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Ishakh Pulakkatu-thodi

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INJURY AND DAMAGE BY THREECORNERED ALFALFA HOPPER,
SPISSISTILUS FESTINUS (SAY), IN GROUP IV SOYBEAN

By

Ishakh Pulakkatu-thodi

A Thesis
Submitted to the Faculty of
Mississippi State University
in Partial Fulfillment of the Requirements
for the Degree of Master of Science
in Agricultural Life Sciences (Entomology)
in the Department of Entomology and Plant Pathology

Mississippi State, Mississippi

May 2010

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By

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SPISSISTILUS FESTINUS (SAY), IN GROUP IV SOYBEAN

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Threecornered alfalfa hopper, *Spissistilus festinus* (Say), is a pest of soybean during vegetative and reproductive stages. The primary damage from this pest is girdling of the main stem during vegetative stages and girdling of the petioles during reproductive stages. Previous research determined that yield losses are greater during reproductive stages than vegetative stages. I hypothesized that some reproductive stages are more vulnerable to damage than other stages. I used field cages infested with different pest densities at five reproductive stages of group IV soybean. A greenhouse study compared the injury and damage caused by the adults and nymphs. The field study showed that the threecornered alfalfa hopper did not significantly impact yields at the growth stages studied. Adults preferred to feed on leaf petioles while nymphs fed mostly on stems. Significant yield reduction was noticed at growth stage R4 in the greenhouse due to adult and nymphs compared to control.

Key words: *Spissistilus festinus*, Threecornered alfalfa hopper, Soybean, Girdles

DEDICATION

I dedicate this document to my beloved parents, Mohammed Pulakkatu-thodi and Ayisha Kalaparambil, and my elder brother Haris Pulakkatu-thodi.

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CHAPTER 1
GENERAL INTRODUCTION

Soybean and U.S. Agriculture

Introduced to the U.S. in the mid-1770s from China as ballast in returning clipper ships, soybean, *Glycine max* (L.) Merrill, has become a major contributor to the agriculture industry of the nation (Smith 1994, USDA- ERS 2008). In 2008, the U.S. harvested 30.2 million hectares of soybean which accounted for a total farm value of \$27.4 billion, the second-highest crop value in the U.S behind corn. Soybean harvested area and production peaked in 2009 with a record-high of 30.99 million hectares and 0.09 billion tonnes, respectively (USDA-NASS 2009). The United States is the largest producer and exporter of soybeans. For example, in 2004, U.S. soybean production accounted for 40% of world production, and it exported about 35% of total production. Further, 90% of total oil seed production in the U.S. is from soybean, while other oil seeds such as cottonseed, sunflower seed, rapeseed and peanut complete the rest of the profile (USDA-ERS 2008). Iowa, Illinois and Minnesota are the three leading states in terms of production value according to 2009 statistics (USDA-ERS 2008). Soybean acreage has shown an increasing trend starting from 1988 to 2009 with the exception of a few years (Fig. 1.1). In addition, soybean yields continue to increase as a result of improved varieties and advanced farming practices. USDA agricultural baseline projections to 2017 predict the trend to continue (Fig. 1.2). Brazil and Argentina are the

other two major soybean exporters. Projections indicate that, in the immediate future, Brazil could replace the U.S. as the top exporter (USDA-NASS 2008).

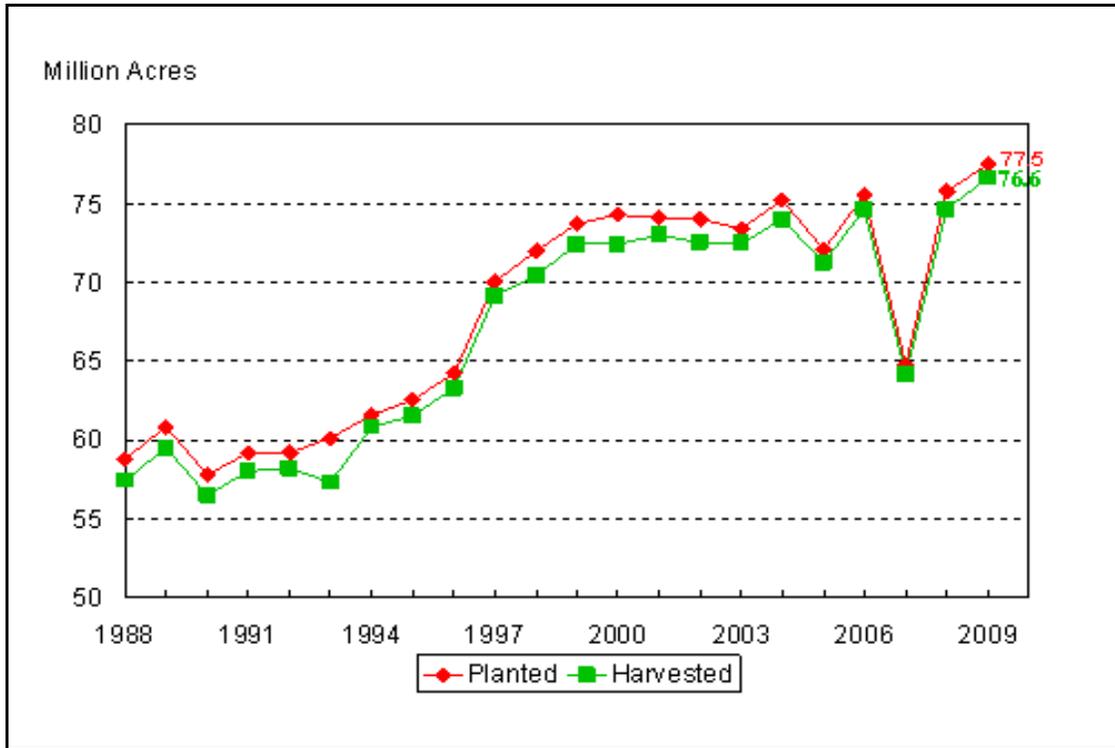


Figure 1.1 U.S. soybean acreage by year (USDA-NASS 2009)

Processed soybeans are the largest source of protein feed and second largest source of vegetable oil in the world (USDA-ERS 2008). Soybeans contain about 40% protein, 21% oil, 34% carbohydrate, and 5% ash (FAO, 2005). Initially, soybean was mainly grown for feed and industrial purposes; however, enhanced research on soybean processing was initiated in the 1940s to replace imported edible oils that were no longer available during World War II. Soybean quickly dominated the edible oil industry (Smith 1994). The current quest for bio fuels has opened up new markets for soybean, and the

potential of soybean as bio fuel is yet to be fully realized. Brazil’s competitive advantage may limit the U.S. share to the global market in the near future, but the U.S. soybean industry is expected to withstand the competition through increased support from domestic markets.

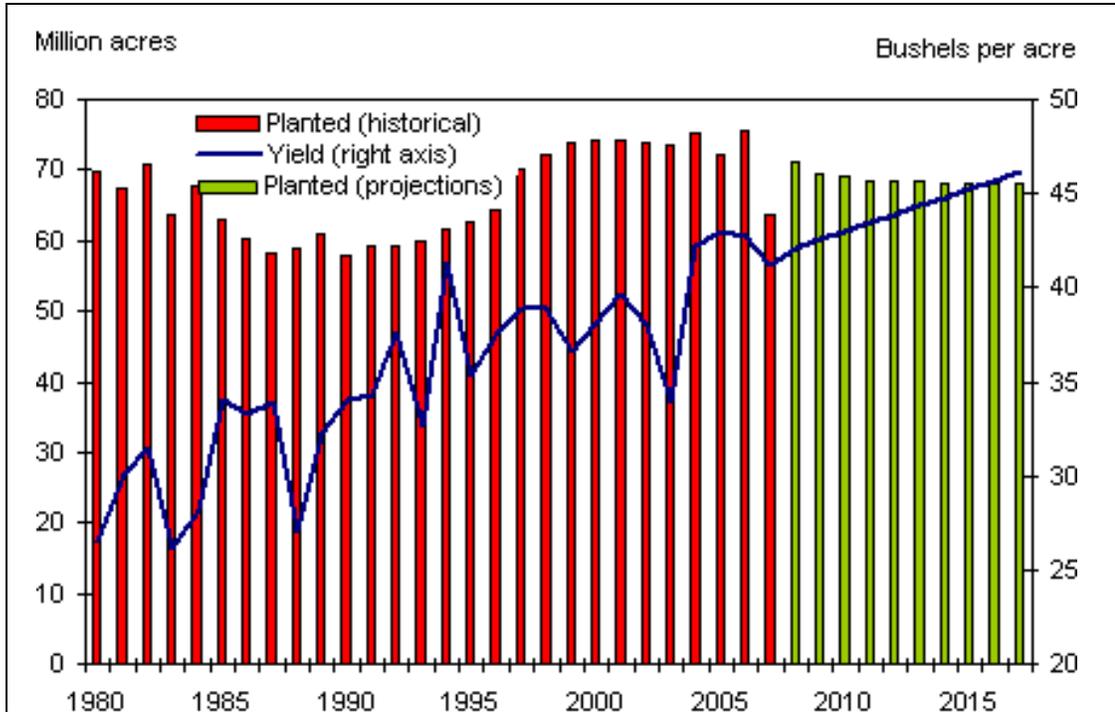


Figure 1.2 U.S. soybean acreage and yield -historical and projections (USDA - NASS 2009)

