EVALUATION OF POSTHARVEST CONTROL AND DIAPAUSE

OF THE COWPEA CURCULIO

by

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(Under the Direction of ALTON N. SPARKS, JR.)

ABSTRACT

The cowpea curculio, *Chalcodermus aenus* Boheman, is the key pest of cowpeas in the Southeastern USA. There is limited published information available on the life history, trapping, and control of this serious pest. Current commercial control tactics are almost entirely foliar sprays of insecticides that target the above-ground adult life phase of the curculio and are failing to provide adequate control. This research evaluated the use of insecticides and biological control agents, applied post-harvest, targeted at the soil phase of this pest. Additional research focused on potential diapause of overwintering females. This research is the first to document diapause in cowpea curculio and to publish field efficacy data on biological and chemical treatment options targeting the control of the curculio in the soil phase in Georgia.

INDEX WORDS: Cowpea curculio, *Chalcodermis aenus*, weevil, diapause, emergence trap, monitoring, biological control, entomopathogenic nematode, entomopathogenic fungi

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DEDICATION

For my parents and sister who encouraged me to pursue this degree and finish what I had started.

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CHAPTER 1

INTRODUCTION

Cowpea, *Vigna unguiculata* L., also referred to as southern pea, crowder pea, blackeyed pea, and field pea, is one of the most culturally significant specialty crops in the southeastern USA (Riley and Sparks 2019). Historically, USA cowpea acreage peaked with nearly 6 million acres in 1937 when it was planted for cow forage as well as human consumption. Nearly all the acreage was in the Southeast at that time (USDA Statistics 1957). In the most intensively cropped areas of the Southeast, losses due to the cowpea curculio, *Chalcodermus aeneus* Boheman, have been so severe in recent decades that large portions of commercial acreage for fresh frozen consumption have moved out of this region to avoid this one pest (Riley et al. 2014). Economically unacceptable losses are occurring even with the most efficacious labeled insecticides available. There is a near zero tolerance for curculio contaminated peas in the frozen pack process (Chalfant 1997).

On the other hand, cowpeas have recently become more popular worldwide now that consumers are concerned with improving their diets. Consumer interest has likely driven the recent increase in global production of cowpeas from 1.3 million tons in 1981 to 7.0 million tons in 2013 (Goncalves et al. 2016). However, in the traditional cowpea cropping area of the United States, production has declined from 41,888 harvested acres in 1997 to 21,942 harvested acres in 2012 (NASS, 1997 and NASS, 2012) even though the commodity price has risen during this time. The majority of this acreage loss occurred in the Southeastern USA. The inability to consistently control the curculio is likely one of the reasons for the decline in cowpea production in this region. Cowpea curculio females can lay nearly three-hundred eggs in a forty-five day window. Adults damage cowpea foliage in addition to damaging the pods through feeding and oviposition (Arant 1938, Capinera 2001); however, the primary damage occurs as the grub develops within the pod and feeds on the seed.

Pyrethroid insecticides are no longer an efficacious option for controlling this pest which has left growers with a lack of chemical control options that adequately manage the cowpea curculio (Riley 2011, Riley and Sparks 2019). Biological control measures such as the use of natural enemies and fungi have been evaluated against the cowpea curculio but more information is still needed to make confident recommendations to cowpea growers (Bell and Hamalle 1990, Capinera 2001, Daoust 1986). Cultural control methods, such as resistant cultivars and good sanitation methods, have also been shown to be ineffective (Arant 1938, Chalfant et al. 1972, Riley 2011). However, use of a Modified Tedders trap has proven an effective means of population monitoring and may provide better timing of control (Riley et al. 2015).

Previously, the primary method of control for the cowpea curculio has been the application of foliar sprays. The development of resistance in the cowpea curculio has since yielded this control method less effective. The development of more effective control is critical for the continued growth of the cowpea industry in the Southeastern United States. Targeting the soil phase of the curculio's development for control has been investigated in recent years and was a goal of this research project. This approach is novel in targeting a portion of the pest's life cycle which has been largely ignored within management programs because it occurs after crop production. Even if highly effective, implementation by growers may be impeded by a perceived lack of direct benefits during the growing season. This approach relies on a long term commitment to cowpea curculio management at a landscape level.

In order to evaluate the efficacy of each of the soil phase control methods emergence traps were developed and evaluated. In previous studies (Riley and Sparks, personal communication) used several different emergence traps. While all of these traps appeared to function in terms of capturing adult curculios, they had not been formally evaluated nor compared. Development of an efficacious emergence trap was needed for evaluation of the proposed post-harvest control. Potential soil phase insecticides and biological controls that were evaluated were chlorpyrifos, *Heterorhabditis spp*., and *Beauveria bassiana* (Harty 2016). Seasonal occurrence and possible diapause of the curculio were also investigated to increase knowledge of the biology of the insect and potentially improve control methods.

The goal of this research is to provide a better understanding of the biology and potential postharvest control of the cowpea curculio, *Chalcodermus aeneus* Boheman. Potential diapause in this insect was studied through seasonal dynamics of the population and dissection of adults. Post-harvest control strategies were studied in small plot field studies using synthetic and biological pesticides targeting the curculio in the soil phase. The hypotheses tested in this thesis are as follows:

Hypothesis 1 (Chapter 3 - Cowpea curculio emergence trap evaluations)

1) One trap out of several tested would be found to be the most efficacious for sampling the emerging population of curculios from a given area of soil surface under similar environmental conditions surrounding the trap.

Hypothesis 2 (Chapter 4 - Efficacy of biological and chemical agents against the cowpea curculio in the soil)

1) Curculio adult emergence capture rates would be lowest with the most efficacious post-harvest treatment indicating more mortality in the soil phase of the life cycle.

Hypothesis 3 (Chapter 5 - Emergence biology and evidence of diapause in the cowpea curculio)

1) Evidence of diapause in terms of reduced egg development would be detected in the fall generation as compared to the summer generation of cowpea curculio.

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CHAPTER 2

LITERATURE REVIEW

Cowpea, the Legume Crop

Increased emphasis on improving dietary decisions has peaked consumer's interest in legume crops, such as, *Vigna unguiculata* L., cowpeas. These low input crops have also improved the lives of subsistence farmers in Africa and South America (Goncalves et al. 2016). Major world producers of cowpeas are India, Nigeria, Haiti, Myanmar, Australia, and the United States (Anusha et al. 2016). Numerous positive physical and nutritional characteristics have likely caused the increase in global production between 1981 and 2013 from 1.3 to 7.0 million tons (Goncalves et al. 2016). However, in the United States, production has declined from 41,888 harvested acres in 1997 to 21,942 harvested acres in 2012 (NASS 1997 and NASS 2012). This is presumably due to increased pest pressure in the southeastern states, the traditional main production area.

Domestication of cowpeas occurred in Africa over 10,000 years ago, where it is still the most common pulse crop. Cowpeas contribute to meeting the dietary needs of millions of people through their edible shoots, leaves, immature pods, green and dried seed, and the seed can also be processed into flour or paste (Goncalves et al. 2016, Rubatzky and Yamaguchi 1997). In the United States, fresh seed are cooked and eaten, or seeds are dried and processed for later consumption (Rubatzky and Yamaguchi 1997).

In the United States, cowpeas were initially grown in the 19th century in the Southeast for forage, grain and human consumption which has declined dramatically since and later in California for dry blackeye bean production where it has continued to increase (Hall and Frate 1996). It is interesting to note that cowpea curculio has not been reported for California to date. Cowpeas are legumes that also have increased drought tolerance, making them a good candidate for crop rotation (Moroke et al. 2011, Verbree et al. 2014). Agricultural water usage has become increasingly controversial and is often the limiting factor for yield. This crop presents a viable option for combatting this issue (Moroke et al. 2011). This drought tolerance is due to the plant exhibiting paraheliotropism (orienting its leaves to receive less heat from the sun). Cowpeas also exhibit stomatal adaptions and reduced leaf area also critical to combatting water loss. Oftentimes, water loss in cowpeas is so minimal that senescence will occur due to a loss of dry weight, not a loss of water (Verbree, et al. 2014).

The increased drought tolerance of cowpea contributes to the crop's value, not only as a cover crop, but as a potentially more reliable food source in the changing climate (Moroke et al. 2011, Goncalves et al. 2016). Cowpea has also been shown to use water more efficiently than soybeans, sunflowers, and grain sorghum due to the plant accessing water at lower depths more effectively. The plant can also outcompete many weed species, due to its fast growing nature (Moroke et al. 2011, Rubatzky and Yamaguchi 1997).

Arthropod Pests of Cowpea

Throughout the world, there are numerous known pests of cowpeas. In India, one of the largest growers of cowpeas, the legume pod borer, leaf hoppers, cowpea aphid, flower bud thrips, tobacco caterpillar, spotted pod borer, and a sucking bug complex are the pests of cowpeas (Anusha et al. 2016). The legume pod borer is also a pest in Ghana as well as flower bud thrips, the pod sucking bug, whitefly, and other sucking pests (Tanzubil et al. 2008). Losses from these insects can be devastating, but vary from 20-100% (Epidi et al. 2005).

