30 samples were taken in 2004.

Likely overwintering sites were arbitrarily selected and monitored for squash bugs. Only 5 were selected in 2003, but 23 were surveyed in 2004 (Appendix 1). These sites included fallen trees, sheltered leaf debris or farm debris (old boards, mulch piles, discarded vegetables). All squash bugs found in the sample sites were marked with acrylic paint (Testors® Acryl®, The Testors Corp., Rockford, IL) and released. The paint was tested for one month on 10 squash bugs reared in a colony to determine its effects on the bugs' behavior, and its adhesion. Bugs painted with the Testors® Acryl® paint did not have altered behavior as seen with bugs marked with oil based paint and with permanent markers. The bugs found in overwintering sites were marked with dots on the pronotum in a unique color and pattern to delineate the site in which they were found. The overwintering sites where bugs were found were monitored and surveyed weekly from late January until May. All locations surveyed were recorded using GPS for defining latitude and longitude.

During the first two weeks of October of 2003, the field with infested squash plants was surveyed twice for adult squash bugs, and these were marked with a specific color of acrylic paint (Orange). If these bugs were found the next year in the field, or in overwintering sites, the distance traveled from the field could be determined.

A total of six squash bugs were collected (n=4 (2003), n=2 (2004)) from the field and dissected to confirm the conditions of reproductive diapause (Appendix 2). Four female field-collected bugs were dissected in 2003, and two were dissected in 2004. Small samples were used because of the very low population of bugs found in overwintering sites. Additionally, the spring movement of these overwintered bugs was part of a separate study, and it was important to have as many painted bugs as possible survive in overwintering sites (see next chapter). Four actively reproducing female bugs were also removed from squash bug rearing cultures at the same time as the field collection and dissected in the same following manner. The bugs were captured and immediately prepared for dissection by removing the wings. The tergites of the abdomen were removed with a razor blade, and the fat body was observed before being removed to view the condition of the ovaries. The bugs were dissected then placed in 70% ethyl alcohol for photographing.

## RESULTS and DISCUSSION

During 2003 we conducted surveys on 17 separate days from February 7, 2003 to May 13, 2003, and in 2004 we conducted surveys on 11 days from February 28, 2004 to May 8, 2004 (Table 1.1, Table 1.2). A total of 149 locations in two years were visually examined in a search for adult squash bugs. Only eight locations were found with squash bugs present. Every location where bugs were found was examined every week thereafter. As outlined in Appendix 1, an area of approximately 6 hectares was searched during each of the two years. The cultivated field comprised approximately 0.60 hectares, the open pasture 3 hectares, and the woodland was about 2.4 hectares in area.

We found no squash bugs along transect lines within the field site, none in the short-grass pasture, and none in the oak-hickory habitats outside the field. See Appendix 1 for an outline of the landscape sampled. The map shows where transect surveys were run, and the locations of habitats that were individually sampled. We found a total of 53 bugs in the selected overwintering sites in 2003 (Table 1.1, Appendix 1), and 17 bugs in 2004 (Table 1.2, Appendix 1). The bugs collected were only painted the first time they were found, so multiple recaptures of any individual was possible each successive sample. The locations of the overwintering sites sampled weekly are also found in Appendix 1. Squash bugs did not move from habitat to habitat in the area sampled, but did move to different parts in the same habitat. For example, if a bug was captured under a piece of bark on the top of a decomposing log, that bug may later be found in a pile of bark beside that same log.

Table 1.1. Number of adult squash bugs marked and recaptured per week at the 4 locations bugs were found in Atoka Co., OK during 2003

| Date | Bugs marked | Bugs recaptured | Total viewed |
|---|---|---|---|
| 2/7/2003 | 1 | 0 | 1 |
| 2/13/2003 | 4 | 0 | 4 |
| 2/14/2003 | 8 | 1 | 9 |
| 2/20/2003 | 7 | 3 | 10 |
| 3/7/2003 | 0 | 5 | 5 |
| 3/14/2003 | 3 | 3 | 6 |
| 3/17/2003 | 1 | 4 | 5 |
| 3/19/2003 | 12 | 8 | 20 |
| 3/21/2003 | 8 | 7 | 15 |
| 3/27/2003 | 1 | 10 | 11 |
| 4/11/2003 | 5 | 15 | 20 |
| 4/17/2003 | 1 | 6 | 7 |
| 4/25/2003 | 2 | 6 | 8 |
| 5/1/2003 | 0 | 7 | 7 |
| 5/8/2003 | 0 | 7 | 7 |
| 5/12/2003 | 0 | 1 | 1 |
| 5/13/2003 | 0 | 0 | 0 |
| Totals | 53 | 83 | 136 |

* Individual recaptured squash bugs were counted multiple times causing more recaptures than marks

Table 1.2. Number of adult squash bugs marked and recaptured per week at the 4 locations bugs were found in Atoka Co., OK during 2004

| Date | Bugs Marked | Bugs Recaptured | Total viewed |
|---|---|---|---|
| 2/28/2004 | 0 | 0 | 0 |
| 3/6/2004 | 1 | 0 | 1 |
| 3/13/2004 | 2 | 0 | 2 |
| 3/20/2004 | 0 | 0 | 0 |
| 3/27/2004 | 3 | 0 | 3 |
| 4/3/2004 | 3 | 2 | 5 |
| 4/10/2004 | 2 | 1 | 3 |
| 4/17/2004 | 3 | 2 | 5 |
| 4/24/2004 | 2 | 2 | 4 |
| 5/1/2004 | 1 | 1 | 2 |
| 5/8/2004 | 0 | 0 | 0 |
| Totals | 17 | 8 | 25 |

* Individual recaptured squash bugs were counted multiple times causing more recaptures than marks

The condition of the overwintered squash bugs as recorded in this study confirm they undergo a reproductive diapause. The adult bugs were physically active, even at very low temperatures when disturbed. Bugs that were dissected were found to have large yellow fat body accumulations and females showed reduced and nonfunctional ovaries (Appendix 2). The overwintering sites selected by squash bugs appear to be limited to objects large enough for them to find a suitable microhabitat. They were not found to overwinter successfully in the soil of a previously infested field. The field was cultivated at the end of the growing season, a practice recommended for the control of squash bugs. There were also no bugs found in the surrounding pasture surveyed that was covered with grass. Squash bugs were not found to overwinter in grass or the root layer of grass. Finally, the areas surveyed under the forest canopy were comprised mostly of decomposing leaf litter, and again, this was not found to be an overwintering site. Logs that were located in this oak-hickory forest also did not harbor squash bugs. Beard (1940) noted that piles of leaves provided high moisture conditions and that squash bugs placed under the piles died and were infected with entomopathogenic fungus. The logs in the wooded area were also notably damp at all times of sampling.

The only habitats in which squash bug adults were found during winter months were decomposing logs, piles of tree bark, and piles of lumber in the open grassland. These locations were elevated above soil level, and the bugs were never found in direct contact with moist decaying areas of wood. The bugs actively moved to dry areas when disturbed. The results of this study indicate that squash bugs may not successfully overwinter in leaf litter. The leaf litter in this study was quite damp, due to the forest area being a low area. Other study sites with drier forest floors should be investigated further.

Even though the bugs may be physically active, they do not leave the immediate area of a suitable overwintering habitat.

The fact that the area surveyed yielded few insects, and of those found and marked only a few were squash plants in the field the following spring (see next chapter) indicates that squash bugs move farther or are concealed better than was discovered. The following chapter details the immigration of adult overwintered squash bugs into a squash farming setting. Very few of the insects marked while in an overwintering site were found in a squash field the following spring. This reinforces that idea that squash bugs travel to overwintering sites that may be very far from fields they had infested the previous year to overwinter.