Soybean Production in the Mid-South

The soybean production system in the Mid-South has changed considerably in the last 15 years. Traditionally, Mid-South farmers planted late maturing determinate cultivars of maturity groups V, VI and VII. Planted mostly in May, these cultivars often suffered from severe drought conditions prevalent during the months of July, August and September, which caused significant yield reductions. Drought, coupled with low prices,

prompted a search for alternative practices. As a result, early planted, early maturing soybeans were adopted in the Mid-South which uses indeterminate cultivars of maturity group IV (Heatherly 1999). In this system, planting is initiated in mid-April and the crop is ready for harvest by the end of August. This approach not only improved yields by avoiding drought, but also reduced pest pressure from many late season defoliators (Baur et al. 2000). The higher yields with the early production system, along with better prices in recent years, have given new optimism for soybean production in the Mid-South. As a result, soybean acreage in MS has been increasing (USDA-NASS 2009).

Growth Stages and Maturity Groups of Soybean

Soybean is an annual legume which responds to photoperiod and temperature. Though classified as short-day plant, cultivars differ significantly in the critical day-length requirement (Garner and Allard, 1920) and temperature (Carlson and Lersten, 1987) needed to stimulate reproductive growth. There are thirteen maturity groups for soybean based on the response to day length conditions which range from 000, 00, 0 and I through X (Poehlman and Sleper, 1995). These are adapted for cultivation in comparatively narrow latitudinal belts (0 to 50°N) of the United States and southern Canada (Johnson and Bernard, 1962). Growth of soybean, from seedling emergence to full maturity of pods has been clearly described to facilitate the description of soybean development (Table 1.1).

Soybean cultivars are further classified as determinate and indeterminate types which are based on growth and flowering habit and genetic variation. Indeterminate types continue to grow from a vegetative terminal bud even after the appearance of flowers on

lower older nodes while vegetative growth stops on determinate types when reproductive growth begins. Flowering in determinate types occurs simultaneously on all the flowering nodes. No additional nodes are formed in determinate types after the initiation of flowering. In contrast, indeterminate soybeans start flowering from older nodes and progress up the plant and new nodes are formed at the top of the plant (Hodges and French 1985).

Table 1.1 Description of soybean growth stages (Fehr et al. 1971)

Stage	Stage Title	Stage Description
VE	Emergence	Cotyledons emerge above soil surface
VC	Cotyledon	Cotyledons fully unrolled.
V(n)	Nth-node	N number of nodes on main stem with fully developed leaves beginning with the cotyledon leaves
R1	Beginning bloom	Flowers open at any node on the main stem
R2	Full bloom	Flowers open at upper two nodes on the main stem with a fully developed leaf
R3	Beginning pod	Pod (0.5 cm) long at one of four uppermost nodes with a fully developed leaf
R4	Full pod	Pod (≈ 2 cm) long at one of four uppermost nodes with a fully developed leaf
R5	Beginning seed	Seed (0.31 cm) long in a pod at one of four uppermost nodes with a fully developed leaf
R6	Full seed	Pod containing a green seed that fills the pod cavity at one of the four uppermost nodes with a fully developed leaf
R7	Beginning maturity	One normal pod on the main stem has reached its mature pod color

Soybean Insect Management

In Mississippi, the major insect pests of soybean consist of a complex of stink bug (Hemiptera: Pentatomidae), bean leaf beetle (Coleoptera: Chrysomelidae) and threecornered alfalfa hopper, (Hemiptera: Membracidae). Stink bug species include southern green stink bug, *Nezara viridula* (L.), green stink bug, *Acrosternum hilare* (Say), brown stink bug *Euschistus servus* (Say), redshouldered stink bug, *Thyanta* spp., and redbanded stink bug, *Piezadorus guildinii* (West) and as a complex are the most damaging pest of soybean for the last several years (Musser and Catchot 2007). Economic losses from stink bugs primarily occur due to yield reductions and pest management costs. A four-year survey conducted by Musser and Catchot (2007) showed that the intensity of soybean pest management has been increasing, with more soybean acreage being scouted and more frequent insecticide applications.

Adoption of early planted indeterminate cultivars of soybean has helped to minimize damage from late season defoliators such as velvetbean caterpillar, *Anticarsia gemmatilis* (Hubner) and soybean looper *Pseudoplusia includens* (Walker). Percentage defoliation by these pests has been significantly reduced in early soybean production system (ESPS) compared to conventional system (McPherson et al. 2001). However, ESPS has favored early colonization of pests such as green stink bug and threecornered alfalfa hopper, *Spissistilus festinus* (Say) (Baur et al. 2000, McPherson et al. 2001). Flowering and pod development start early in these cultivars which are attractive structures for stink bug feeding. Because flowering and pod development are sequential, there is a constant supply of maturing pods for stink bugs to feed on. Overwintering

threecornered alfalfa hoppers can infest early planted soybeans shortly after breaking their reproductive diapause, avoiding traditional winter hosts such as clover and vetch (Baur et al. 2000).

Biology and History of Threecornered Alfalfa Hopper

Three-cornered alfalfa hopper, a small triangular insect of the order Hemiptera (formerly Homoptera) and family Membracidae, was first described as *Membracis festina* by Thomas Say in 1831(Say 1831 cited by Wildermuth 1915). It was later included in the genus *Stictocephala* by Stal (Stal 1869 cited by Wildermuth 1915) and was cited as *Stictocephala festina* (Say) in literature until Caldwell (1949) reclassified and placed it in the genus *Ceresini*. Later revisions, based on his studies on male genitalia of the tribe ceresini, identified the insect in the genus *Spissistilus*, and described it as *Spissistilus festinus* (Say) (Davis 1969).

The insect is common throughout the southern U.S and is found in limited numbers throughout the northern half of the United States. The name ‘threecornered alfalfa hopper’ was used by farmers referring to its shape and the host plant upon which it was commonly found. Preferred hosts are members of Leguminosae, but the hopper feeds and reproduces on a wide range of hosts including alfalfa, bermudagrass, johnsongrass, wheat, barley, oats, sweet clover, red and bur clovers, cowpeas, soybean, sunflower, cocklebur and vetch (Wildermuth 1915). Adult hoppers are about 6.2 to 6.8 mm in length, yellowish green in color and triangular in shape when viewed from above or the sides (Wildermuth 1915, Davis 1969). Adult males (Fig. 1.3) are characterized by the

presence of a reddish line down the dorsum of the prothoracic shield by which sexual distinction can be easily made (Fig. 1.3 and 1.4) (Wildermuth 1915, Davis 1969).

Females (Fig. 1.4) generally exhibit a pre-oviposition period of 5-16 days after their final molting (Davis 1969, Meisch and Randolph 1965). They insert white crescent-shaped eggs about 1 mm long into slits made by the ovipositor, just below the epidermis or deep into the stem (Wildermuth 1915). On soybeans, adult females lay eggs at the base of the lower petioles and lower main stem (Moore and Mueller, 1975). Greenhouse studies on alfalfa and soybean have revealed that preferred oviposition sites are at the base of the main stem, often just below the soil surface and occasionally on higher parts of the stem (Meisch and Randolph 1965). Later studies on oviposition on soybean clarified that the majority of eggs are laid in nodes, with greater numbers in unifoliate and trifoliate nodes than in cotyledonous nodes. As the plant advances to late vegetative phases, most eggs are laid on the upper nodes. A significant number of eggs are found on the terminal, indicating that the hopper uses all parts of the stem for oviposition (Rice and Drees, 1985).