The insect pests of cowpea in Georgia include thrips, aphids, Lepidoptera larvae as defoliators, stinkbugs, Lygus bugs, cowpea weevil (as a stored grain pest) and cowpea curculio (Chalfant 1985). The key pest of cowpea in the Southeastern United States is the cowpea curculio, *Chalcodermis aenus* Bohemon (Figure 2.1). In the above-ground or plant phase of its life cycle, the curculio crawls to the plant, feeds on leaves and flower buds, and oviposites in the developing fruiting structures. Cowpea curculio females can lay nearly three-hundred eggs in a forty-five day period (Arant 1938, Capinera 2001). These eggs are white, oval in shape, and are less than a millimeter in length (Capinera 2001). Females oviposit eggs into pods and consequently damage the seed (Arant 1938). The duration of the egg stage is between three to six days. The duration of the four larval instars is temperature dependent, but normally between six and nine days (Capinera 2001). The grub has been observed to remain in the pod to feed and develop for up to twenty-seven days (Arant 1938). The larval stage is the principle source of damage to the crop because it feeds directly on the growing pea, but is not generally a target of insecticidal control as it is protected from direct contact by the pod (N'Guessan and Chalfant 1990).

The soil phase of the curculio's life cycle begins after the curculio larvae emerges from the pod. The larvae fall onto the soil and burrow to a depth of approximately three inches, form a pupal chamber, and pupate for up to nineteen days (Arant 1938, Capinera 2001). The larvae and pupae are light yellow in color, and the pupae look similar to the adult stage (Capinera 2001). Once the insect has developed into the adult stage, it has been reported to remain in the soil between one and sixteen days (Arant 1938), but this likely refers to the spring-summer generation. Wings are fully developed; however, flight is rarely observed, so the curculio likely crawls to its host plant when it leaves the soil phase (Capinera 2001).

After overwintering, adults are starved in the spring when the first cowpeas are planted and will feed directly on the plant prior to fruiting. Thus, in addition to damaging the pods through feeding and oviposition, adults also damage cowpea foliage and flower buds. Developmental time for curculio ranges between twenty-three and fifty-three days. Arant (1938) collected what was believed to be overwintered adults that then survived until the following winter and asserted that the longevity of the curculio to be up to one year. However, this was the only report of this occurrence.

Cowpea Curculio Management

Cultural control methods for the cowpea curculio include keeping the field clear of any alternate hosts of the insect (Capinera 2001). The curculio feeds on young cotton, string beans, lima beans, strawberries, English pea, common vetch, wild bean, sheep sorrel, sow thistle, and evening primrose (Arant 1938, Sudbrink et al. 1998). The curculio has also been observed feeding on sicklepod but prefers cowpeas as a host over the other hosts listed. Cowpea serves as the only known reproductive host of significance. If there are no hosts for the rarely flying pest, the population of the adult curculios may decrease. Pod wall thickness has been proven to provide some resistance to the curculio, but at increased curculio populations, the crop is overcome by the insect (Capinera 2011). Even the most resistant cultivars can be severely damaged by the curculio (Chalfant et al. 1972).

Population monitoring of the cowpea curculio has been limited by the lack of trap development. A Modified Tedders trap has been evaluated to monitor the seasonal movement of cowpea curculio populations (Riley et al. 2015). The inability to monitor the cowpea curculio limits effective timing of control which has the potential to increase yield loss. The first recommended insecticidal control for the cowpea curculio was lead arsenate (Arant 1938), which is no longer legal to apply due to the adverse health effects that it can have on humans. Chemical sprays have been used to target the adult stage of the curculio. These sprays are applied prior to and during pod development in an effort to protect yields (Chalfant 1973, Riley and Sparks 2019).

Due to insecticide resistance issues, traditionally used chemical controls, such as pyrethroids, are no longer efficacious in control of this pest (N'Guessan and Chalfant 1990, Riley and Sparks 2019). There has been a lack of chemical control options that adequately manage this pest for some time (Riley 2011). Resistance to pyrethroid insecticides was first documented nearly thirty years ago (N'Guessan and Chalfant 1990). This was determined to be the result of cross resistance to chlorinated hydrocarbons, such as DDT. During that time, it was recommended to incorporate other methods of control to decrease growers' reliance on insecticides. N'Guessan and Chalfant (1990) also suggested incorporating host plant resistance, other cultural control measures, and rotating insecticides.

Biological control measures have also been evaluated against the cowpea curculio. Bell and Hamalle (1971) reported low mortality rates when larvae were treated with *Metarrihizium anisopliae*, which was thought to be a potentially efficacious foliar control method. *Beauvaria bassianna* and *Metarhizium anisopliae* are not yet widely used in commercial cowpea production but have shown promising results in lab testing (Schmidt et al. 2018). Agostino Bassi first observed *B. bassiana* infecting silkworm in 1835, and recently, *B. bassiana* has been shown to be efficacious against a variety of pests. The boll weevil, Colorado potato beetle, coffee berry borer, pepper weevil, aphids, whiteflies, armyworms, European corn borer, pine caterpillars, codling moth, diamondback moth, grasshoppers, and thrips are all hosts of this pathogen (Hajek 2004).

B. bassiana was discovered to be naturally occurring in grain crops in the 19th century in the Midwestern United States (Lord 2005). It has also been applied for many years to millions of hectares in China. *B. bassiana* has a long shelf life, is relatively inexpensive to grow and many programs have utilized its release (Hajek 2004). Some research suggests that *B. bassiana* can survive on dead curculios for up to 2 weeks with no decline in virulence, and on other insect's bodies for up to six months. Sunlight is the main limitation to the growth of the fungus, although some fungi have been shown to persist in the soil for 2 years (Daoust 1986).

Soil applied entomopathogenic nematodes, such as *Heterorhabditis spp*. have been shown to be efficacious against some insect pests. Host mortality is fairly rapid because of the mutualistic association with the bacteria *Photorhabdus* (Vashisth et al. 2013). Chemoreceptors aid the nematode in finding the host, it enters the host through a weakened or open portion of the body cavity, the bacterium rapidly kills the insect host, and the nematode feeds on the insect. This control method is attractive not only due to the speed of action, but also the ease of production, the lack of environmental implications, and the ease of application. Nematodes can be applied with equipment that most growers are already using, making the transition to using them very simple. However, the efficacy of entomopathogenic nematodes decreases in extreme environmental conditions. Ideal temperatures for their growth and development are between 20-30°C (Vashisth et al. 2013). *Heterorhabditis spp.* was shown to be more effective than fungi in some laboratory bioassays and showed a high rate of virulence and infection rates (Schmidt et al, 2018).

Diapause in Weevils

Sudbrink et al. 1998 provided the most complete survey of alternate hosts of the cowpea curculio and documented overwintering adults near dry broomsedge (*Andropogon* sp.), but did not indicate this as a food host. Because cowpea serves as the primary host for the curculio and does not survive freezing temperatures, the cowpea curculio that survive the winter, generally must do so without an apparent food source. A weevil with similar biology and geographical origin, the boll weevil, *Anthonomus grandis* Boheman, was reported to exhibit diapause 60 years ago (Brazzel and Newsom 1959) which helped to explain how this weevil could survive months during the winters when no cotton was present after hard freezes in the southern USA (Slosser et al. 1996). By the late 1960s, photoperiod induction was determined to be the primary driver of diapause in the boll weevil (Mangum et al. 1968). Slosser et al. 1996 showed how diapause provided protection of overwintering boll weevil from freezing temperatures. Summy et al. (1993) demonstrated classic diapause, but also showed that dormancy in boll weevil was not always associated with those weevils found in typical overwintering sites. Perez-Mendoza et al. (2002) reported that one of the best indicators of true diapause is the lack of egg follicle development or ovarian age-grading determined through abdominal dissections.

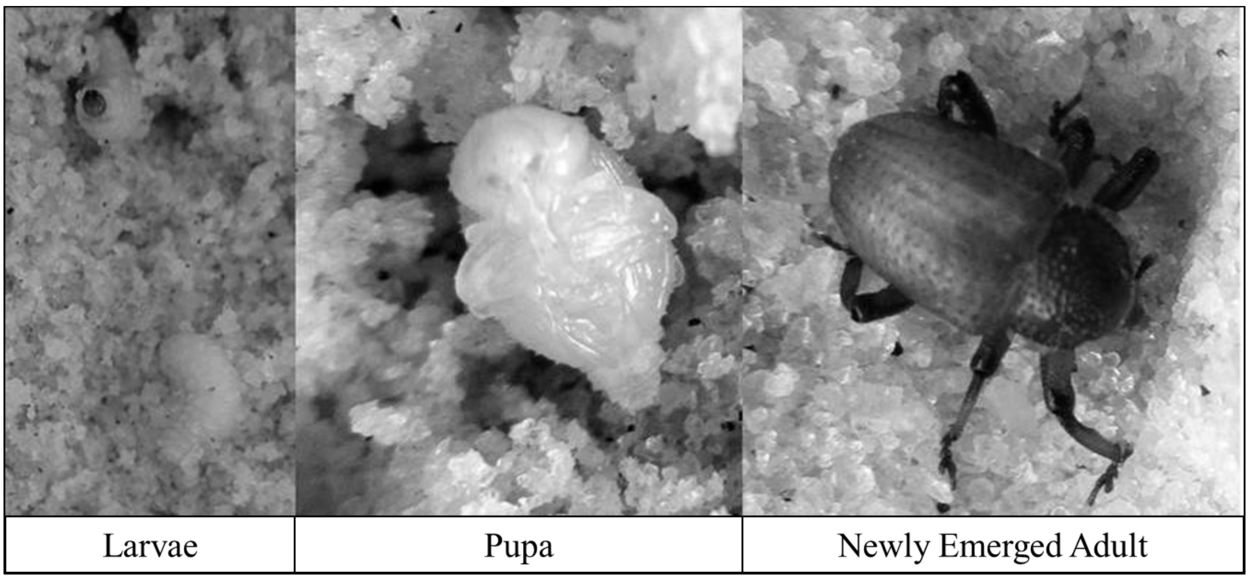

Figure 2.1. The life stages of the cowpea curculio in the soil phase.