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# CHAPTER III

# TRACKING THE SPRING IMMIGRATION OF THE OVERWINTERED ADULT SQUASH BUG, *ANASA TRISTIS* (DEGEER)

## INTRODUCTION

The squash bug, *Anasa tristis* is a pest throughout the US, and can cause yield losses in many cucurbit crops, including pumpkins, squash, and watermelon. The squash bug feeds on the plant xylem and blocks the vessels when stylets are removed (Neal 1993, Neal and Cook 1999). In this way, the bugs not only damage the plant by feeding on the resources, but they block water movement up the stem. Especially susceptible to this feeding are young seedling plants (Edelson et al. 2002). Mature squash plants can tolerate more damage (Fargo et al. 1988, Fargo and Bonjour 1988). The damage done by bugs in the early growing season is also greater because of the low temperatures found during these times (Edelson 2002). The more stresses on the plant, the more damage a squash bug can do.

Adult squash bugs overwinter in protected habitats, but these habitats have never been systematically sampled. The types of habitats have been anecdotally described as early as 1899, and field rearing of bugs in artificial habitats have repeatedly failed (Beard 1940). A new investigation into the natural overwintering habitats of the squash bugs is needed to help pest managers control this harmful pest. The overwintering adults emerge as early as the last week of April in southern Oklahoma (Dogramaci et al. 2004), and these bugs begin searching for host plants (Fargo et al. 1988). At this time of year, the plants suitable for feeding in the south central U.S. include watermelon and other cucurbit crops planted in the early spring. The seedling plants cannot tolerate squash bug feeding, and it has been shown that a density of one bug per plant can cause growth and yield reduction (Edelson 2002).

Population dynamics of the squash bug have been studied in squash by Fargo et al. (1988). Immigration and emigration are important aspects of any study of population dynamics, but it is often overlooked or ignored. There are established techniques to determine the movement of insects in and out of a specific area including crop fields in agroecosystems. Young and Young (2003) compiled numerous techniques of statistical analysis for both closed and open populations. It has been assumed that after adult squash bugs migrate into a field in the spring that the area then contains a closed population (Fargo et al. 1988).

In this study we attempted to study the immigration patterns of the squash bug after the adults emerge from their overwintering locations. The minimum emergence date in southern Oklahoma is not known. Also, it is not known if there is one mass migration into a crop setting in the spring as conditions permit, or if there is continuous immigration. There is also no information about the emigration rates or distances of the squash after they have found suitable host plants.

## METHODS AND MATERIALS

### Sentinel Plants

Squash plants (*Cucurbita pepo* L., yellow crook neck squash, 'Lemondrop') were planted on the perimeter of a field that was naturally infested with squash bugs during the fall of the previous year at the Wes Watkins Agricultural Research and Extension Center (WWAREC) in Lane, OK in late March, 2003. Ten squash plants were planted in a greenhouse weekly in 12.7 cm diameter pots filled with Scotts Redi-Earth® (Scotts-

Sierra Horticultural Products Company, Marysville, OH) and each was fertilized with Osmocote® (15-15-15). The plants were grown to the four true leaf stage before placement in the field. Individual plants of each group were maintained in the greenhouse in order to have the same size plants for replacement of plants in the field. Eight squash plants were placed around the periphery of the fields, with two plants on each of the four sides of the field on March 10, 2003 and March 25, 2004. The plants were placed in the soil at ground level in their pots. After the soil temperature reached 15.56° C, the plants were removed from pots and planted in the soil. These plants were examined three times weekly until the rest of the field was planted with a crop of squash and thereafter the sentinel plants were surveyed every weekday. All squash bugs found were marked on the pronotum with a pattern of dots of paint (Testors® Acryl®, The Testors Corp., Rockford, IL) in a way to indicate the week when they were found using different colors and patterns of dots (Appendix 3). The paint was tested for one month on 10 squash bugs reared in a colony to determine its affects on the bugs behavior, and its adhesion. Bugs painted with the Testors® Acryl® paint did not have altered behavior as seen with bugs marked with oil based paint and with permanent markers.

**Field Plants**

Beginning in late April, 2003, three rows of squash (total of 95) were direct-seeded into the field. The emergent plants were thinned to two meter intervals in the rows and each row had approximately 32 plants. The rows in 2003 were widely spaced at approximately 18 meters. In 2004 the rows were closer together at 3.5 meter intervals.

There were 100 transplanted seedling squash plants in 2004. These plants were monitored daily and all squash bugs found on the plant material and in the area shaded by the leaves were marked with a color code to indicate the week they arrived. The data obtained for each plant every day were recorded, including number of male and female adult bugs, and the color with which each of the bugs was marked. In this way, all squash bugs found in the field (95 plants in 2003, and 100 plants in 2004) and on the eight sentinel plants were counted, marked, and released on the same plant. Bugs were only painted once, there was not attempt made to repaint a previously marked individual. The plants were surveyed between 7 and 11 AM on each date to catch the bugs while they were least active. The cooler temperatures allowed easy capture and ensured the bugs did not move immediately when they were released on the same plant.

In the previous chapter, the marking of overwintering bugs in overwintering sites was discussed. If these bugs were found, they were recorded, and their date of movement to the field was noted (Table 2.6 and Table 2.7)

**Degree Day Model**

Fargo and Bonjour (1988) studied the developmental rate of the squash bug under varying temperatures to create a degree day model. They found that it takes an average of 277.5 Celsius degree days with a minimum threshold of 15.6° C for the squash bug to progress from an egg through all five instars to the adult form. To confirm the approximate time that the first generation of newly emerging adults were found in the field, the date of the first eggs found in the field was noted. The average daily temperatures for WWAREC were logged by an on site weather station (MESONET, Oklahoma Climatological Survey 2004). By using the daily average temperatures, a simple degree day model analysis was performed. The threshold temperature was subtracted from the daily average temperature and the resulting number was accumulated daily. When the degree days exceeded 277.5 Celsius degree days, sampling for adult overwintered squash bugs was ceased. After the date there was no way to ensure that the adults being marked were from the overwintering population. The degree day model adult emergence prediction matched with the occurrence of many field observed fifth instar insects.

**Statistical Analysis**

To determine movement trends through time in the field, the number of marked bugs and total field population abundance were analyzed by averaging the total bugs found week using PROC GLM (SAS Institute Inc. 1997). To determine the relationship between immigration and emigration in the field through time, the trends of increasing or decreasing recaptures through time was checked by chi-square by PROC FREQ (SAS Institute Inc. 1997).

## RESULTS and DISCUSSION

The adult overwintered squash bugs were first noted on sentinel plants in the field at Lane on May 7 in 2003, and on April 27 in 2004 (Table 2.1, Table 2.2, Figure 2.1). During 2003, a total of 1,438 adult squash bugs were marked after emergence from overwintering habitats before the first generation of squash bugs molted to the adult form (Table 2.1, Figure 2.1, Figure 2.2). After the first squash bug eggs were noted, degree days were calculated to predict when the F1 generation of adults would emerge using the model developed by Fargo and Bonjour (1988). Surveys were terminated when the new generation of adults was predicted to appear, and this coincided with field observations of many fifth instar nymphs, some of which were found in the molting process. In 2004, 1473 adult squash bugs were marked after emergence and before the first generation of adults were found (Table 2.2, Figure 2.2). The total number of squash bugs in the field slowly increased from May 7 until May 17, 2003 when a very large migration occurred (Figures 2.1, 2.3, and 2.8). This level of infestation was constant until June 16, when there was another increase in adult numbers (Figure 2.3). During 2004, the total number of squash bugs increased slower than in 2003 (Figures 2.1, 2.4, and 2.8). There were not as many bugs in the fields during May 26, 2004. However, before the large immigration, from April 27 to May 26, 2004, there was a higher average of bugs than in 2003 before that year's large migration. The large increase in newly molting first generation adult bugs during the week of June 16, 2003 can be seen on Figure 2.5 and Table 2.1.