The total life cycle of threecornered alfalfa hopper proceeds from egg, through five nymphal instars, and to adult. The incubation period of eggs and nymphal duration are found to vary considerably depending upon the temperature, photoperiod and type and availability of food sources (Mitchell and Newsom 1984a, Davis 1969, Meisch and Randolph 1965, Wildermuth 1915). For instance, the total life cycle of the threecornered alfalfa hopper on soybean is completed within 36-38 days under Mississippi conditions (Davis 1969); however greater variability of 35-114 days was observed at Tempe, Arizona on alfalfa (Wildermuth 1915). In Arkansas, it took an average of 18.7 days for

the 1st instar to emerge as an adult on soybean, while a longer period of 26.8 days was required on cocklebur under a mean temperature of 32.5 °C (Moore and Mueller 1975). First instars are usually straw colored and turn light green as they develop through further instars. The size of each instar, which ranges from 1.4 to 6.7 mm, the presence of characteristic spines on their body surface, and the development of wing pads are the key characters used to identify each nymphal stage (Wildermuth 1915, Davis 1969, Meisch and Randolph 1965).



Figure 1.3 Adult male of the threecornered alfalfa hopper (Photo: Scott Justis)

Threecornered Alfalfa Hopper and Soybean

Threecornered alfalfa hopper has traditionally been considered an early season pest of soybean (Mueller and Dumas 1975, Tugwell and Miner 1967, Tugwell et al. 1972). On young plants, the hopper feeds around the stem (Fig. 1.5) in a regular and continuous fashion just above the soil line, girdling the main stem. This feeding makes the plant weaker and may cause breakage or gradual death (Wildermuth 1915). The first report of a heavy infestation of threecornered alfalfa hopper on soybean was given by Jordan (1952) in Louisiana. Prior to that, Wildermuth (1915) and Packard (1951) reported that the pest could be injurious to soybean. Typically, the adult and nymph feed on the host plant by inserting their sharp mouth parts deep into the main stem to extract plant juices. Continuous and regular puncturing around the circumference of the stem forms a girdle which may lead to lodging, breakage or even plant death (Mueller and Dumas 1975). First and second instars generally do not form a girdle as they feed by single random punctures, but third to fifth instars and adults are capable of girdling a young plant (Spurgeon and Mueller 1992). Fourth instars are the most injurious stage. A single fourth instar nymph can completely girdle a plant within 24 hours (Moore and Mueller 1975). Stylet insertions usually reach vascular bundles and thus block and disrupt nutrient translocation (Mitchell and Newsom 1984b). As the plant grows and the main stem thickens, it becomes less favorable for feeding, so they move upward into the canopy and cause girdles on leaf petioles (Fig. 1.6) and feed on developing pods (Bailey et al. 1970, Mitchell and Newsom 1984b). A radiotracer study using ^{14}C labeled glucose showed that petiole girdling blocked movement of assimilates in the phloem (Hicks et al. 1984). The

presence of girdles increased severity of stem blight and stem anthracnose in some cultivars and reduced the germination percentage of seeds (Russin et al. 1987).



Figure 1.4 Adult female of the threecornered alfalfa hopper (Photo: Lynette Schimming)

Caviness and Miner (1962) conducted a three-year study to estimate the impact of threecornered alfalfa hopper damage on soybeans. They simulated hopper damage by manually clipping soybean plants at random to create 3 levels of stand reduction (15%,

30% and 45%), each at three different growth stages (two weeks before bloom, at bloom and two weeks after bloom). Yields of these treatments were compared to plots with no stand reduction. Fifteen and 30 percent stand reductions did not reduce yield significantly in any of the growth stages. Removal of 45% of the plants 2 weeks before bloom did not reduce yields significantly based on a 3-year average. However, 45 and 30% stand reduction at bloom and post bloom respectively resulted in a yield reduction. This was further supported by studies from Mueller and Jones (1983), when they found that damage during early vegetative stages did not affect plot yields unless 65 to 70% of plants were girdled. Tugwell et al. (1972) observed a comparable result as they found no significant difference in yields between unsprayed plots and plots in which alfalfa hoppers were controlled with insecticide applications during vegetative growth. These findings, supported by evidence from studies by Sparks and Newsom (1984), led to the establishment of the current threshold at 1 threecornered alfalfa hopper/sweep for plants greater than 25 cm in height.

Yield losses due to feeding by this pest depend on soybean stand and environmental stresses faced by plants during later stages of development. In many cases, there is no yield loss from losing a few plants due to girdling (Mueller and Dumas 1975). One of the main reasons for this is the ability of soybeans to compensate for low plant populations. During early vegetative stages, healthy plants branch profusely and occupy the spaces of adjacent damaged plants and make up for the loss. It has been well demonstrated that soybeans can withstand up to 45% stand reduction during vegetative growth, without any substantial yield reduction (Caviness and Miner, 1962). Another reason some stand loss is generally not a problem is the use of a high initial seeding rates.

For example, the recommended seeding rate in Arkansas is about 39 seeds per row meter to give a growing density of 26 plants per row meter (Mueller and Dumas, 1975). However, the minimum plant requirement for a maximum yield is 9 to 12 healthy plants per row meter (Wiggins 1939, Wright et al. 2002). Thus, it is not surprising that yields are not affected by early season infestation of the pest unless >65% of the main stems are girdled (Mueller and Jones 1983).



Figure 1.5 Soybean main stem showing threecornered alfalfa hopper girdling (Photo: Ishakh Pulakkatu-thodi)

Population Dynamics

Previous studies suggest that 3-4 generations of threecornered alfalfa hopper are possible per season (Mitchell and Newsom 1984a, Jordan 1952, Wildermuth 1915). At least two complete generations occur in soybean (Heatherly 1999). Individuals of the last

generation in soybeans exhibit hypertrophied fat bodies, indicating the beginning of reproductive diapause (Mitchell and Newsom 1984a). Adults have been found overwintering under pine trees and feeding on pine needles on warm winter days. Under the thick cover of fallen pine needles, these hoppers can survive temperatures as low as -12 °C. At the onset of spring, threecornered alfalfa hoppers move to leguminous hosts such as clover and vetch and start to reproduce on them (Newsom et al. 1983). Recent observations (Baur et al. 2000, McPherson et al. 2001) indicate that the population dynamics of threecornered alfalfa hopper are changing due to the adoption of the early soybean production system. Overwintering populations of many arthropods, including threecornered alfalfa hopper, colonize early planted soybeans, avoiding their traditional spring hosts, and thus enabling a higher density of pests during sensitive soybean growth stages.



Figure 1.6 A leaf petiole showing girdling injury (Photo: Ishakh Pulakkathodi)

Rearing of Threecornered Alfalfa Hopper

To facilitate research on the threecornered alfalfa hopper, many researchers have tried to rear the pest in the greenhouse or laboratory. They can be reared on potted soybean plants (Meisch and Randolph 1965, Mitchell and Newsom 1984a) in the greenhouse and on green beans, *Phaseolus vulgaris* F., in glass jars in the laboratory, or by using a combination of both (Mitchell and Newsom 1984a, Mueller and Jones 1983). Potted soybean plants are covered with screen cages of appropriate sizes and field collected adults are introduced to the cages for oviposition. Plants are observed for nymphal emergence after which they are transferred to glass jars with green beans as a food source. Under long-day conditions (26.5 °C and photoperiod of LD 14:10) created in the laboratory, a single field collected female is capable of producing an average of 223

first-instars with a mean reproductive life span of 38.6 days (Mitchell and Newsom 1984a).