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CHAPTER 3

COWPEA CURCULIO EMERGENCE TRAP EVALUATIONS

ABSTRACT A Modified Tedders trap has been developed and used for the seasonal monitoring of cowpea curculio, *Chalcodermus aeneus* Boheman (Coleoptera: Curculionidae), however, an emergence trap to monitor adult emergence from the soil from a specific area to evaluate the efficacy of treatments targeting the soil phase of this insect was needed. These traps may also prove useful in monitoring the emergence patterns of curculio following a crop. Environmental conditions underneath the trap must be taken into consideration as they can affect the survival rate or length of time that it takes the weevil to complete its lifecycle. This report documents the efficacy of three Modified Heliothis traps (Hartstack trap), a Pyramid Trap within a cage, and a Bucket Trap for monitoring emergence of cowpea curculio from the soil. Overall, the most effective emergence traps were shown to be the Modified Heliothis traps. In a second comparison done between Plastic-Covered and Uncovered Modified Heliothis Traps, a higher number of weevil emergence was observed in the Covered Traps. These traps seem to provide a precise monitoring of cowpea curculio emergence from the soil that could be used in the assessment of various biocontrol and chemical control methods targeting the soil phase of this pest.

Introduction

Population monitoring of the cowpea curculio is difficult due to the weevil's ability to feign death and drop off of the plant when approached (Arant 1938). Populations of the cowpea curculio have been shown to change seasonally (Chalfant 1997) and the ability to predict early seasonal occurrence of the insect could aid growers in better timing of control. The lack of an effective means of trapping has been an issue for the entire history of cowpea production. A Modified Tedders trap has been developed to monitor the seasonal movement of cowpea curculio populations (Riley et al. 2015), however, an effective emergence trap has not yet been developed.

An effective emergence trap was recently developed for the western corn rootworm, *Diabrotica virgifera virgifera* LeConte, which also pupates in the soil and emerges as an adult. The novel trap was pyramidal in shape and was 99% effective at recapturing deployed beetles (Rauch et al. 2016). Cone-type emergence traps have also been developed for the root weevil, *Diaprepes abbreviates,* and are successful at providing data on newly emerging weevils (Nigg et al. 2002). However, environmental conditions underneath the emergence trap must also be considered. Southwood (1965) indicated that the quality of data collected using an emergence trap depends on how different conditions are underneath the trap. Growth and survival rates of the curculios may be affected due to increased temperatures or changes in moisture underneath the trap.

Any emergence trap developed for the cowpea curculio must be effective at capturing newly emerged adults and have environmental conditions underneath the trap similar to the surrounding area. In this study, six methods of cowpea curculio emergence monitoring were tested. It was proposed that one trap out of several tested would be found to be the most efficacious (highest curculio count per soil unit sampled) for sampling the emerging population of a given area of soil surface under similar environmental conditions surrounding the trap.

Materials and Methods

Emergence traps. Five different emergence traps were evaluated including: a bucket trap, a Modified Tedders trap inside an A frame cage, and four Modified Heliothis traps (Figure 3.1). Bucket traps consisted of an inverted five-gallon bucket with the conical portion of a boll weevil trap affixed above an approximately 10cm hole in the bottom of the bucket. These traps were installed by pushing them into the freshly tilled soil. The Modified Tedders trap was approximately one foot in height, had the conical portion of a boll weevil trap on top, and was covered with a three feet by four feet A-frame screened cage. The first Modified Heliothis trap, the Heliothis Trap – Riley Modification, was made from the conical base with fine wire mesh attached inside and a complete boll weevil trap affixed to the top. The second trap, the Heliothis Trap – Sparks Modification, had a conical base frame made from hog wire fencing, an outer conical portion made from window screen, and the conical portion of a boll weevil trap affixed to the top. Hog wire fencing was used for the base, covered by the window screen with the conical portion of the boll weevil trap being glued between the window screen and the hog wire base. The third Modified Heliothis trap, the Plastic Covered Modified Heliothis Trap – Sparks Modification, was identical to the first Sparks Modification but covered with white plastic mulch. It was theorized that the white plastic might maintain a higher humidity within the trap to prevent desiccation, avoid excessive heating that would occur with black or clear plastic, and restrict light to the top of the trap resulting in additional attraction of adults to the top of the trap. The Modified Heliothis Trap – Sparks Modification with Seed included a seed bait (dried southern peas that had been soaked for at least one hour) in the boll weevil trap that was affixed to the top. All of the Modified Heliothis traps were approximately three feet in diameter and installed by covering the edges of each trap with soil.

Trap efficacy was evaluated by introducing 15 adult weevils under each trap and monitoring capture over time. Two traps of each type were established on each date with three establishment dates. Traps were moved to a different location and new weevils were deployed under each trap on each date. Tests were initiated on May 17, 2016, June 8, 2016, and July 5, 2016. Traps were monitored periodically and captured weevils were counted and removed. Cumulative capture at one, three and six days after test initiation were evaluated with ANOVA procedure ($P<0.05$). Where significant differences were detected, means were separated with LSD ($P=0.05$). Once a trap type had been selected for use in further studies, additional tests were conducted to compare environmental conditions under this trap with and without the plastic cover.

Source and handling of cowpea curculio adults. Adult weevils were collected from Modified Tedders traps on UGA Tifton's Horticulture Farm, Lang Farm, and Tifton Vegetable Park. The curculios were maintained on a diet of blackeyed peas, which had been soaked in water for at least one hour, until enough weevils were collected to begin tests. Fifteen adults were deployed under each trap and their capture was monitored over several days until the majority had been captured. These tests were replicated three times.

Temperature and moisture monitoring. A second series of tests evaluated the environmental conditions under two of the Modified Heliothis traps (which had been selected for use in future studies) with comparison to ambient conditions. Monitors were established under the covered and non-covered Sparks Modified Heliothis traps. One trap of each type was established on bareground on May 10, June 5, June 26, and July 17, 2017 and monitored for two to three weeks. The 3ft by 3ft area under each trap was drenched with 3l. of water prior to installing each of the traps. Soil moisture and soil temperature at approximately two inches below the soil surface, and ambient temperature were monitored under each of the traps and compared to conditions outside of the traps. This was accomplished through the use of Decagon EC-5 soil moisture sensors (2365 NE Hopkins Court Pullman, WA 99163) and Watchdog B Series Button Loggers (Spectrum Technologies, Aurora, IL).

Results and Discussion

Emergence Traps. The cumulative number of adult weevils collected at 1, 2 and 6 days after placement in the traps is presented in Table 3.1. Combining all trap types, approximately half of the weevils placed under traps were captured by 6 days after placement, with the Modified Heliothis traps ranging from 58 to 78 percent capture (8.7 to 11.7 adults of 15 introduced). The pyramid trap within the screen cage and the bucket trap captured far fewer individuals than any of the Modified Heliothis traps. The majority of weevils captured were collected within the first two days of trapping (66.6 and 86.5 percent through day one and day two respectively; combined for all trap types). All of the Modified Heliothis traps performed statistically similar, with minor exceptions. The covered trap did collect fewer individuals in the first day of trapping as compared to the trap with seeds, but performed similarly thereafter.

The poor performance of the bucket trap is likely related to difficulty for the weevil to climb up the vertical surface of the smooth plastic and traverse the flat horizontal surface to the collection devise in the center. This trap has proven useful for monitoring emergence in the field when deployed with a wooden stake in the center of the bucket to facilitate climbing by the curculios into the trap (Riley, unpublished data). The pyramid trap inside of the cage also performed poorly. Weevils likely encountered other surfaces and distributed throughout the cages rather than being collected in the pyramid traps. The Modified Heliothis traps all consisted of a screen cage which allowed for easy climbing and a conical design such that all vertical surfaces lead directly to the collection device.

Although all of the Modified Heliothis traps performed similarly, the covered and non-covered Sparks modification was selected for further study. While placement of seeds into the collection device did appear to provide some potential benefit, this approach was eliminated because of issues with the seeds deteriorating which would require frequent replacement. The Riley modification was eliminated for the second set of experiments because the trap modification was more difficult and expensive than the Sparks modification.

Temperature and Moisture Monitoring. Maximum, minimum and temperature differentials are shown in Tables 3.2, 3.3 and 3.4, respectively. These are also shown in Figures 3.1, 3.2 and 3.3 respectively. Maximum ambient temperatures were higher than ground temperatures under both Heliothis traps, but ambient temperatures were lower than soil temperatures on the bareground. This likely is the result of the traps providing shading of the soil. Ambient temperature was highest under the Covered trap. Minimum ambient temperatures were similar for all trap types. While ambient temperatures did vary among trap types, ambient temperature would likely have minimal impact on cowpea curculio emergence as the pest is located within the soil. Maximum soil temperatures were greatest in the bareground, followed by the uncovered and then covered Heliothis traps, with a difference of 12 to 14 degrees. Minimum ground temperatures varied in the opposite direction, with the bareground showing the lowest temperature followed by the uncovered trap and the covered trap with the highest temperature, but with a differential of only approximately 2 degrees. The higher variation in maximum temperatures and similar minimum temperatures resulted in greater maximum to minimum temperature differentials for the bareground followed by the uncovered trap and the minimal differential for the covered trap.