Table 2.1: Number of the squash bugs marked and recaptured in 2003 and calculated percentage of marked adult bugs

| Date (2003) | Number Marked | Number Recaptured | Total counted | Percent (%) of Total Newly Marked |
|---|---|---|---|---|
| 5/6 | 0 | 0 | 0 | 0 |
| 5/7 | 1 | 0 | 1 | 100 |
| 5/8 | 6 | 0 | 6 | 100 |
| 5/9 | 0 | 2 | 2 | 0 |
| 5/12 | 8 | 5 | 13 | 61.5 |
| 5/13 | 10 | 8 | 18 | 55.6 |
| 5/14 | 3 | 11 | 14 | 21.4 |
| 5/15 | 5 | 5 | 10 | 50 |
| 5/16 | 18 | 11 | 29 | 62.1 |
| 5/19 | 158 | 13 | 171 | 92.4 |
| 5/20 | 133 | 65 | 198 | 67.2 |
| 5/21 | 16 | 74 | 90 | 17.8 |
| 5/22 | 13 | 95 | 108 | 12 |
| 5/23 | 37 | 107 | 144 | 25.7 |
| 5/27 | 31 | 50 | 81 | 38.3 |
| 5/28 | 49 | 74 | 123 | 39.8 |
| 5/29 | 45 | 73 | 118 | 38.1 |
| 5/30 | 90 | 94 | 184 | 48.9 |
| 6/2 | 157 | 121 | 278 | 56.5 |
| 6/3 | 50 | 146 | 196 | 25.5 |
| 6/4 | 98 | 140 | 238 | 41.2 |
| 6/5 | 76 | 183 | 259 | 29.3 |
| 6/6 | 22 | 238 | 260 | 8.5 |
| 6/9 | 149 | 107 | 256 | 58.2 |
| 6/10 | 40 | 101 | 141 | 28.4 |
| 6/11 | 107 | 81 | 188 | 56.9 |
| 6/12 | 79 | 147 | 226 | 35 |
| 6/13 | 37 | 164 | 201 | 18.4 |
| 6/17* | 475 | 144 | 619 | 76.7 |
| 6/18* | 405 | 321 | 726 | 55.8 |
| 6/19* | 248 | 408 | 656 | 37.8 |
| 6/20* | 294 | 479 | 773 | 38 |
| Totals | 2860 | 3467 | 6327 | Avg 45% |

*These counts were not included in statistical analyses because the large increase in adults marked may have been due to occurrence of new adults of the F1 generation

Table 2.2: Number of the squash bugs marked and recaptured in 2004 and calculated percentage of marked adult bugs

| Date (2004) | Marked | Recaptured | Total | Percent (%) of Total Newly Marked |
|---|---|---|---|---|
| 4/25 | 0 | 0 | 0 | 0 |
| 4/27 | 4 | 0 | 4 | 100 |
| 4/29 | 3 | 1 | 4 | 75 |
| 5/1 | 6 | 1 | 7 | 85.7 |
| 5/4 | 36 | 6 | 42 | 85.7 |
| 5/6 | 11 | 17 | 28 | 39.3 |
| 5/8 | 17 | 7 | 24 | 70.8 |
| 5/10 | 18 | 15 | 33 | 54.5 |
| 5/11 | 2 | 8 | 10 | 20 |
| 5/12 | 13 | 27 | 40 | 32.5 |
| 5/13 | 9 | 23 | 32 | 28.1 |
| 5/14 | 8 | 22 | 30 | 26.7 |
| 5/17 | 55 | 22 | 77 | 71.4 |
| 5/18 | 15 | 12 | 27 | 55.6 |
| 5/19 | 51 | 18 | 69 | 73.9 |
| 5/20 | 47 | 24 | 71 | 66.2 |
| 5/21 | 40 | 32 | 72 | 55.6 |
| 5/24 | 44 | 23 | 67 | 65.7 |
| 5/25 | 13 | 15 | 28 | 46.4 |
| 5/26 | 54 | 26 | 80 | 67.5 |
| 5/27 | 30 | 19 | 49 | 61.2 |
| 5/28 | 38 | 25 | 63 | 60.3 |
| 5/31 | 267 | 16 | 283 | 94.3 |
| 6/1 | 221 | 41 | 262 | 84.4 |
| 6/2 | 151 | 60 | 211 | 71.6 |
| 6/3 | 35 | 42 | 77 | 45.4 |
| 6/4 | 106 | 65 | 171 | 62 |
| 6/7 | 108 | 61 | 169 | 63.9 |
| 6/8 | 71 | 60 | 131 | 54.2 |
| Totals | 1473 | 688 | 2161 | Avg 61% |

The total number of bugs in the field per week was not constant over time in 2003 (P= <0.0001), nor in 2004 (P= <0.0001) as indicated from comparisons using the PROC GLM procedure (Table 2.3). The results of this analysis indicate that the total populations of weeks 1 and 2 were not significantly different (P= 0.4783). Neither were the populations from weeks 3 and 4 (P= 0.4841). In 2004, the total number of bugs in the field per week also did not hold constant. The totals slowly increased over time, with the first 3 weeks holding consistent (P= >0.05), and weeks 2, 3, 4, and 5 holding consistent (P= >0.05). The average populations in the field during the final weeks (6 and 7) were not different (P= 0.1228).

Table 2.3. Comparison of the average daily total population of adult squash bugs in the field after spring emergence (PROC GLM)

| Week After Emergence | Avg. Total 2003 | Avg. Total 2004 | Avg. Total Combined |
|---|---|---|---|
| 1 | 1.8 **a** | 3.8 **a** | 2.7 **a** |
| 2 | 16.8 **a** | 31.3 **ab** | 22.3 **a** |
| 3 | 142.2 **b** | 29 **ab** | 85.6 **b** |
| 4 | 126.5 **b** | 63.2 **b** | 91.3 **bc** |
| 5 | 246.2 **d** | 57.4 **b** | 151.8 **c** |
| 6 | 202.4 **c** | 200.8 **c** | 201.6 **c** |
| 7 | N/A | 150 **c** | 150 **bc** |

Values within a column followed by the same lower case letters are not significantly different (Fisher's protected significant difference procedure $\alpha = 0.05$)

The total number of bugs marked per week did not hold consistent through time in 2003 (P= <0.01) or 2004 (P= <0.0001). In 2003, the number of bugs marked during the first two weeks did not vary (Table 2.4). The final four weeks also had similar numbers marked (P= >0.05). In 2004, weeks 1 through 5 showed similar numbers marked (P= >0.05). Finally, week 7 had similar numbers of bugs marked as weeks 4, 5, and 6. This shows that there was not one mass migration during the first few weeks, but there was a consistent immigration into the field.

Table 2.4. Comparison of the average daily population of marked adult squash in 2003 and 2004 after spring emergence (PROC GLM)

| Week After Emergence | Avg. # Marked 2003 | Avg. # Marked 2004 | Avg. # Marked Combined |
|---|---|---|---|
| 1 | 1.4 **a** | 3.3 **a** | 2.2 **a** |
| 2 | 8.8 **a** | 21.3 **ab** | 13.5 **ab** |
| 3 | 71.4 **b** | 10 **a** | 40.7 **ab** |
| 4 | 53.8 **ab** | 41.6 **ab** | 47 **ab** |
| 5 | 80.6 **b** | 35.8 **ab** | 58.2 **ab** |
| 6 | 82.4 **b** | 156 **c** | 119.2 **c** |
| 7 | N/A | 89.5 **bc** | 89.5 **bc** |

Values within a column followed by the same lower case letters are not significantly different (Fisher's protected significant difference procedure $\alpha = 0.05$)

The results of the Chi Square test indicated that the ratio of newly marked to recaptured squash bugs was different in 2003 and 2004 (Table 2.5). In 2003, the ratio of bugs in the field newly marked decreased as time progressed ($P<0.05$). This means the likelihood of recapturing bugs increases with time. In 2004 the opposite was seen, with the ratio of marked bugs increasing with time ($P<0.05$). The likelihood of recapturing bugs actually decreases as time progresses in 2004. These differences may have been due to increased death of squash bugs in the field during 2004. An adult bug parasitoid, *Trichopoda pennipes* (Fabricius), was first noticed and collected in 2004, even when similar collecting efforts were made the previous year. The parasitoid is a large (1.5 cm) brightly colored fly that hovers above squash bugs while laying eggs, and is easily noticed. The fly also feeds on nectar from milkweed plants. These plants were present on the south side of the field, and were easily monitored daily. Finally, the egg laid by *T. pennipes* is yellow, and should have been seen while the bugs were being panted and sexed if it was present. Even though about 1450 bugs were marked during the same time span both years, there were many less recaptures of these marked bugs in 2004.