Significance of the Current Study

Numerous studies were conducted on *S. festinus* addressing early season damage, impact of feeding on soybean yield, biology and seasonal occurrence from 1965 to 1984 (Meisch and Randolph 1965, Mueller and Dumas 1975, Tugwell et al. 1972, Bailey 1975, Moore and Mueller 1975, Mitchell and Newsom 1984a, Hicks et al. 1984). However, the impact of late season damage was neglected until researchers initiated several studies at Louisiana State University and Arkansas in the 1980s. They found that yield losses from threecornered alfalfa hopper were actually more common from late season infestations than from early infestations (Sparks and Newsom 1984, Sparks and Boethel 1987, Sparks et al. 1987, Spurgeon and Mueller 1992). Their research is part of the basis for our current threshold of one threecornered alfalfa hopper/sweep on plants greater than 25 cm tall (Catchot 2007). Sparks and Boethel (1987) noted that yield reductions occurred with a threecornered alfalfa hopper population at a density 60% of the current threshold and suggested that the existing threshold should be modified. Dr. Gordon Andrews (MSU DREC, Stoneville, MS) concluded that threecornered alfalfa hopper sometimes causes soybean yield losses without reaching the current threshold. At the joint conference of the MS Pest Management Associations (Feb. 26, 2008 Stoneville, MS), Andrews stated that “yield losses were evident even when threecornered alfalfa hopper populations never exceeded 50% of current threshold. Given the current high price of soybeans and low cost of pyrethroid insecticides, preventing almost any yield loss would be economical”.

However, applying insecticides is not merited unless it is justified by the return on the investment. Indirect returns through natural enemies should also be considered while making a decision because they often help to maintain pest population levels below thresholds if not disturbed by insecticide applications.

Since the establishment of the current treatment threshold in the 1980s, soybean production in the Mid-South has changed substantially. Today most growers in Mississippi have adopted the early soybean production system which uses early-maturing varieties and earlier planting dates (Heatherly 1999). This change has improved yields and reduced late-season insect pressure by defoliators. However, pressure from sucking pests like the threecornered alfalfa hopper has increased during early to mid-season under the ESPS (Baur et al. 2000). With yields and prices dramatically higher than when the threecornered alfalfa hopper research was conducted, the economic relationship between crop loss and insect control has changed. This may alter the current economic threshold for threecornered alfalfa hopper. Because the soybean growth stages are well defined (Fehr et al. 1971) and predictable (Zhang et al. 2005), depending upon the maturity group, more accurate pest management decisions are possible if we could determine the growth stage or stages that are most vulnerable to threecornered alfalfa hopper damage.

Immature threecornered alfalfa hoppers, especially late instars, cause similar injury as adults (Moore and Mueller 1975). An effort was made by Sparks and Boethel (1987) to compare the damage and yield reduction caused by adults and nymphs separately. Their experiment, which was conducted in the open field, used insecticide applications at various stages of *S. festinus* development to control the second generation of the pest and eliminate portions of late-season damage. Comparisons among treatments

allowed determination of yield reductions by nymphs versus adults. However, the pest density was based on a natural infestation and the growth stage of soybean was not controlled as they had to wait for nymphal instars to reach different stadia. A field cage or greenhouse experiment in which population levels and time of infestation could be controlled is a better way to understand the injury and yield loss due to adults and nymphs. The results from these experiments would be useful for better decision making on timing of insecticide applications. By sampling adults and nymphs during soybean reproductive stages, any shift in the population dynamics of threecornered alfalfa hopper in relation to different growth stages could be studied and pest management decisions could be made accordingly.

Besides alfalfa and soybean, which are two conventional hosts, the threecornered alfalfa hopper is a major pest of peanuts in Georgia (Brown et al. 2000) and Alabama where insecticide application is recommended when >30% of plants are damaged (Weeks 2006). In South Carolina up to 89% of peanuts were girdled in 2003 (Rahman et al. 2007). The reports on this pest being injurious to other crops and the scarcity of published research on this pest after the late 1980s makes it important to gain more information about this insect.

The current experiment was an initial attempt to understand the dynamics of threecornered alfalfa hopper infestation density, subsequent injury and resulting yield responses on indeterminate maturity group IV soybeans. A series of field cage and greenhouse experiments were conducted to investigate these relationships. To address this, the following objectives were proposed.

- I. To assess injury and damage due to different pest densities of threecornered alfalfa hopper adults at reproductive stages R3 through R7 in maturity group IV soybean
- II. To compare injury and damage due to adults and nymphs of threecornered alfalfa hopper

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CHAPTER 2
GIRDLING INJURY AND YIELD IMPACT BY THREECORNERED ALFALFA
HOPPER ADULTS IN GROUP IV SOYBEAN

Abstract

Field cages experiments were conducted in 2008 and 2009 at Starkville and Stoneville, MS, to understand the effect of different densities of threecornered alfalfa hopper at critical reproductive stages of group IV soybean. The experiment was successful in creating three different injury levels at maturity stages from R3 through R7. The injuries mainly consisted of leaf petiole girdles and they were distributed around the middle of the soybean plant. Significant yield reductions were not observed by these pest densities either overall or at any of the soybean growth stages. The mean thousand seed weight from Starkville in 2008 field was found to vary significantly at R6 but this was not consistent in any other fields or maturity stages. The mean weight of individual seeds collected from the nodes on the upper half of the plant showed an increasing trend with the increase in pest density. The seed quality in terms of percentage protein and fat content generally was not impacted by the threecornered alfalfa hopper.

Introduction

Injury caused by threecornered alfalfa hopper, *Spissistilus festinus* (Say) on soybean has previously been referred to as girdles (Mitchell and Newsom 1984,

Wildermuth 1915), which block nutrient translocation along stem and leaf petioles and cause lodging of early infested plants (Hicks et al. 1984, Mueller and Dumas 1975). The current treatment threshold for this pest in soybean is one threecornered alfalfa hopper/sweep (Catchot 2010) during all reproductive stages and it is based on the research by Sparks and Newsom (1984). They, like other researchers (Tugwell et al. 1971, Sparks and Boethel 1987), used insecticidal applications to achieve different densities of threecornered alfalfa hopper during early and late stages of soybean growth. The distinction between early and late season was often based on plant height and included both vegetative and reproductive stages. Experiments with a known density of threecornered alfalfa hopper were conducted by Bailey (1975) using screen cages placed on two row sections of soybean. In his study, soybeans were at their early vegetative stage (3.5 to 5 cm tall) and yield reductions occurred due to lodging of girdled plants later in the season. Yield losses from early season main stem girdling were also observed by Mueller and Dumas (1975). However, later studies by Mueller and Jones (1983) showed that soybeans recuperated from early season injuries by plant compensation and re-growth of girdled plants. A similar finding was also reported in Arkansas from simulated stand reduction experiments (Caviness and Miner 1962).

Being a photosensitive crop, soybean responds strongly to daylength and temperature in its development (Garner and Allard, 1920, Carlson and Lersten, 1987). Daylength responses vary greatly among varieties. There are thirteen maturity groups for soybean based on the response to day length conditions which range from 000, 00, 0 and I through X (Poehlman and Sleper 1995). Soybean growth stages beginning from emergence to maturity are well described based on plant morphology (Fehr et al. 1971).

Reliable estimates about the date at which a soybean field enters a particular growth stage are possible based on growth phenology models for various maturity groups (Hodges and French 1985).

Since the establishment of the current treatment threshold of one threecornered alfalfa hopper/sweep in 1984, soybean cultivation in the Mid-South has changed considerably (Heatherly 1999). Mississippi farmers now plant earlier maturing varieties in maturity groups IV and V for higher yield potential and reduced pest pressure from late season defoliators. These varieties are planted in April and early May as compared to traditional planting times of late May and June. Up to 80% of soybeans in MS are planted early when weather conditions are favorable at the time of planting (Koger 2009, personal communication). This shift has resulted in higher densities of some pests like threecornered alfalfa hopper and stink bugs in soybean (Baur et al. 2000, McPherson et al. 2001).

Sparks and Newsom (1984), suggested that the maximal benefit of control is not realized by sampling adults because they developed through nymphal stages which were equally injurious. Later, scientists investigated the role of nymphs in yield reduction and suggested adjusting sampling methods to include damage by nymphs (Sparks and Boethel 1987). The concerns expressed by Sparks and Newsom (1984) and Sparks and Boethel (1987) were never addressed. Now that the early soybean production system (ESPS) has been widely adopted in MS and the profitability has increased due to better yields and prices, further study of threecornered alfalfa hopper damage in soybeans during reproductive growth is merited. The reports on the changing population dynamics of threecornered alfalfa hopper due to the ESPS (Baur et al. 2000, McPherson et al. 2001)

and recent studies on infestation of threecornered alfalfa hopper in peanuts (Rahman et al. 2007) have made such an effort even more critical.