Soil moisture data are presented in Figure 3.4. In general, the covered traps clearly maintained moisture levels during dryer portions of the trial. Rain events are reflected in the bareground and uncovered traps with increases in moisture followed by declines in moisture between rains. Moisture declined most rapidly in the bareground treatments.

Overall, both Heliothis traps did influence temperatures and soil moisture. The cooler soil temperatures under the traps could slow development of the curculio in the soil. However, these temperatures may actually better reflect their true environment which would be covered with plant debris. The covered trap also maintained consistent soil moisture which does effect mortality. Rearing studies conducted in temperature chambers experienced extremely high mortality when moisture was not maintained (Figure 3.5). For these reasons, the covered Heliothis trap was selected for the field studies on post-harvest treatments for control of the cowpea curculio.

Table 3.1. Mean number of cowpea curculio adults captured at indicated days after placement under emergence traps over all dates (n=6) at Tifton, GA in 2016.

Trap type	Cumulative number of weevils captured			Percent of weevils captured
	Day 1	Day 2	Day 6	
Uncovered Heliothis Trap – Sparks Modification with seed	8.3 a	9.3 a	10.7 a	71.3
Uncovered Heliothis Trap – Riley Modification	7.3 ab	10.7 a	11.7 a	78.0
Uncovered Heliothis Trap – Sparks Modification	6.0 abc	8.0 a	8.7 a	58.0
Covered Heliothis Trap – Sparks Modification	4.3 bc	6.3 ab	8.7 a	58.0
Pyramid in Cage	2.7 cd	2.7 bc	2.7 b	18.0
Bucket	0.0 d	0.3 c	0.7 b	4.7
F	6.47	5.19	8.31	
df	7, 10	7, 10	7, 10	
P	0.0045	0.01	0.0017	

* Means within columns followed by the same letter are not significantly difference (LSD, $P<0.05$).

Table 3.2. Maximum temperatures recorded by data loggers used with temperature sensors 15 cm above the ground and 5 cm below ground at Tifton, GA in 2016.

Location	11 May – 2 June	6 June – 26 June	27 June – 17 July	18 July – 31 July	11 May – 31 July
Ground - Bareground	96.5 a	95.5 a	100.7 b	98.3 b	97.7 b
Ambient - Bareground	89.0 b	88.4 b	93.3 c	93.3 c	90.8 cd
Ground - Uncovered Heliothis Trap – Sparks	90.4 b	87.6 b	92.1 d	91.0 d	90.2 d
Ambient - Uncovered Heliothis Trap – Sparks	89.3 b	88.4 b	94.2 c	93.8 c	91.2 c
Ground - Covered Heliothis Trap – Sparks	82.9 c	83.9 c	86.5 e	86.8 e	84.8 e
Ambient - Covered Heliothis Trap – Sparks	96.4 a	95.6 a	103.0 a	102.1 a	98.9 a
F	42.16	32.16	71.12	40.10	47.32
df	27, 110	25, 100	25, 100	18, 65	83, 390
P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

* Means within columns followed by the same letter are not significantly difference (LSD, $P<0.05$).

Table 3.3. Minimum temperatures recorded by data loggers used with temperature sensors 15 cm above the ground and 5 cm below ground at Tifton, GA in 2016.

Location - trap	11 May – 2 June	6 June – 26 June	27 June – 17 July	18 July – 31 July	11 May – 31 July
Ground - Bareground	72.6 c	73.9 c	78.4 c	78.5 b	75.5 c
Ambient - Bareground	67.1 e	70.1 e	72.9 e	73.5 c	70.6 e
Ground Uncovered Heliothis Trap – Sparks	73.4 b	74.5 b	79.1 b	78.7 b	76.1 b
Ambient Uncovered Heliothis Trap – Sparks	67.5 de	70.4 e	73.0 e	73.7 c	70.8 e
Ground Covered Heliothis Trap – Sparks	74.3 a	75.8 a	80.6 a	79.8 a	77.4 a
Ambient Covered Heliothis Trap – Sparks	67.9 d	71.0 d	74.3 d	74.2 c	71.5 d
F	99.43	115.11	54.03	34.78	102.37
df	27,110	25, 110	25, 110	18, 65	83, 390
P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

* Means within columns followed by the same letter are not significantly difference (LSD, $P<0.05$).

Table 3.4. Difference in temperatures recorded by data loggers used with temperature sensors 15 cm above the ground and 5 cm below ground at Tifton, GA in 2016.

Location	11 May – 2 June	6 June – 26 June	27 June – 17 July	18 July – 31 July	11 May – 31 July
Ground - Bareground	23.9 b	21.6 b	22.3 b	19.9 b	22.2 b
Ambient - Bareground	21.8 c	18.3 c	20.4 c	19.9 b	20.2 c
Ground Uncovered Heliothis Trap – Sparks	17.1 d	13.1 d	13.0 d	12.3 c	14.1 d
Ambient Uncovered Heliothis Trap – Sparks	21.8 c	18.0 c	21.3 bc	20.2 b	20.4 c
Ground Covered Heliothis Trap – Sparks	8.6 e	8.2 e	5.8 e	6.9 d	7.5 e
Ambient Covered Heliothis Trap – Sparks	28.4 a	24.7 a	28.7 a	28.0 a	27.4 a
F	56.09	35.99	69.12	52.58	51.11
df	27, 110	25, 100	25, 100	18, 65	83, 390
P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

* Means within columns followed by the same letter are not significantly difference (LSD, $P<0.05$).

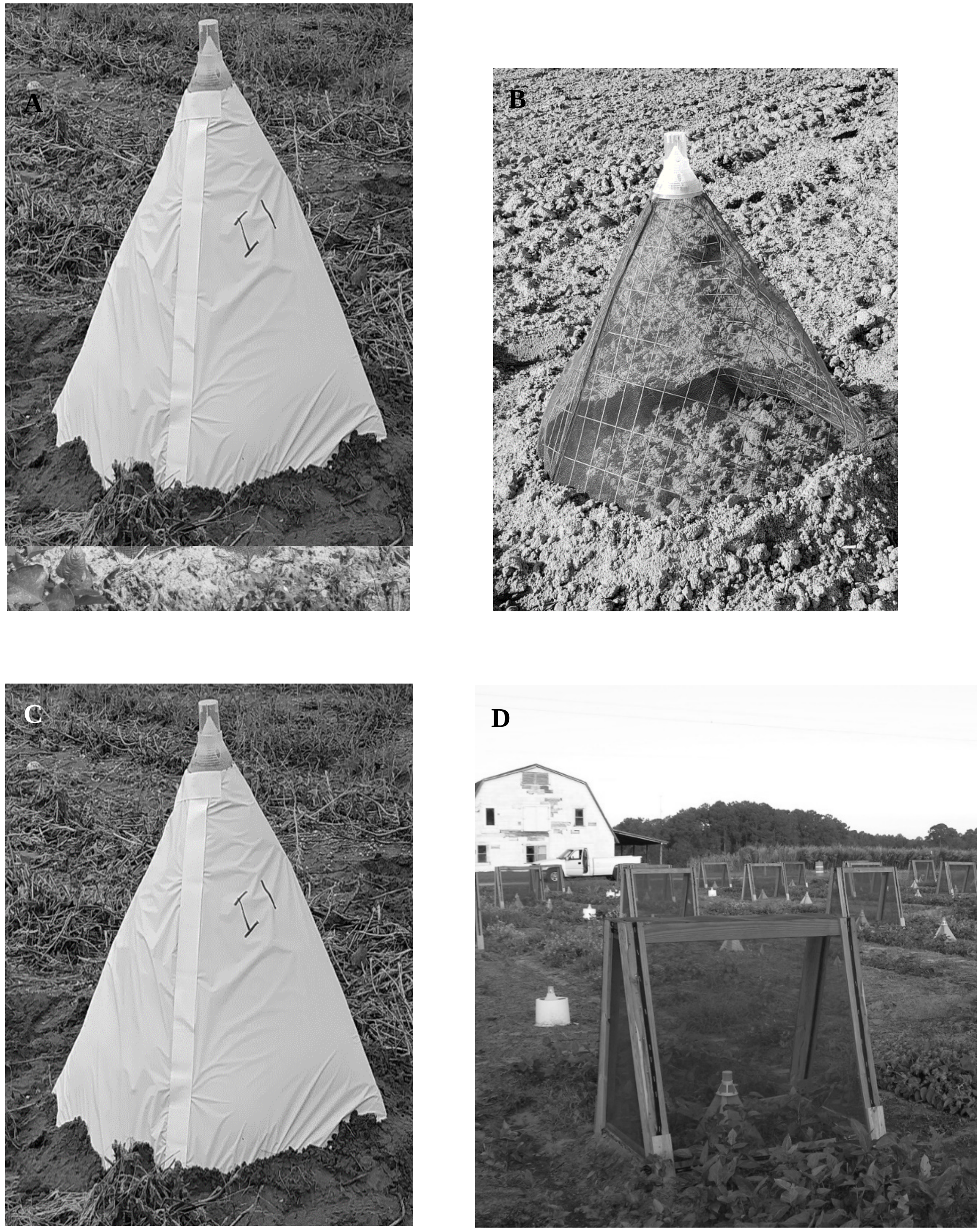

Figure 3.1. Cowpea curculio emergence traps tested in 2016, (A) Heliothis Trap – Riley Modification, (B) Heliothis Trap – Sparks Modification, (C) Covered Heliothis Trap –

Sparks Modification and (D) Modified Tedders trap in a cage (note bucket trap in background).