Table 2.5. Comparison of the average weekly percent of the total population that were newly marked after emergence (PROC FREQ)

| Week After Emergence | Avg. % Newly Marked 2003 | Avg. % Newly Marked 2004 |
|---|---|---|
| 1 | 77.78 | 86.67 |
| 2 | 52.38 | 68.09 |
| 3 | 50.21 | 34.48 |
| 4 | 42.49 | 65.82 |
| 5 | 32.74 | 62.37 |
| 6 | 40.71 | 77.69 |
| 7 | N/A | 59.67 |
| Average | 40.47 | 68.16 |

Values within a column followed by the same lower case letters are not significantly different (Chi Square $\alpha = 0.05$)

The length of time that squash bugs remain in a given field is shown on Tables 2.6 and 2.7. During 2003 (Table 2.6) there were many more recaptures than in 2004 (Table 2.7). The bugs remained in the field for as long as five weeks at a time during 2003 and 2004. Even though there are differences during the two years of this study, it still indicates that many of the bugs immigrate into the field over time. There is not one immigration into the spring when some "unknown optimum condition" is reached. The number of bugs recaptured, with markings indicating the week they were collected, is inflated each week because each individual marked bug certainly was recaptured more than once.

Table 2.6 – Spring 2003: Seasonal recapture matrix of bugs marked from overwintering habitats and six sampling periods in a squash field of about 100 plants through spring 2003. All bugs captured during a single week in the crop field were marked identically. Values indicate the number of recaptured bugs per week. Individual recaptured bugs were likely counted multiple times

| Marking Period (# initially marked that week) | Total Recaptured Bugs Observed Each Time Period | | | | | |
|---|---|---|---|---|---|---|
| | May 7 – May 9 | May 12 – May 16 | May 19 – May 23 | May 27– May 30 | June 2 – June 6 | June 9 – June 13 |
| Feb. – May* (53) | 0 | 0 | 1 | 2 | 4 | 1 |
| May 7 – May 9 (7) | 2** | 19 | 9 | 0 | 0 | 0 |
| May 12 – May 16 (44) | -- | 21** | 41 | 15 | 9 | 9 |
| May 19 – May 23 (357) | -- | -- | 303** | 201 | 196 | 46 |
| May 27– May 30 (215) | -- | -- | -- | 73** | 224 | 67 |
| June 2 – June 6 (403) | -- | -- | -- | -- | 395** | 196 |
| June 9 – June 13 (412) | -- | -- | -- | -- | -- | 281** |
| Total Recaptured | 2 | 40 | 354 | 291 | 828 | 600 |

* Marked while in winter habitat during dates indicated, two individuals were recaptured multiple times in the crop field

** Bugs marked early in the week could be recaptured and counted later that same week

Table 2.7 – Spring 2004: Seasonal recapture matrix of bugs marked from overwintering habitats and seven sampling periods in a squash field of about 100 plants through spring 2004. All bugs captured during a single week in the crop field were marked identically. Values indicate the number of recaptured bugs per week. Individual recaptured bugs were likely counted multiple times

| **Marking Period (total # initially marked that period)** | **Total Recaptured Bugs Observed Each Time Period** | | | | | | |
|---|---|---|---|---|---|---|---|
| | April 27 – May 1 | May 4 – May 8 | May 10 – May 14 | May 17 – May 21 | May 24 – May 28 | May 31 – June 4 | June 7 – June 8 |
| Feb. – April* (17) | 0 | 0 | 0 | 5 | 4 | 4 | 0 |
| April 27 – May 1 (13) | 2** | 13 | 7 | 1 | 0 | 0 | 0 |
| May 4 – May 8 (64) | -- | 17** | 45 | 25 | 7 | 6 | 0 |
| May 10 – May 14 (50) | -- | -- | 43** | 31 | 3 | 0 | 0 |
| May 17 – May 21 (208) | -- | -- | -- | 46** | 51 | 15 | 9 |
| May 24 – May 28 (179) | -- | -- | -- | -- | 43** | 29 | 10 |
| May 31 – June 4 (780) | -- | -- | -- | -- | -- | 170** | 62 |
| June 7 – June 8 (179) | -- | -- | -- | -- | -- | -- | 40** |
| Total Recaptured | 2 | 30 | 95 | 108 | 108 | 224 | 121 |

* Marked while in winter habitat during dates indicated, three individuals were recaptured multiple times in the crop field

** Bugs marked early in the week could be recaptured and counted later that same week

During the first two weeks of infestation, the field had a high density of bugs because there were only 8 early planted sentinel squash plants to support the entire population moving into the field (Figure 2.7, Figure 2.8). The drop in numbers of bugs per plant indicates when the soil temperature was warm enough for the rest of the field plants to emerge. The bugs then moved from sentinel plants into the field proper. There must have also been immigration into the field. Because bugs were found through the field every day, the bugs appear to fly into the field instead of slowly moving into the center of the field as one would expect with insect with walking immigration. The average number of bugs per plant then increases to an average of 1-2 bugs per plant during both years as more and more bugs consistently immigrate into the field.

Because all the bugs were sexed while being marked it was possible to confirm the sex ration of squash bugs of 52% female. By marking all adult squash bugs in one area daily, it was possible to determine the number of bugs moving into the field daily. The recaptured bugs were an indication of how many weeks these bugs remained in the field, which overwintering site they came from, how far they can travel, and how long they can live. Recaptured bugs could and were counted multiple times, which explains how one could consistently have more recaptures than marked bugs through time. The insects that were marked during the winter, before they emerged from overwintering sites, were never the first bugs noticed in the field. This could mean that these bugs did not emerge from overwintering habitats early in the season, or they emerged and survived on non-suitable hosts that were not sampled, or they were feeding in a cucurbit field that was farther from their overwintering site. The field surveyed in this study was the closest cucurbit field to all the overwintering sites that in which squash bugs were found and marked. The descriptive nature of this information is important to describe the behaviors of the adult squash bug which can help provide missing information about this insect's life history. As the knowledge of behaviors and life history of this important pest is broadened, it is more likely that new control tactics will be found to safely and more effectively manage this bug.

The earliest migration of the bug was detected in the field during 2004, on April 27. This coincided with the average soil temperature increasing to a level above 18.3° C (Figure 2.1). The bugs were present in the field for 6 weeks each year before the next generation of adults matured. The degree day model used to predict the development of squash bugs (Fargo and Bonjour 1988) was confirmed in the field by observing the first eggs laid each year and the presence of fifth instar nymphs. Squash bugs require 277 Celsius degree days to mature. Sampling was ended during 2003 at 279 Celsius degree days, and sampling was ended during 2004 at 290 Celsius degree days.

The population of bugs in the field during about 6 weeks of sampling did not increase exponentially as would be expected with a constant immigration and no emigration or death. The population fluctuations were due to weather restricting bug movement or high temperatures leading to increased activity.

The number of marked and recaptured bugs each year indicates that overwintered squash bugs move continually through the spring season (Table 2.5). It was previously assumed that the immigration during the spring occurred once or twice depending on temperatures. This is certainly not the case. There is a constant immigration into the field, but in this study the rate increased one year and decreased the other year. This still tells us that immigration was constantly occurring. Emigration did also occur, because the insects marked in the squash field were found in other cucurbit research fields up to 1 kilometer away. These other fields where marked squash bugs were found had crops ranging from squash, watermelon, and cantaloupe. By using Neal's (1988) diapause termination model of 13.4 hours of daylight, it was possible to determine the earliest that the bugs could begin mating, but not begin movement. In Oklahoma a day length of 13.4 hours occurs about April 20. The squash bug movement found in this study indicates that squash bugs may require a minimum soil (overwintering habitat) temperature of 18.3° C for a few days to initiate movement (Figure 2.1). That temperature is also close to the minimum temperate for most cucurbits to initiate growth.