The current experiment investigated the effect of different densities of threecornered alfalfa hopper at various growth stages of soybean to quantify damage at each stage. I hypothesized that the girdling injury by threecornered alfalfa hoppers could be more harmful to some growth stages than to others. To test this hypothesis, I investigated the effects of injury caused by threecornered alfalfa hopper adults at various reproductive stages of soybean using 3 different pest densities.

Materials and Methods

The experiment was conducted during 2008 and 2009 at the R. R. Foil Plant Science Research Center in Starkville, MS and the Delta Research and Extension Center in Stoneville, MS. Maturity group IV soybeans were planted on 22 April in Starkville and 30 April in Stoneville during 2008. In 2009 the planting dates were 28 April in Starkville and 20 April in Stoneville. In 2008, DK4651 Roundup Ready soybean was planted at both locations, while in 2009, both locations were planted with AG4605. Seeding density was between 26 and 33 plants per row meter in all fields. Adequate rainfall during early vegetative stages and furrow irrigation late in the season ensured a good soybean stand. Each location had 15 treatments which were replicated three times. Each treatment consisted of a 1.8 m section of two rows of soybeans planted in 76 cm rows contained inside a 1.8 m x 1.8 m x 1.8 m cage of 32 mesh screen placed over a galvanized iron pipe frame. The trial was laid out in a randomized complete block design. The treatments consisted of three pest densities (0, 1 and 3 threecornered alfalfa

hoppers/plant) caged on soybeans for one week beginning at each of five 5 growth stages (R3, R4, R5, R6 and R7). Two buffer rows were maintained between plots. Adult threecornered alfalfa hoppers were collected from soybean fields using a 38-cm diameter sweep net and maintained on soybean plants until they were released onto the plants. The dates of infestation in 2008 were 23 June, 1 July, 21 July, 14 August and 29 August at R3, R4, R5, R6 and R7 stages, respectively, for the Starkville location. At the Stoneville location, the infestation dates were 2 July, 9 July, 16 July and 14 August for R3, R4, R5, and R6, respectively. The infestation at R7 was omitted at the Stoneville location in 2008 due to a late season charcoal rot and dectes stem borer infestation. In 2009, the treatment dates were 29 June, 6 July, 20 July, 4 August and 26 August in Starkville and 25 June, 10 July, 23 July, 6 August and 17 August in Stoneville for R3 to R7 stages, respectively. One week after infestation, cages were removed and 10 plants were collected from the treatments to assess girdling injury by the pest. Samples were frozen to minimize wilting and leaf shed until all observations were recorded. Each leaf petiole, internodal space, pod and peduncle were carefully observed for girdling. The number and location of girdles were recorded with reference to the main stem node. Girdles present on the leaf petiole of the main stem were labeled as leaf petiole girdles (LPG) and girdles present on the main stem were referred as main stem girdles (MSG). All treated plots were sprayed with Karate Z 2.08 CS (Syngenta Crop Protection, U.S.) at the rate of 75 ml/ha to eliminate threecornered alfalfa hopper after cage removal. The experimental field was sampled using a 38 cm sweep net at each maturity stage from R3 to R7 to assess the level of natural infestation of threecornered alfalfa hopper and other pests such as stink bugs and bean leaf beetles. In 2008, the Starkville field received an insecticidal spray to

control southern green stink bug immediately after the R5 treatment was completed. In 2009 no additional insecticidal applications were required as all pests were below thresholds.

The plots were harvested manually on 3 September in Stoneville and 18 September in Starkville in 2008. In 2009, the Stoneville field was harvested on 28 August and the Starkville field was harvested on 15 September. The harvested plants from individual plots were bundled and dried to make threshing easier. From each bundle, 10 plants were taken out and kept frozen to do plant mapping. The remaining plants were threshed using a 'small bundle thresher' (ALMACO, Nevada). The seeds were cleaned using a fractional aspirator. Yield, percentage moisture, thousand seed weight, and test weight were recorded. The yield was expressed in kg/ha after standardizing to 12% moisture. Seed samples were analyzed for protein and crude fat content at H.W. Essig Nutrition Laboratory, Mississippi State University, Mississippi State, MS.

Plant mapping was conducted by partially adopting the concept used by Gai et al. (1984). The number of pods, number of seeds and seed weight from each node of 10 soybean plants from each replication were recorded. Data from lateral stems were not mapped because the plants were not uniformly branched and development on lateral stems could not be associated with main stem development. Plant mapping was done because I expected that if the girdling injury had a direct effect on any yield component, it would be reflected in areas of highest insect injury.

The data collected on injury, yield/ha, yield/plant, thousand seed weight, test weight (kg/hL), and percentage protein and fat content were analyzed using the PROC GLM and PROC MIXED procedures of SAS 9.2 (SAS Institute 2009).

Results and Discussion

Girdling Injury

The mean number of all girdles (MSG and LPG) per soybean plant varied significantly with the threecornered alfalfa hopper density ($F= 25.31$; $df= 2, 145$; $P= 0.0001$) (Fig. 2.1). Growth stage did not affect the mean number of girdles ($F= 1.21$; $df= 4, 147$; $P= 0.306$) except in Starkville during 2009 ($F=14.59$; $df=4, 30$; $P=0.0001$). There was no interaction between growth stage and pest density at any location ($F= 0.36$; $df= 8, 147$; $P= 0.939$). Overall all locations, the infestation density had a significant impact on the total number of girdles at growth stages R3 ($F= 8.7$; $df= 2, 31$; $P= 0.001$), R4 ($F= 15.05$; $df= 2, 31$; $P= 0.0001$), R5 ($F=7.41$; $df= 2, 31$; $P=0.0023$) and R7 ($F= 10.86$; $df= 2, 14$; $P= 0.0014$). The soybeans were exposed to natural infestations of threecornered alfalfa hopper except at the period of the treatment and suffered some injury from these natural population (Fig. 2.1). The overall mean number of girdles/plant was highest in Starkville during 2008 (4.41/plant) followed by Starkville in 2009 (1.95/plant). The soybean plants in Stoneville suffered less girdling injury (1.11 and 1.38 girdles/plant during 2008 and 2009, respectively). The variation in the amount of injury among locations may be due to the differences in the natural densities of threecornered alfalfa hopper. For example, during R3 stage, the natural density in Starkville during 2008 was 11.8 threecornered alfalfa hoppers/ 25 sweeps, but only 4.2 threecornered alfalfa hoppers/ 25 sweeps were caught in Stoneville at the R3 stage during 2008.

A comparison between LPG and MSG densities are shown in Fig. 2.2. There were few MSGs compared to LPGs at all growth stages. Previous studies have shown that threecornered alfalfa hopper change their feeding location with the change in soybean

phenology (Bailey et al. 1970, Mitchell and Newsom 1984). During reproductive stages they move upward and feed on leaf petioles, pods and peduncles. However, occurrence of girdles on reproductive structures is extremely rare (Mitchell and Newsom 1984). Our observation on the location of girdles is in agreement with previous reports. A graphical representation of the number of girdles on a soybean plant and their positions with respect to plant node is shown in Fig. 2.3. The data combines all five growth stages studied. Each data point represents about 270 plants. The distribution of leaf petiole girdles (LPG) and main stem girdles (MSG) under the highest pest density (3 threecornered alfalfa hoppers/plant) is compared with the control (0 threecornered alfalfa hoppers/plant). The majority girdles caused by the threecornered alfalfa hopper during reproductive growth were distributed between 8 and 17 nodes of the soybean plant. The number and distribution of MSGs, even under high pest pressure, was very low and comparable with the control, which indicates that threecornered alfalfa hopper preferred non-stem structures for feeding. The presence of girdles on the control plants suggests pressure from natural populations of threecornered alfalfa hopper that likely occurred during earlier growth stages. There were no MSGs observed at the first 5-6 nodes from the base of treated soybean and control. This indicates that maturity group IV soybeans, which were planted earlier than conventional soybeans, avoided early season main stem girdling. The analysis of girdles gives a general idea about threecornered alfalfa hopper activity during reproductive stages and their preferred feeding areas in the canopy. However, because they can feed on pods and peduncles (Mitchell and Newsom 1984), girdles are not the only indication of pest pressure.