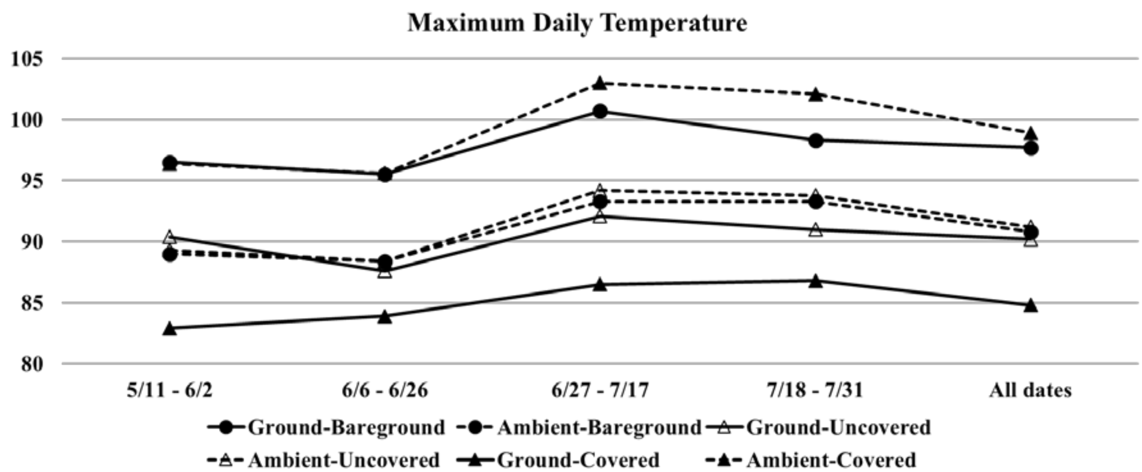

Figure 3.2. Maximum temperatures recorded by data loggers used with temperature sensors 15 cm above the ground and 5 cm below ground.

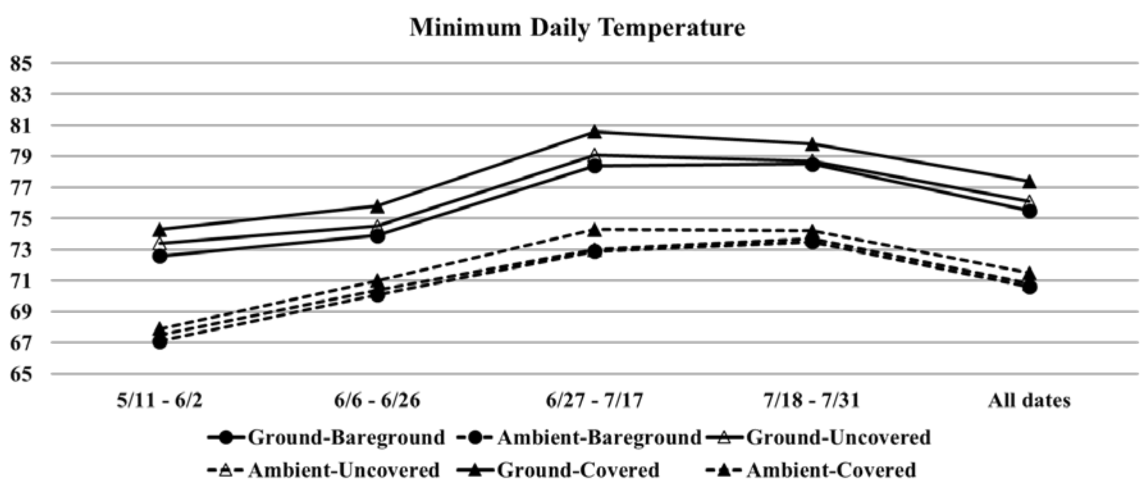

Figure 3.3. Minimum temperatures recorded by data loggers used with temperature sensors 15 cm above the ground and 5 cm below ground.

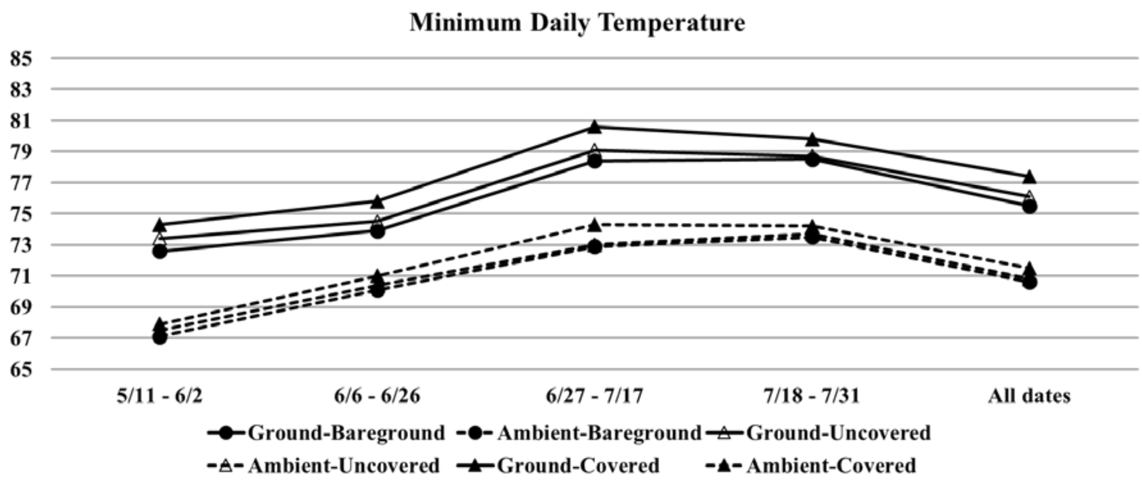

Figure 3.4. Difference in temperatures recorded by data loggers used with temperature sensors 15 cm above the ground and 5 cm below ground.

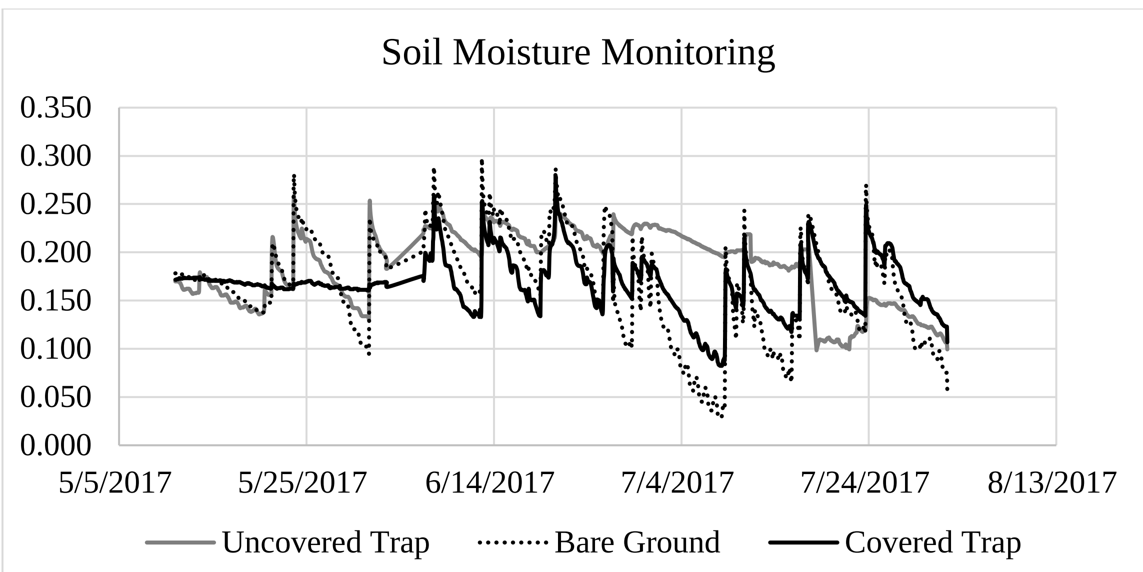

Figure 3.5. Soil moisture data (measured in Volumetric Water Content) collected from underneath the bare ground, the Uncovered Heliothis Trap, and the Covered Heliothis Trap.

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CHAPTER 4

EFFICACY OF BIOLOGICAL AND CHEMICAL AGENTS AGAINST THE COWPEA CURCULIO IN THE SOIL

ABSTRACT There are currently no options for the effective control of *Chalcodermus aeneus* Boheman (Coleoptera: Curculionidae). Foliar sprays for control of adults were most recently the most consistent method of controlling this pest, however, the weevil has shown resistance to each of the previously known methods of insecticidal control. The soil phase biology of this pest may allow for effective soil applied insecticide applications or biological controls targeting the grub and pupal stages. The soil phase begins when the cowpea curculio grub emerges from the pea pod, falls to the ground, and burrows into the soil to pupate. In these experiments we evaluated an insecticide (chlorpyrifos), an entomopathogenic fungi (*B. bassiana*), and an entomopathagenic nematode (*Heterorhabditis spp*.) applied to the soil for management of cowpea curculio. Each treatment was evaluated as a pre- and post-infestation treatment in a field bioassay. Results showed no significant differences among treatments which could be due to an error in trial installation, a lack of rainfall or a need for irrigation to keep the curculio, nematodes and fungi viable for the completion of the soil phase of the curculio life cycle.