To control the early emerging squash bugs it has been apparent for years that multiple spray controls are necessary to protect seedling plants. This study has shown that this is the case primarily due to the constant and season-long immigration of overwintered adult squash bugs. During warm days many bugs were seen flying through the field. The farthest any marked bugs were found from the experimental squash field capture site was 1.078 Kilometers. The two bugs found at this distance were feeding on cantaloupe plants. This one example shows that the squash bug, previously thought to only fly very short distances between plants, are strong and active fliers. Control efforts should take this new information into account when sampling for and controlling the populations of squash bugs. Sampling for squash bugs should commence during April in southern Oklahoma, and continue through the spring as immigration occurs.

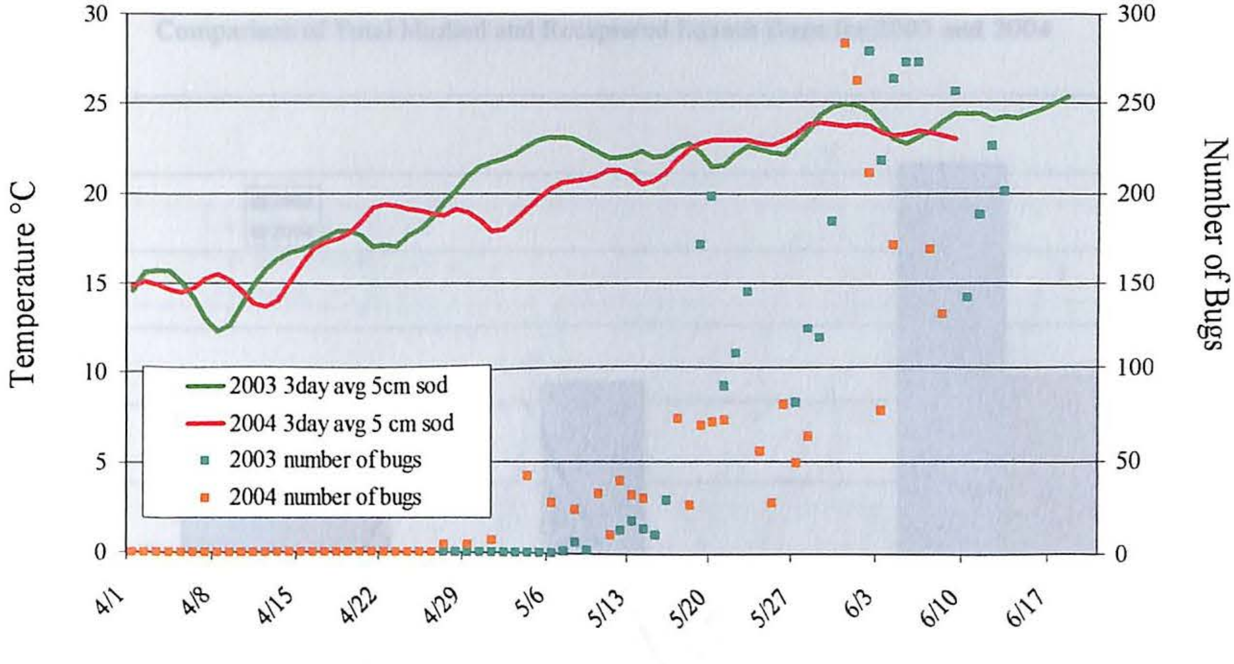


Figure 2.1. Squash bug populations during 2003 and 2004 with specific reference to the average soil temperature

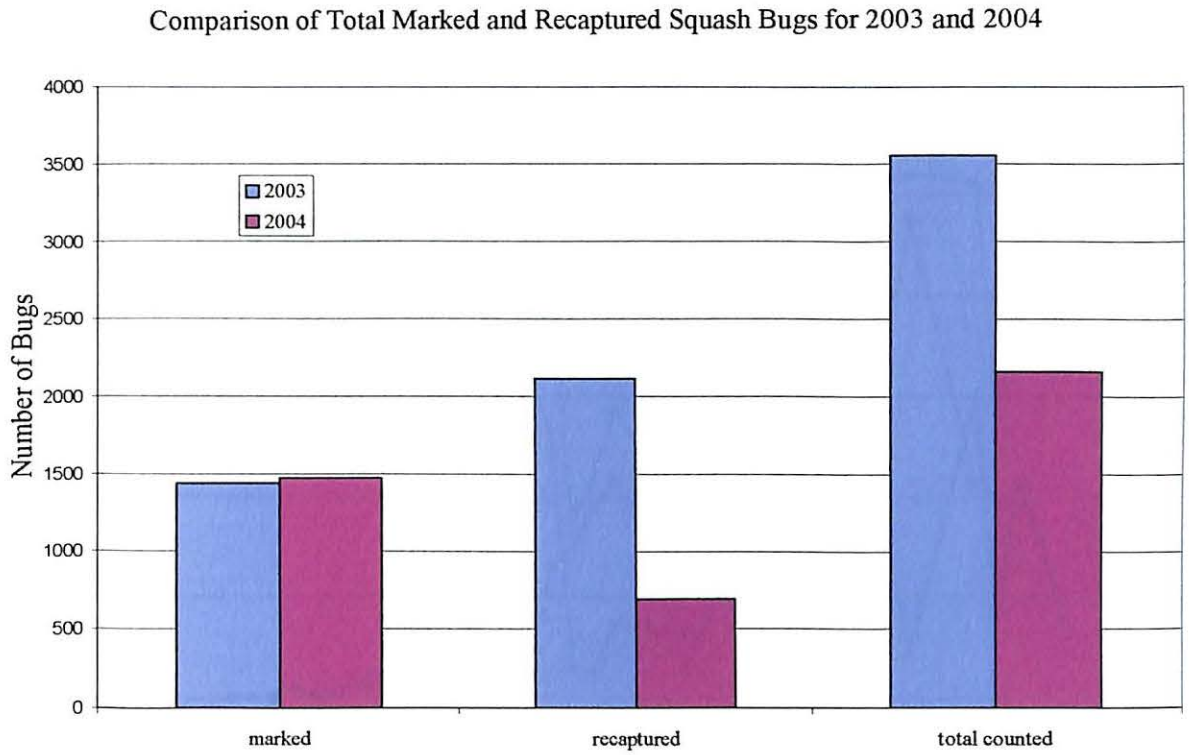


Figure 2.2. Comparison of total marked and recaptured overwintered squash bugs during the springs of 2003 and 2004