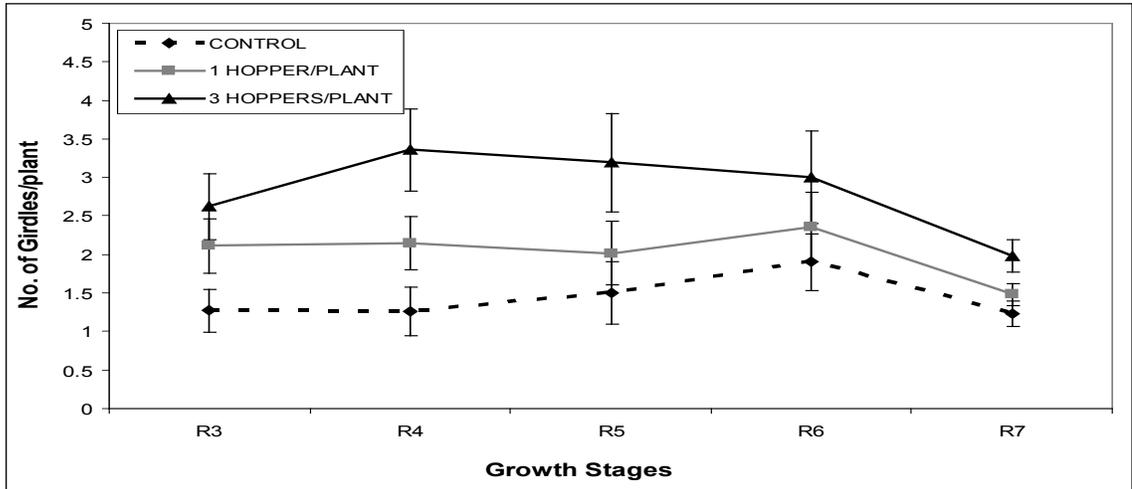


Figure 2.1 Mean girdles \pm SEM/plant when soybeans were infested with 3 different densities of threecornered alfalfa hopper at growth stages R3 through R7 for one week (Starkville 2008, 2009 and Stoneville 2008, 2009)

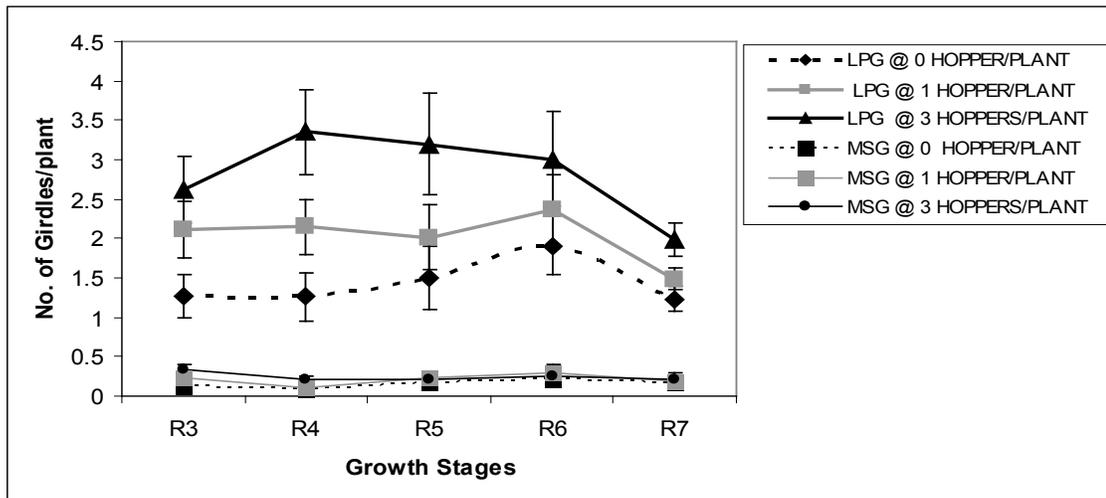


Figure 2.2 Mean leaf petiole girdles (LPG) and main stem girdles (MSG) \pm SEM/plant from threecornered alfalfa hopper infestations made at growth stages from R3 through R7 for one week (Starkville 2008, 2009 and Stoneville 2008, 2009)

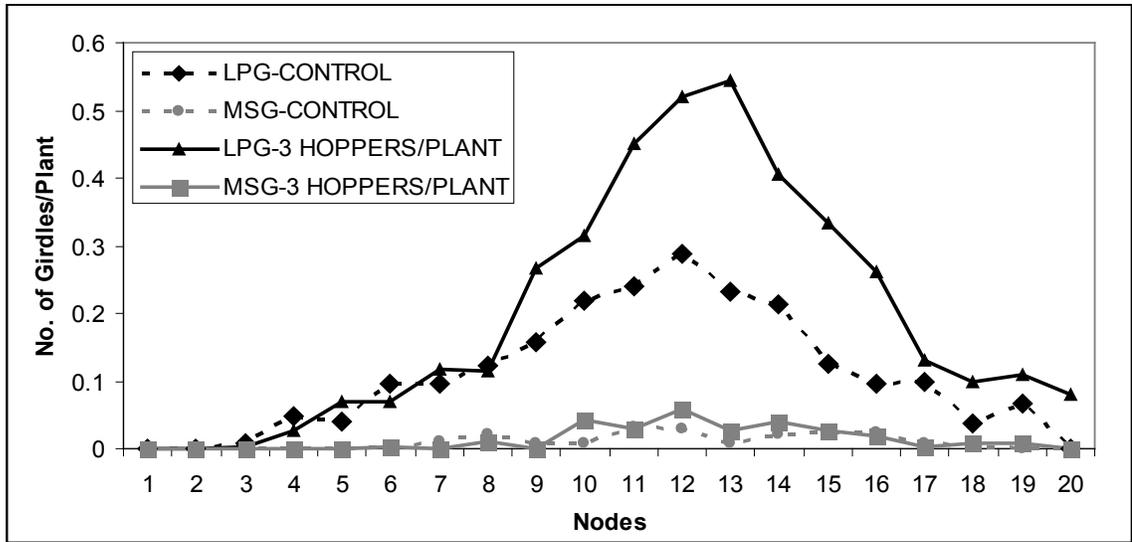


Figure 2.3 Distribution of main stem girdles (MSG) and leaf petiole girdles (LPG) under two pest densities- 0 hopper/plant (control) and 3 hoppers/plant (Starkville 2008, 2009)

Yield and Yield Components

The mean yield did not differ significantly among treatments, either overall or in any of the individual fields (Overall: $F=0.04$; $df= 2, 119$; $P= 0.96$). Similarly mean yield did not differ significantly with threecornered alfalfa hopper density at any of the growth stages (Table 2.2). However, overall mean yield varied with the growth stage infested ($F= 3.47$; $df= 4, 119$; $P= 0.01$). The interaction between growth stage and threecornered alfalfa hopper density was not significant ($F= 0.53$; $df= 8, 119$; $P=0.83$). Because the overall yield did not vary significantly with the threecornered alfalfa hopper density and there were no significant interactions between pest density and growth stage, it can be concluded that yield was not impacted by the threecornered alfalfa hopper. Fig. 2.4 shows a declining trend in yield between R3 and R6 irrespective of pest pressure. The pod and seed formation stages (R3 to R6) are very critical to soybean. The length of photoperiod

has a positive effect on number of pods and seeds. It has been shown that the extended photo period increases the R3-R6 stages which in turn increase the number of pods and seeds in indeterminate soybean (Kantolic and Slafer 2004). Therefore the yield response from infestations at different growth stages may have been due to the effects of the shade caused by the field cages.