Introduction

Lead arsenate, the first chemical control tactic used against the cowpea curculio is no longer legal to apply due to human health issues (Arant 1938). Foliar chemical sprays were traditionally used during pod development to target the adult stage and limit yield loss (Chalfant 1997). Recently, foliar sprays targeting the adult stage of development have been shown to be ineffective in controlling the cowpea curculio because of insecticide resistance. Due to insecticidal ineffectiveness, other methods of control must be evaluated (N'Guessan and Chalfant 1990). Targeting the soil phase of development has not been previously attempted and may be an efficacious alternative. After the curculio emerges from the pod the larvae falls onto the soil and burrows in to pupate. The pupal stage of development can last nearly three weeks (Arant 1938, Capinera 2001). Once the insect has developed into the adult stage it may remain in the soil for over two weeks (Arant 1938). Soil treatments may also have some effects on adults above ground, due to their contact with the soil during the warmer portions of the day (Capinera 2001).

B. bassiana, a proposed method for the control of the cowpea curculio, works by infecting the host through the cuticle. This control method is attractive due to the reduced threat it poses to natural enemies and non-target organisms. However, fungi are typically slow-acting, and this has inhibited extended adoption by growers (Lacey et al. 2015). Daoust and Pereira (1986) have reported that conidia from *B. bassiana* can survive and remain viable on the cadavers of the cowpea curculio for up to sixteen weeks. However, once exposed to the elements, many of the conidia were removed from the insect cadavers. Slow activity and the stability of the fungi in field conditions remain the largest constraints for the use of foliar applied *B. bassiana* for successful control of the cowpea curculio (Dauost and Pereira 1986). In laboratory bioassays *B. bassiana* any separation from the host and the fungi was shown to be a problem. Once this was realized curculios were first deployed into the container and the fungi was then applied. Efficacy increased once the fungi was applied in this manner (Schmidt et al. 2018).

Entomopathogenic nematodes, such as *Heterorhabditis spp*. have been shown to affect their hosts quickly, in as little as two days. This quick activity is due to the mutualistic association with the bacteria, *Photorhabdus*. The nematode finds its host through the use of its chemoreceptors, enters the host through a weakened or open portion of the body cavity, the bacterium rapidly kills the insect host, and the nematode feeds on the insect. This control method is attractive not only due to the speed of action, but also the ease of production, the lack of environmental implications, and the ease of application. Nematodes and fungi can be applied with equipment that most growers are already using, making the transition to using them very simple. However, the efficacy of entomopathogenic nematodes decreases in extreme environmental conditions. Ideal temperatures for their growth and development are between 20-30°C (Vashisth et al. 2013). *Heterorhabditis spp.* was shown to be more effective than fungi in some laboratory bioassays and showed a high rate of virulence and infection rates (Schmidt et al. 2018).

This study sought to compare chlorpyrifos, *Beauvaria bassiana*, and *Heterorhabditis spp*. as soil applied post-harvest treatments to control the cowpea curculio. To do so, post-harvest treatments were applied to bareground plots, curculio infested pods were placed on these plots, Modified Heliothis traps were set in plots, and adult capture was recorded. The hypothesis was that curculio adult emergence capture rates would be lowest with the most efficacious post-harvest treatment indicating more mortality in the soil phase of the life cycle.

Materials and Methods

Experimental Plots. These tests were located at the University of Georgia's Horticulture Farm in Tifton, Georgia, and were completed during the summer and fall of the 2016 and the summer of the 2017 growing season. The tests were initiated in July and August of 2016 and July of 2017. The experimental design was a randomized complete block design with seven treatments, replicated four times, and the entire test repeated three times. The seven treatments that were evaluated include applications of: chlorpyrifos (4 pt./a) prior to infestation (ChlorpyrifosPRE), *Heterorhabditis spp*. (25,000 ij's/ft^2) prior to infestation (NematodesPRE), Botanigard ES (3 qt./a) prior to infestation (BeauveriaPRE), chlorpyrifos (4 pt./a) two weeks after infestation (ChlorpyrifosPOST), *Heterorhabditis spp*. two weeks after infestation (NematodesPOST), Botanigard ES (3 qt./a) two weeks after infestation (BeauveriaPOST), and water (CHECK).

Land was tilled, 5' by 5' plots were established and infested pods were collected from harvest-mature, non-treated cowpeas grown nearby. Twenty-five curculio infested pods were placed near the center of each plot. Chlorpyrifos, *Heterorhabditis spp*., and B. bassiana in 3.0l water were then applied in the pre-infestation plots. Emergence traps (Modified Heliothis Traps) were installed in each of the plots by first putting down the hog wire cone as the base. Next, the window screen with boll weevil trap was placed over the hog wire base. Lastly, the trap was wrapped in plastic mulch and taped at the seam (Figure 4.1). Soil was then placed around the edges of the trap. An additional 50 infested pods were held in two emergence boxes (25 pods per box) in the laboratory to obtain estimates of infestation levels. For Post-infestation treatments, traps were removed and each treatment was applied over the pods that were previously distributed over the plot. Each of the traps were then replaced over the plots in the manner described above. Emergence was monitored three times per week until no cowpea curculios were captured for at least a week. The number of curculio adults caught in each trap were summed and compared with ANOVA ($P<0.05$).

Results and Discussion

In Test 1 the average adult emergence in the emergence box was 2.7 weevils per pod, or an estimated 67.5 grubs per trap, indicating that infestation level should have been high in the field test. However, adult emergence captures were low in the field test and no significant difference was shown in the data (Table 4.2). This could be due to some unforeseen mistake in how the test was set up or a result of the dry weather and need for rain or irrigation to maintain the viability of the curculio, nematodes and Botaniguard. Emergence was higher in Test 2; however, variability was great and no differences were detected among treatments. Similarly, test three captures were low with no differences among treatments.

Data was more consistent with what had been anticipated for the other tests in Test 3. The higher amount of rain received and subsequently lower temperatures beneath the traps may have allowed for increased viability of the biological treatments. Ninety-three weevils were collected from the emergence box, with an average emergence of 0.70 larvae per pod, proving that the deployment of weevils under the traps was successful. Throughout each of the tests Lorsban performed the best numerically with Botaniguard in most cases having the second highest emergence and nematode treated plots having the third highest emergence. The slight difference in the effectiveness of Lorsban could be due to the persistence of chlorpyrifos in the soil and the lack of need of moisture that the other treatments seemingly require. There also seemed to be more success in applying treatments prior to the weevils being introduced. This was consistent with the findings of previous laboratory bioassays (Schmidt et al, 2018).

Figure 4.1. Below is an example of the Covered Modified Heliothis Traps used to monitor cowpea curculio adult emergence.

Table 4.1. Mean number of cowpea curculio adults captured by treatment, soil insecticide post-harvest efficacy test, Tifton, GA 2016-2017.

Treatments	Test 1	Test 2	Test 3
ChlorpyrifosPRE	0.50a	4.75a	1.25a
NematodesPRE	3.50a	20.25a	4.25a
BeauveriaPRE	3.25a	14.25a	3.00a
ChlorpyrifosPOST	0.75a	8.25a	1.75a
NematodesPOST	1.00a	14.25a	2.50a
BeauveriaPOST	1.50a	12.00a	1.50a
Check	0.00a	7.75a	5.75a
F	0.43	1.01	0.86
df	9, 18	9, 18	9, 18
P	0.90	0.47	0.57

* Means within columns followed by the same letter are not significantly difference (LSD, $P<0.05$).

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CHAPTER 5

EVIDENCE OF DIAPAUSE IN THE COWPEA CURCULIO

ABSTRACT *Chalcodermus aeneus* Boheman (Coleoptera: Curculionidae), has been the most destructive insect pest of blackeyed peas or cowpeas, *Vigna unguiculata* L., over the last century in the southeastern USA. The historical distribution of this semitropical pest would lead one to believe that diapause must be present in the insect to allow it to overwinter in parts of the USA where its main crop host plant cannot. The data in this report contributed to the first documentation of biological evidence for diapause in this insect. This was observed by assessing larval emergence from cowpea pods in the summer to fall growing seasons, and dissecting adults and measuring egg development in females over the first (summer) and second (fall) curculio generation. There was a clear reduction in larval emergence from field collected pods from summer to fall. Also, egg development in the female curculio dropped off dramatically by September. Any future regional management of cowpea curculio will have to take into account the ability of this insect to diapause, thereby increasing its capacity to overwinter in regions where the cowpea crop, a warm-season, semitropical plant, is terminated with winter freezing temperatures.

Introduction

There are few published reports available regarding the reproductive biology of the cowpea curculio, *Chalcodermus aeneus* Boheman (Coleoptera: Curculionidae) (Ainslie 1910, Arant 1938, Dupree and Beckham 1955, Riley and Sparks 2019). The most comprehensive biological information in this area was published by Arant (1938). He observed that developmental time for curculio ranged between 23 and 53 days and recorded some adults living up to one year. Arant (1938) did discuss hibernation of weevils, but only referred to overwintering adults as "quiescent" and still susceptible to starvation. He documented eggs being oviposited into pods and consequently developing larvae damaging the seed. The duration of the egg stage is oftentimes between three to six days. The duration of the four larval instars is between six to nine days. Arant (1938) also observed weevils remaining in the pod to feed and develop for up to 27 days. Once the larvae emerges from the pod, they then fall onto the soil and burrow in to pupate for up to nineteen days at a depth of approximately three inches (Arant 1938) living for variable lengths of time in a soil phase (Riley 2016). Arant (1938) reported that once mature, adults can remain in the soil for up to sixteen days, however, Riley (unpublished data) found adults remaining for longer periods during the winter months.