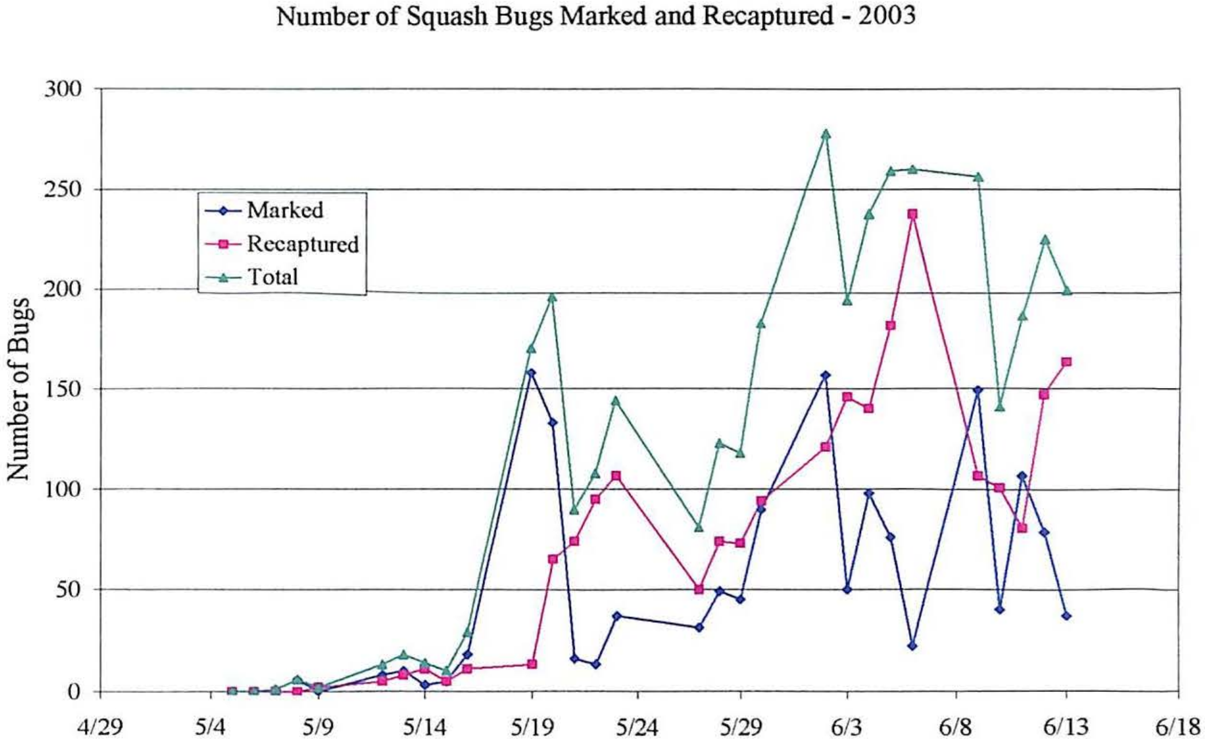


Figure 2.3. Numbers of overwintered squash bugs marked, recaptured, and totals through time, 2003

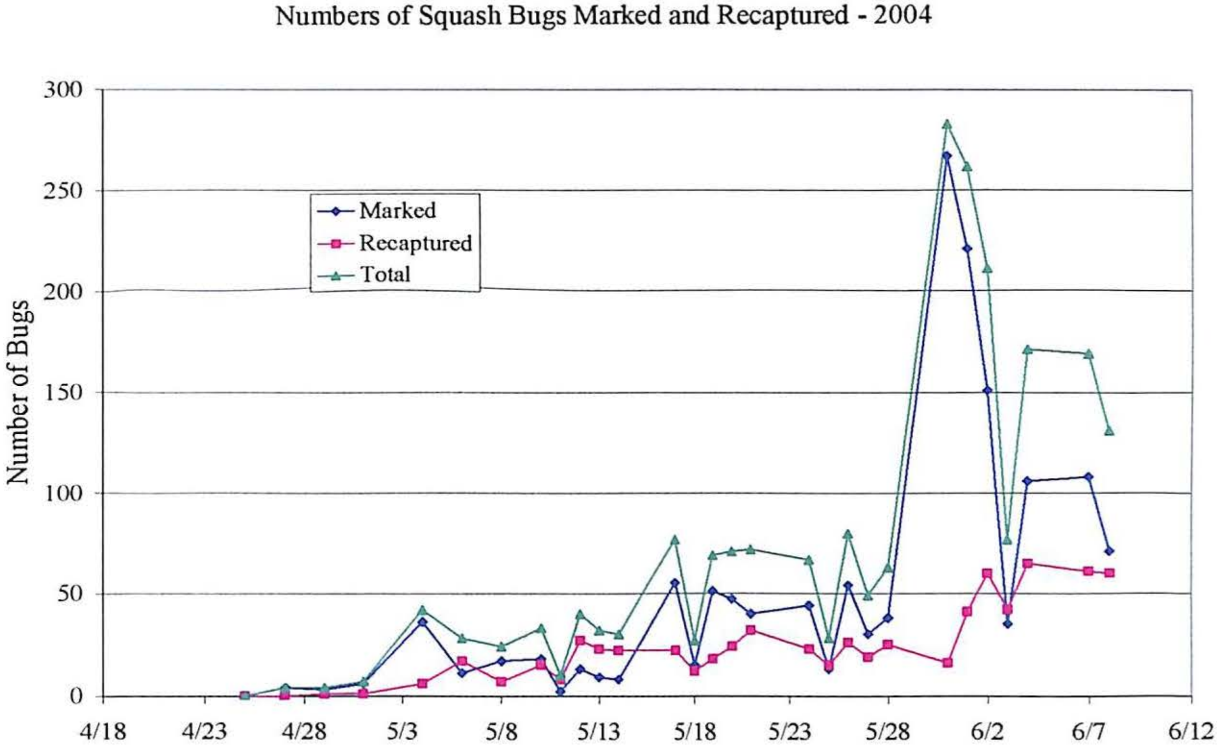


Figure 2.4. Numbers of overwintered squash bugs marked, recaptured, and totals through time, 2004

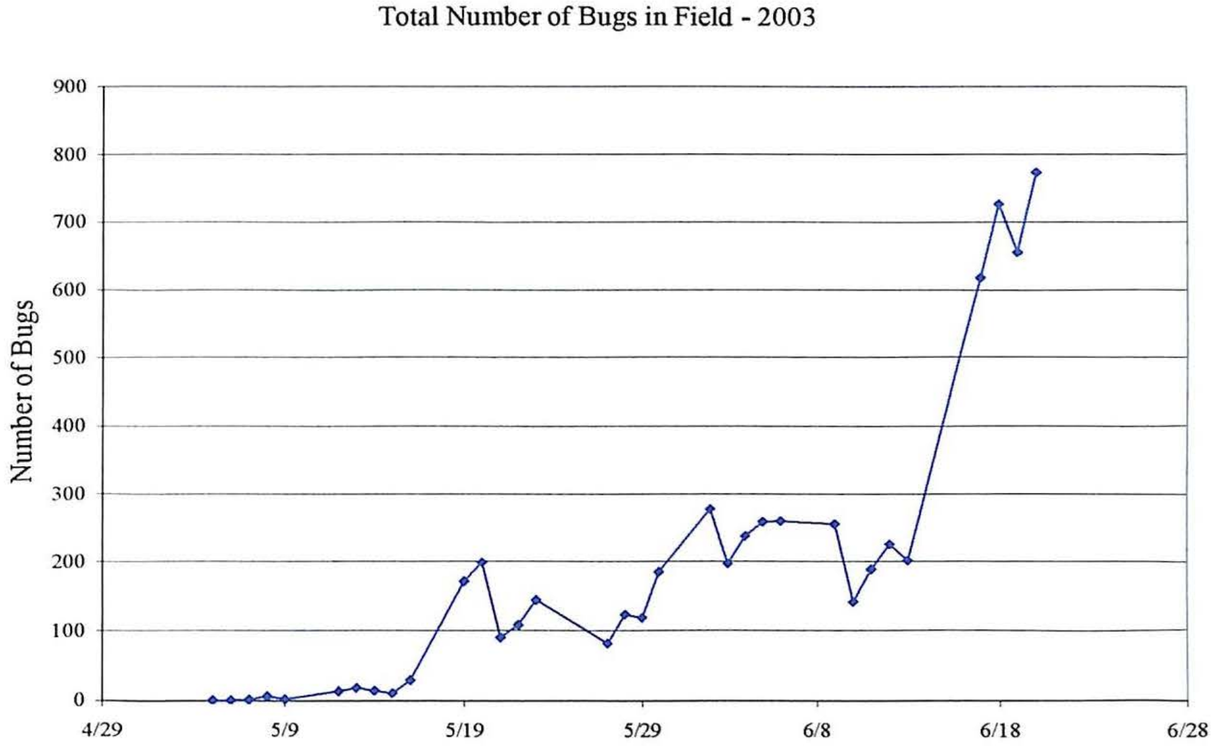


Figure 2.5. Total number of bugs present in the squash field. The last week shows the large population increase that indicates adults of the F1 generation emerging, 2003