Table 2.1 F-statistics of the yield and thousand seed weight of soybean infested with 3 different densities of threecornered alfalfa hopper at growth stages R3 through R7 for one week (Starkville 2008, 2009; Stoneville 2009)

Growth Stages		F	df	P
Yield	R3	0.74	2, 22	0.48
	R4	0.72	2, 21	0.49
	R5	0.38	2, 22	0.69
	R6	0.99	2, 22	0.38
	R7	1.23	2, 22	0.31
Thousand seed weight	R3	1.07	2, 22	0.35
	R4	1.49	2, 21	0.24
	R5	0.38	2, 22;	0.68
	R6	0.72	2, 22	0.49
	R7	0.01	2, 22	0.98

The analysis of thousand seed weight showed similar results as the yield data (Fig. 2.5). Threecornered alfalfa hopper density did not have a significant effect on mean seed weight either overall or at any of the maturity stages (Overall: $F=0.53$; $df= 2, 119$ $P= 0.59$) (Table 2.1). However, the growth stage infested was a significant factor of seed weight ($F= 7.03$; $df = 4, 119$; $P= 0.0001$). Though not significant overall, threecornered

alfalfa hopper density significantly reduced seed weight from infestations during R6 in Starkville during 2008 ($F=8.86$; $df = 2, 6$; $P= 0.0162$). Test weights were uniform among all parameters measured (2008: $F= 0.27$; $df= 2, 29$; $P= 0.76$, 2009: $F= 1.98$; $df= 2, 30$; $P= 0.15$). Regression analysis was used to examine the trend of yield reduction at growth stages having more girdles (Fig. 2.1 and Fig. 2.4). However, the number of girdles was not a significant factor of yield ($F= 1.01$; $df= 1, 121$; $P= 0.31$).

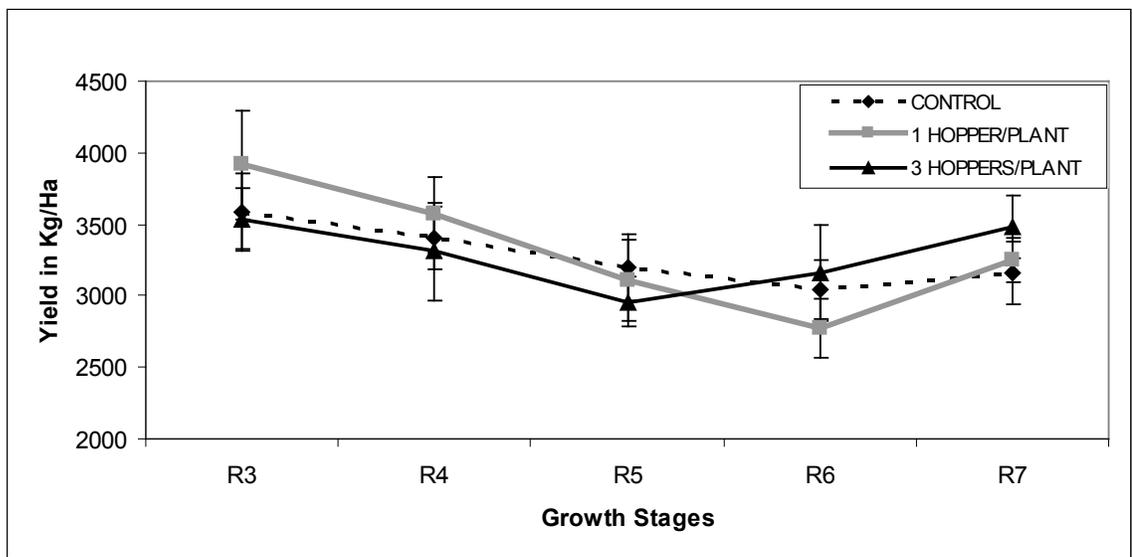


Figure 2.4 Mean yield \pm SEM (kg/ha) when soybeans were infested with 3 different densities of threecornered alfalfa hoppers for one week at growth stages R3 through R7 (Starkville 2008, 2009; Stoneville 2009)

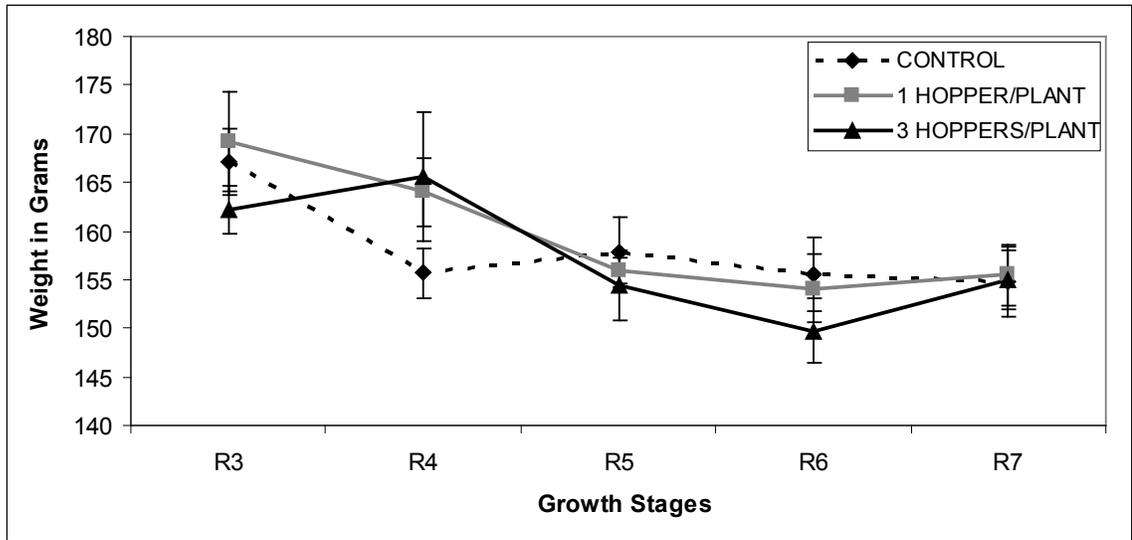


Figure 2.5 Mean weight of thousand seeds \pm SEM (g) when soybeans were infested with 3 densities of threecornered alfalfa hoppers for one week at growth stages R3 through R7 (Starkville 2008, 2009; Stoneville 2009)

Plant Mapping

Mean weight of a single seed at each node was calculated from the data collected using plant mapping. Only data from the highest (3 threecornered alfalfa hoppers/plant) and lowest (control) pest densities were compared. The three fields tested (Starkville 2008, 2009 and Stoneville 2009) showed a trend of increased seed weight with the highest pest pressure from the nodes 11 to 17 which had suffered the highest number of girdles (Fig. 2.3 and Fig. 2.6). The mean weight of seed from 11 through 17 nodes were statistically analyzed but they were not significantly different due to the treatment or growth stage (Treatment: $F= 1.86$; $df= 1, 79$; $P= 0.17$, Growth stage: $F= 1.87$; $df= 4, 79$; $P= 0.12$). The number of pods/plant, seeds/plant and seed weight/plant were analyzed but there were no significant differences observed in any of these yield components due to threecornered alfalfa hopper density or soybean growth stage. Sparks and Boethel (1987)

did not observe any significant reduction in number of pods or seeds per row meter between control and adult threecornered alfalfa hopper treatments, but observed a significant reduction in 100 seed weight in the control treatment. However, because they observed a significant yield reduction due to the threecornered alfalfa hopper treatment at the field level, this observation was not given much attention. It may be possible that plants compensate for injury at the seed level, but this requires further research. I didn't observe any girdling on peduncles, pedicels or on the pods, which agrees with the observation of Mitchell and Newsom (1984). Yield reduction does not appear to be the result of pod shedding or seed abortion due to the girdling because they rarely make girdles on reproductive structures.

Analysis of Seed Quality

The percent of protein content was significantly affected by threecornered alfalfa hopper density at R6 in Starkville during 2009 ($F= 7.33$; $df= 1, 4$; $P= 0.05$). Other than this location and year, the combined data did not show any other factor being significant for crude protein (Fig. 2.7). Analysis of variance on percent crude fat content showed an overall significant difference due to growth stage ($F= 6.91$, $df= 4, 77$; $P= 0.0001$) (Fig. 2.8). Individual fields in Starkville during 2008 ($F=4.11$; $df=4, 19$; $P= 0.01$) and Stoneville during 2009 ($F=4.82$; $df= 4, 20$; $P=0.006$) showed similar results. However, there were no significant differences due to threecornered alfalfa hopper density.