Sudbrink et al. 1998 provided the most complete survey of alternate hosts of the cowpea curculio and documented overwintering adults near dry broomsedge (*Andropogon* sp.), but did not indicate this as a food host. Plants identified as food hosts generally do not survive winter temperatures in southern Georgia. Thus, the cowpea curculio that survive the winter, generally must do so without an apparent plant food source. A weevil with similar biology and geographical origin, the boll weevil, *Anthonomus grandis* Boheman, was reported to exhibit diapause 60 years ago (Brazzel and Newsom 1959) which helped to explain how this weevil could survive months during the winters when no cotton was present after hard freezes in the southern USA. By the late 1960s, photoperiod induction was determined to be the primary driver of diapause in the boll weevil (Mangum et al. 1968). Slosser et al. 1996 showed how diapause provided protection of overwintering boll weevil from freezing temperatures. Summy et al. (1993) demonstrated classic diapause, but also showed that dormancy in boll weevil was not always associated with those weevils found in typical overwintering sites. Mendoza et al. (2002) reported one of the best indicators of true diapause is the lack of egg follicle development or ovarian age-grading determined through abdominal dissections.

The goal of this study was to add to the literature on the reproductive biology of the cowpea curculio, particularly as it relates to diapause. The specific objectives were to: 1) assess curculio emergence from sequentially planted cowpea field plots to observe seasonal changes in reproduction on the plant; and 2) dissect and measure egg development in female adults over the first (summer) and second (fall) curculio generation. The hypotheses tested were: 1) an increase in diapause in the fall should result in reduced ovipositions/larval development in the fall as compared with the spring and summer; and 2) the number of eggs and follicle development in female curculios in the fall should be significantly less than the summer generation of weevils if diapause is occurring.

Materials and Methods

Sequential planting experiment. Cowpeas, var. Pinkeye Purple Hull, were direct seeded into 1.8 m x 30 m plots at the University of Georgia - Tifton Campus

Vegetable Park using standard cultural practices over two years. The biweekly planting dates in 2016 and 2017 started on 20 April and 19 April and ended 24 August and 14 August, respectively. In order to monitor for subsequent curculio larval development and grub emergence for each planting date, 50 mature light green pods were randomly collected from at peak-pod harvest of each planting block and put into two emergence boxes in the laboratory at air conditioned temperatures (21-24°C). Emergence boxes measuring 15.72cm x 15.72cm x 12.14cm were made using plastic Ziploc® containers with a wire mesh shelf to hold the peas above the container floor. Pea pods were placed on the shelf made from 1/4 inch mesh hardware cloth (Lowes Home Improvement, Mooresville, NC) and put inside the containers so that larvae could emerge and fall to the bottom of the container. The bottom of each container was lined with a moist paper towel to maintain humidity and prevent desiccation of the pods. Emergence from the pods was monitored through peak emergence and until at least three consecutive days with no emergence. The means were plotted over time to represent the emergence patterns. Monthly emergence was compared with ANOVA ($P<0.05$) means separated with LSD ($P=0.05$).

Curculio dissection experiment. Adult cowpea curculios were collected from a Modified Texas Style Cone Trap (Haystack and Witz 1981) at the Lang-Rigdon Farm in Tifton, GA from July 8, 2017 to November 3, 2017. The traps used consisted of the 76 cm diameter x 64 cm high wire mesh cone from the fore mentioned trap with reinforcement ring on the bottom, but replaced the collection cage on top with a commercial boll weevil trap (Leggett and Cross 1971) which was foam sealed (Dow Great Stuff 12-oz Spray Foam Insulation, Lowes Home Improvement, Mooresville, NC) onto the top of the cone in order to only allow weevils crawling up from the inside of the cone to enter the collection container on top. These "weevil emergence cone traps" were placed over a harvest-ready cowpea (var. Pinkeye Purple Hull) field that was harrowed into the soil. The bottom edge of the cone was pressed into the loose soil approximately 5 cm and an extra 5 cm loose soil layer was used to cover the edge and pressed down to seal the weevil emergence cone trap into the soil. Both male and female weevils emerging from the 0.454 m^2 of soil surface (the area covered by the trap) were forced to accumulate into the top sealed boll weevil trap and potentially mate. Weevils were collected and dissected weekly.

Approximately 25 adults were dissected each week. Live weevils were placed in ETOH and dissected under the microscope where female reproductive structures could be examined (Figure 5.1). The number of developing egg follicles was recorded for each female to determine physiological changes throughout the summer and fall. All intact vitellaria (maximum 4 per female dissection) were examined and all eggs in the dissection petri dish (includes mature eggs dislodged during dissection or from ruptured vitellaria plus developing follicles in intact ovarioles) were counted as a total egg count. Monthly egg production was compared with ANOVA ($P<0.05$) and means separated with LSD ($P=0.05$).

Results and Discussion

Larval emergence from pods. Larval emergence declined throughout the season, June to November, in the emergence boxes. The two generations represented by trapped adult counts that occur at Tifton, GA have been reported to increase approximately five-fold from the May-June peak to the August-September peak (Riley et al. 2015).

Assuming this increase in population occurred, the larger fall generation population appeared to be producing fewer numbers of larvae per female. Based on monthly averages, a significant decrease in larval emergence from pods was detected for the months of October and November compared to June and July ($F = 4.88$, $df = 5, 17$, $P<0.01$; June = 7.5a, July = 7.4a, August = 3.5bc, September = 4.8ab, October = 2.9bc, November = 0.9c). Months between July and October were variable possibly due to the interaction between the two generations, but ultimately decreased from June and early July (Figure 5.2). Anecdotal evidence suggests that there is a benefit of reduced curculio damage when planting peas in the 1st to 2nd week in August in southern Georgia, but it was unknown as to why, given the greater number of curculio adults available from August to September. This larval development data would suggest that the reduced damage in the fall may result from weevils interring reproductive diapause.

Curculio dissections. The number eggs per female dramatically decreased from July to late September (Figure 5.3). Based on monthly averages, a significant decrease in eggs per female was detected for the fall months compared to the summer months ($F = 44.51$, $df = 4$, $P<0.0001$; July = 10.512a, August = 9.942a, September = 6.444b, October = 0.000c, November = 0.000c). There was an observed reduction in ovary size and appearance for C. aeneus was very similar to that documented for diapausing A. grandis (Brazzel and Newsom 1959). We also observed some individuals during the summer months that did not exhibit developing egg follicles, which we assumed meant that they were unmated, but, as with A. grandis, diapause as a condition is not 100% excluded at any time of year (Segers et al. 1987). The total number of eggs in the female abdomen fell dramatically at the end of September, coincidentally as day lengths where shortening

(Figure 5.3). Eggs were positively correlated with day length (Figure 5.3). Thus, as days shortened to 12 hours of day light, the number of eggs per female declined to approximately zero. The photoperiods shown to induce diapause in A. grandis bracketed this amount of daylight, i.e. 13 hours did not induce diapause, but 11 hours did (Mangum et al. 1968).

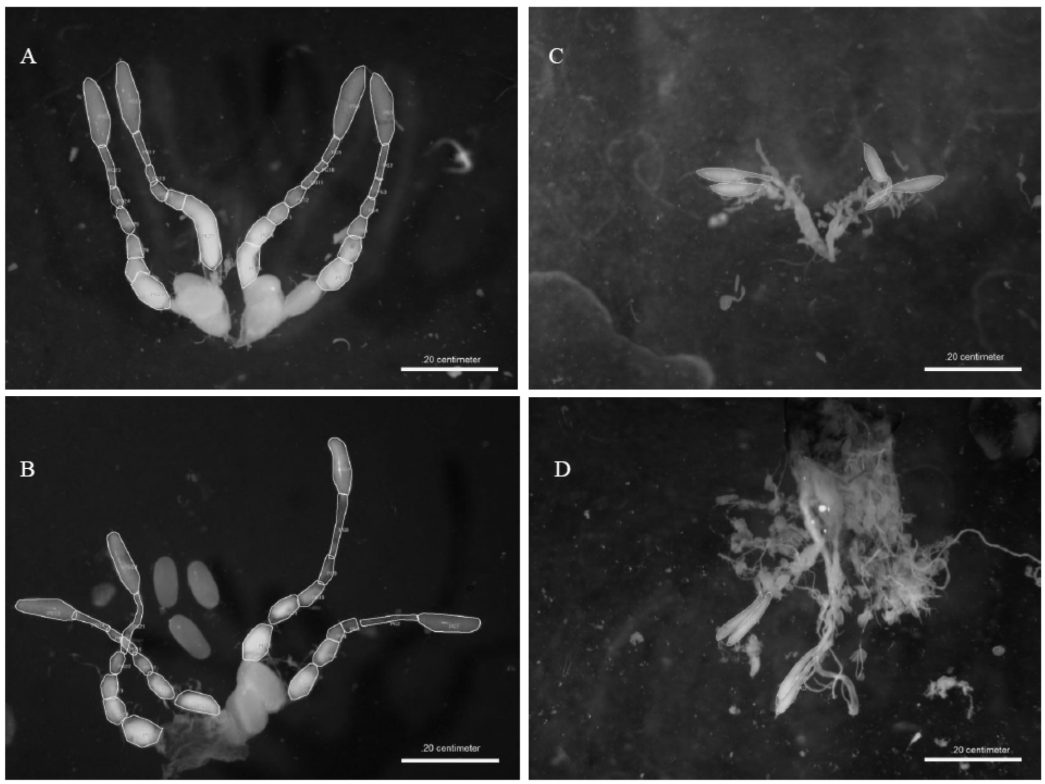

Figure 5.1. Selected images of dissected ovaries of the cowpea curculio, Chalcodermus aeneus, with a polygon overlay on individual developing egg follicles to measure area using Image-Pro Plus® software. Example ovaries from suspected non-diapausing curculios collected on July 8, 2017 (A, B) and suspected diapausing curculios collected on September 17 and October 26 (C and D, respectively).