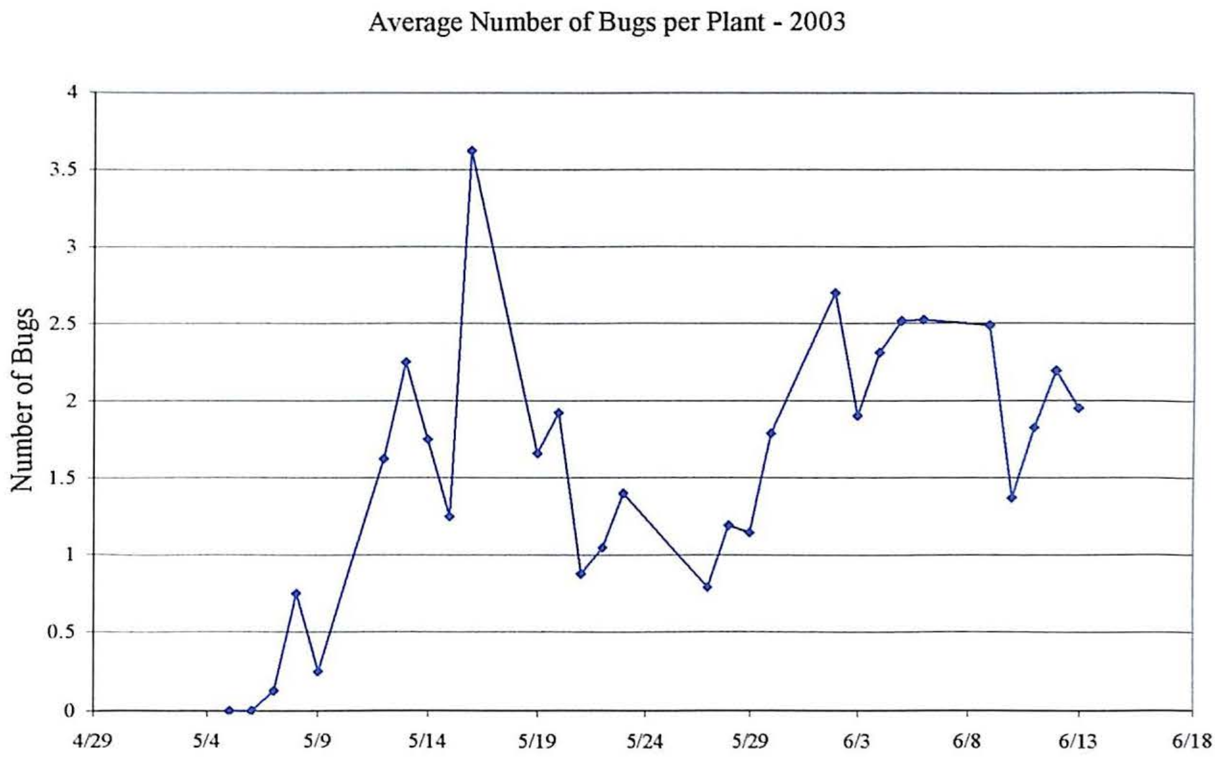


Figure 2.6. Average density of adult squash bugs per plant, 2003

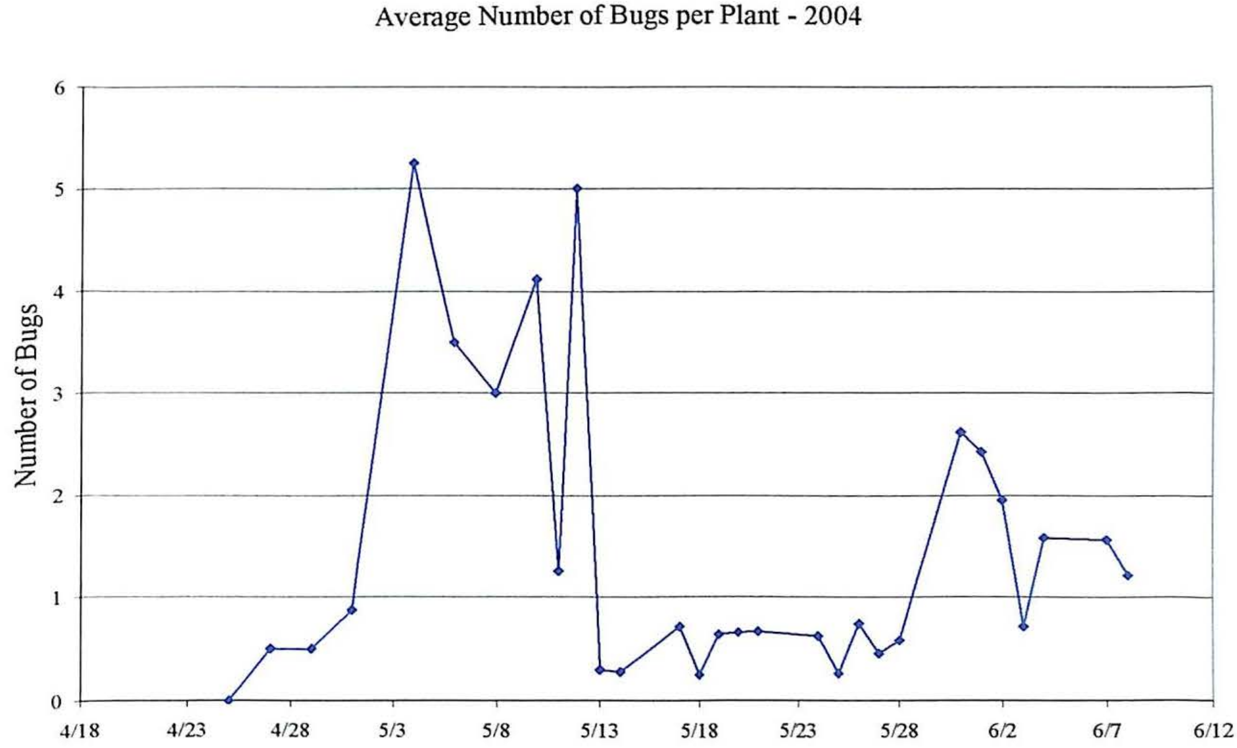


Figure 2.7. Average density of adult squash bugs per plant, 2004

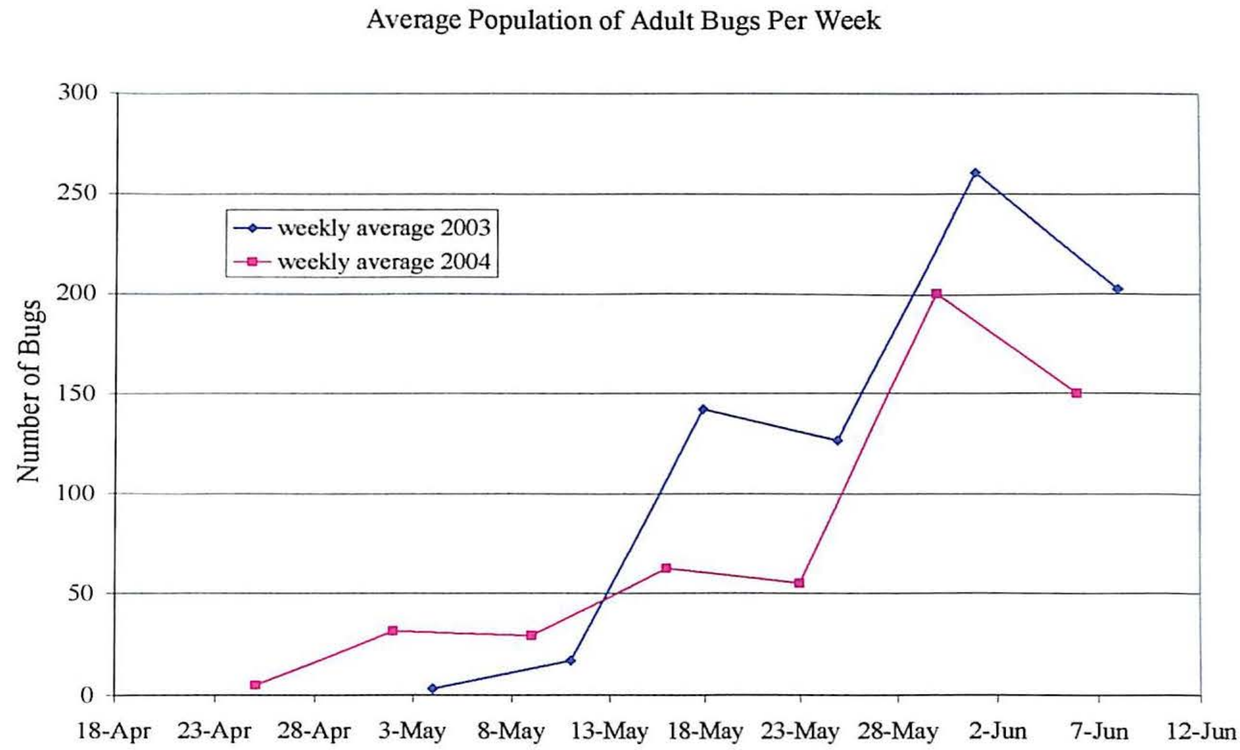


Figure 2.8. Average adult squash bug population present in the field per week during 2003 and 2004

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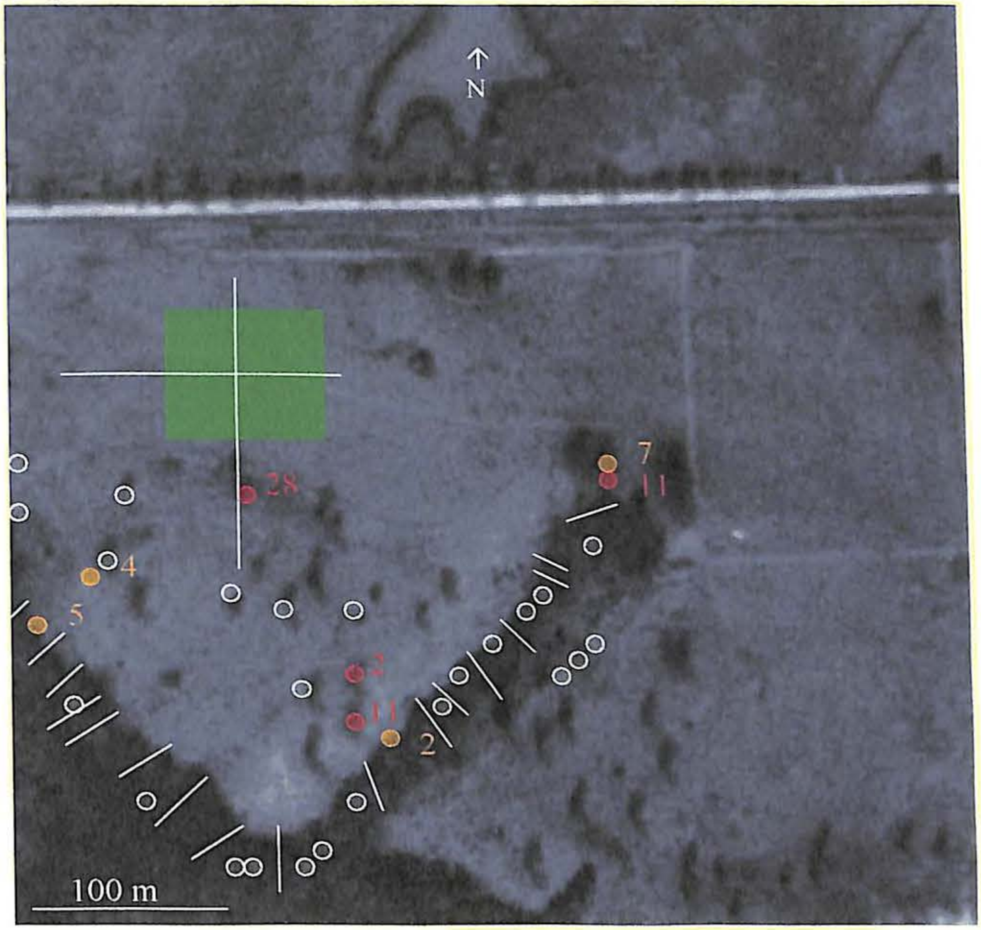
# APPENDIX

Appendix 1: Satellite of landscape surrounding squash field sampled by transects and by selectingoverwintering habitats. Point coordinates provided by handheld GPS during 2003 and 2004




*Numbers indicate the number of bugs found in a location and in which year

Likely overwintering habitats – Bugs Present (2003)

Likely overwintering habitats – Bugs Present (2004)

Likely overwintering habitats – No Bugs Found

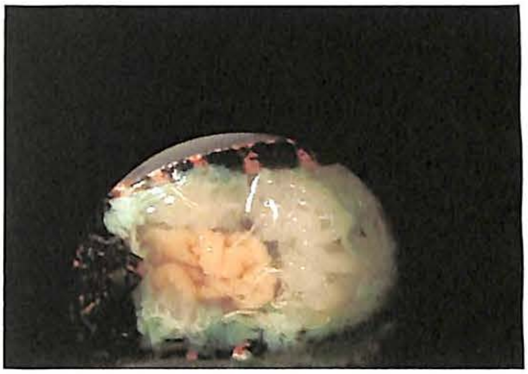
Habitat transect line sampling