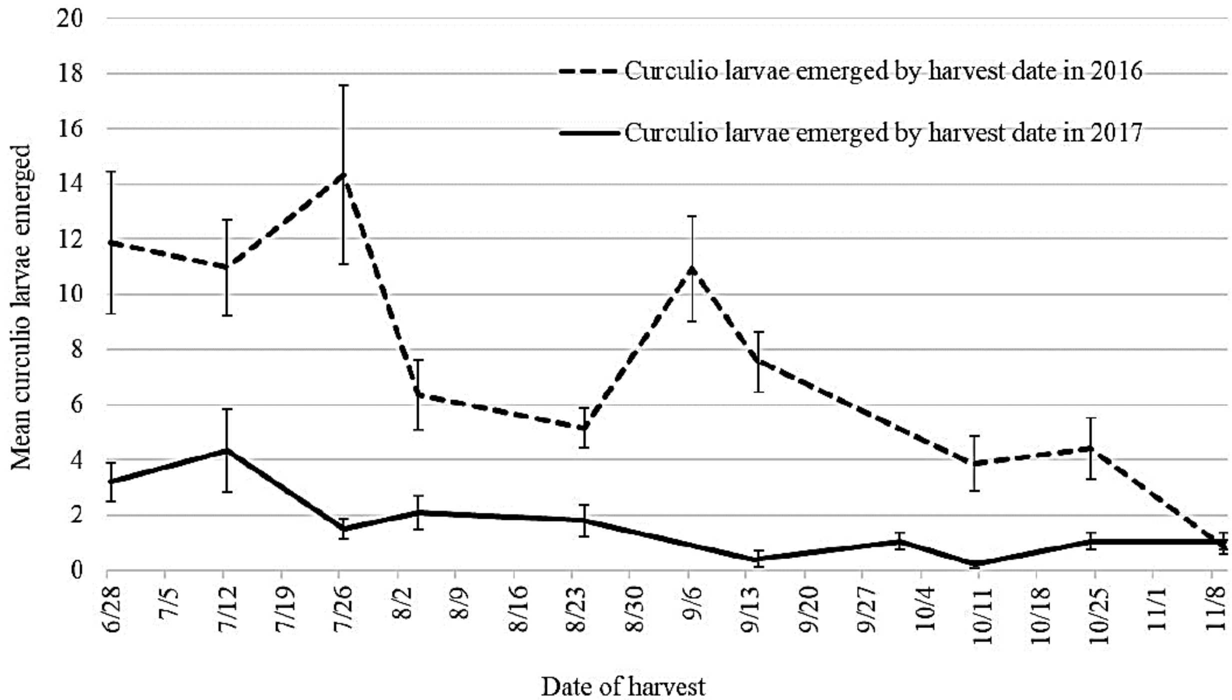

Figure 5.2. The mean ± std of the pod emergence patterns of C. aeneus larvae from mature cowpea pods from sequential field cowpea plantings at Tifton, GA, USA in 2016 and 2017.

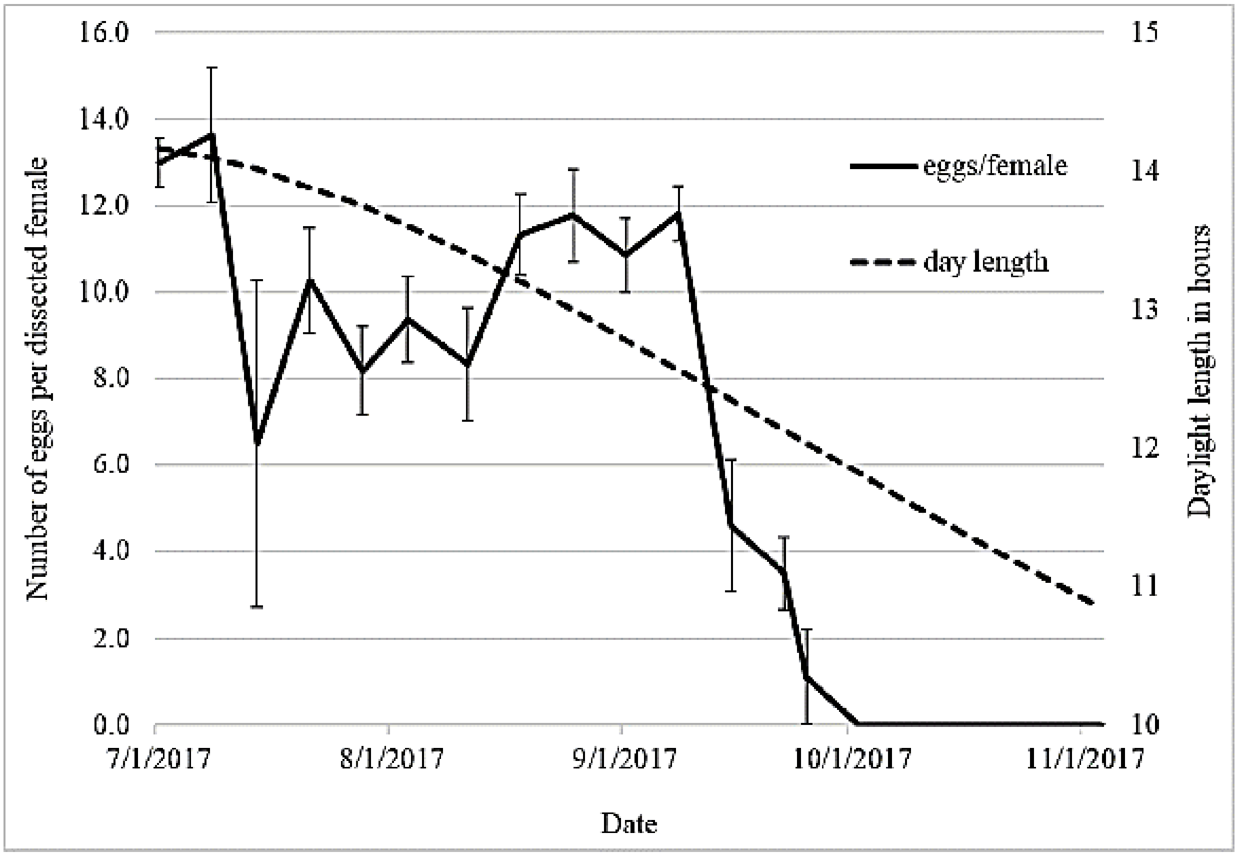

Figure 5.3. The mean ± std of total eggs and developing follicles of C. aeneus females collected from weevil emergence cone traps compared to day lengths at Tifton, GA, USA in 2017.

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SUMMARY

Three objectives were established in order to develop a better understanding of the biology and post-harvest control of the cowpea curculio. The first was to determine the best option for adult emergence monitoring through testing several different emergence traps through evaluating adult capture, ease of construction, environmental conditions, and cost of construction. The second was to test the efficacy of chlorpyrifos, *B. bassiana*, and *Heterorhabditis spp*., applied to the soil post-harvest, against the cowpea curculio by determining which treatment had the highest rate of mortality, or lowest adult capture. The final objective was to determine if and when cowpea curculio females exhibit diapause by quantifying egg development throughout the season.

Monitoring the cowpea curculio, *Chalcodermus aenus*, has been an issue throughout the production of cowpeas, partially due to the weevil's ability to feign death when approached (Arant. 1938). Prior to this work a Modified Tedders Trap was the only proven method for the seasonal monitoring of the cowpea curculio (Riley, et al. 2015). However, an emergence trap has now been developed for the purpose of monitoring adult emergence from the soil. Several emergence traps were tested, including; a bucket trap, pyramid trap in a cage, and different Modified Heliothis traps. The pyramid trap and bucket traps captured significantly fewer adults than the Modified Heliothis traps, thus, more work was done to determine the most effective method from the remaining traps. Each of the Modified Heliothis traps performed similarly, therefore, the least expensive trap was chosen.

The Modified Heliothis trap provided a potential way to evaluate the efficacy of insecticides and biocontrol agents against the soil phase of the cowpea curculio.

However, experiments using this approach were unsuccessful. The data were highly variable and there were no significant differences among the treatments, however, trends suggested that chlorpyrifos performed better than the biological control agents in these experiments. There also seemed to be increased success in applying the treatments prior to infestation. Additional investigation into the best methodology for this experiment is justified.

The final objective of this work, investigating potential diapause, contributed to the first record of biological evidence of diapause in the cowpea curculio. Anecdotal evidence suggested this phenomenon may occur. Larval emergence in emergence boxes declined from June to November and there were clear differences between the summer and fall generations, supporting potential diapause. Dissection data showed that the number of eggs per female also significantly declined during the same timeframe, reaching zero in October, expectedly as day lengths became shorter. These data contributed to the first publication on diapause in the cowpea curculio (Riley et al. accepted).