Squash field (winter wheat during winter)

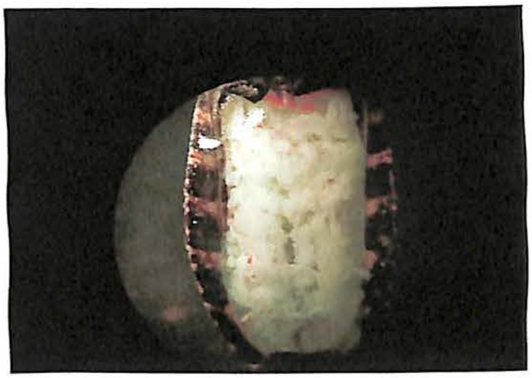
Short-grass pasture

Oak-hickory forest

Appendix 2. Comparison of squash bug internal morphology indicative of reproductive diapause of field collected and lab reared female squash bugs

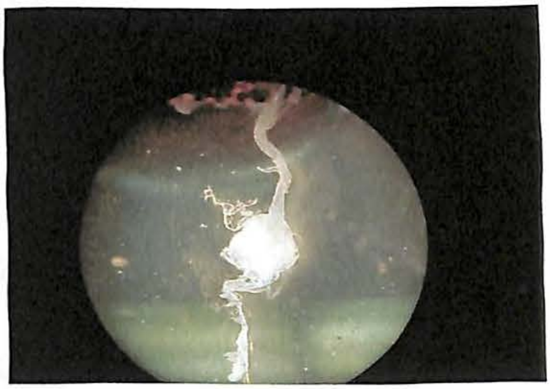


Colony Reared Fat Body



Field Collected Fat Body

Colony Reared Ovary



Field Collected Ovary

Appendix 3. Examples of the Testors® Acryl® paint used to mark adult squash bugs in the winter and spring of 2003 and 2004

Example of the type of paint marking used on adult squash bugs

Three bugs in overwintering site – 2 yellow marks – March 2004

# VITA

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Candidate for the Degree of

Master of Science

**Thesis:** CHARACTERIZING THE OVERWINTERING AND EMERGENCE BEHAVIORS OF THE ADULT SQUASH BUG, *ANASA TRISTIS* (DEGEER)

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