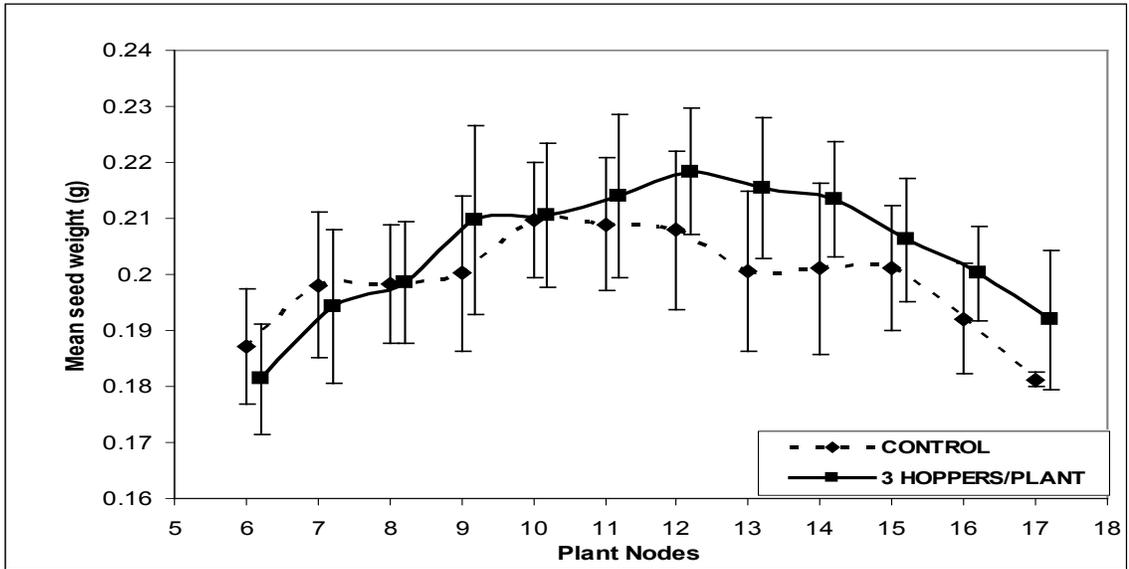


Figure 2.6 Mean weight of individual seed (grams) \pm SEM at each soybean node. Data are averaged for threecornered alfalfa hopper infestations made at growth stages R3 through R7 (Starkville 2008, 2009 and Stoneville 2009)

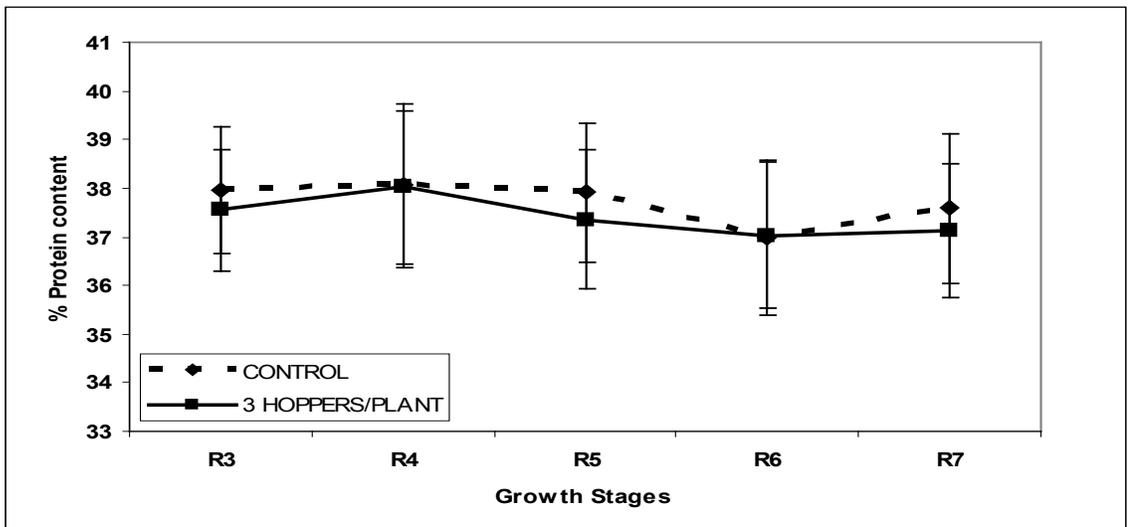


Figure 2.7 Percent of protein content \pm SEM of soybean infested with highest (3 hoppers/plant) and lowest (Control) pest density at reproductive stages R3 through R7 (Starkville 2008 and 2009, Stoneville 2009)

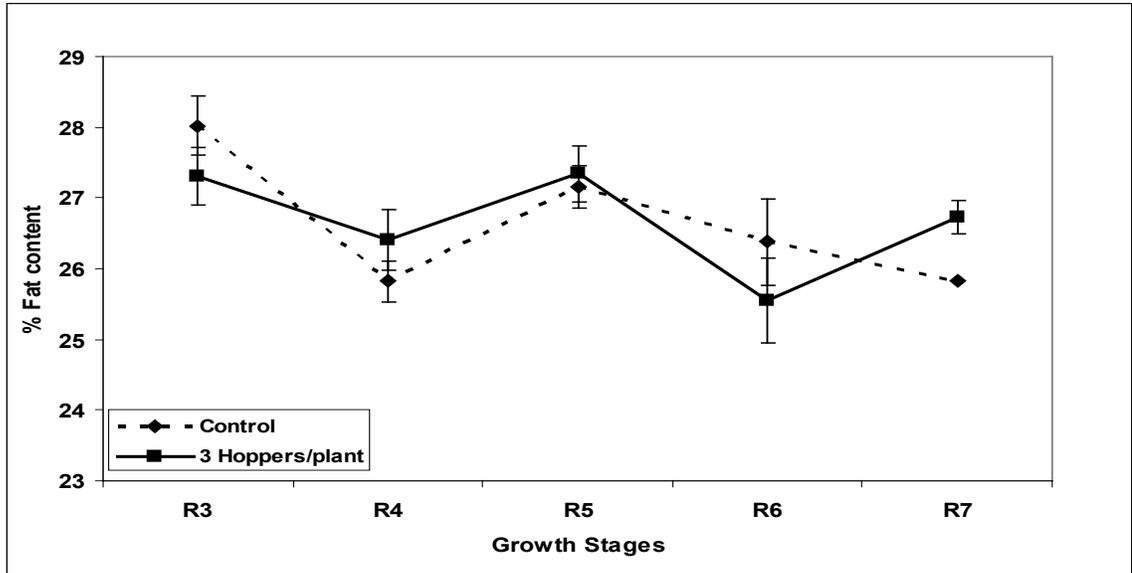


Figure 2.8 Percent fat content \pm SEM of soybean infested with highest (3 hoppers/plant) and lowest (Control) pest densities at reproductive stages R3 through R7 (Starkville 2008 and 2009, Stoneville 2009)

The significant difference in number of girdles by different pest densities tested indicates that the threecornered alfalfa hopper infestations were successful in establishing different levels of injury (Fig. 2.1). The average number of trifoliolate leaves on the main stem of a fully grown soybean plant ranged between 18 and 21 depending upon the variety and location. Based on the analysis of the injury (Fig. 2.3), girdles mainly consisted of LPGs and the number of MSGs was low. The highest mean number of girdles (6.8/plant) was observed in Starkville 2008 field when 3 threecornered alfalfa hoppers/plant were used at R5 stage. At this time, between 32 and 37 percent of leaves on the main stem were girdled. Defoliation thresholds are set at 20% defoliation during bloom, pod formation and pod filling stages in Mississippi based on observed yield reductions from higher levels of defoliation (Catchot 2010). However, with threecornered alfalfa hopper feeding, the leaves which sustained girdling injury were normally retained

on the soybean plants for several weeks and the response due to the girdling is most likely different than for defoliation. Sparks and Newsom (1984) observed significant yield reductions when the number of LPGs/plant ranged between 6.2 to 15.7 and the corresponding maximum level of threecornered alfalfa hopper density ranged between 1.43 and 4.76 per sweep. The relationship between threecornered alfalfa hopper/plant and threecornered alfalfa hopper/sweep needs to be further refined to fully utilize the results of this experiment. Considering the girdles only on the main stem, the soybean plants in the high pest density treatment sustained more injuries than what would be expected from the current threshold of 1 threecornered alfalfa hopper/ sweep. However a yield response was not observed, probably because plants were only infested for 1 week. Thus it is apparent that a higher density of threecornered alfalfa hopper or an infestation over a longer period of time is required to cause economic losses in soybean.

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CHAPTER 3
DISTRIBUTION OF INJURY AND YIELD IMPACT FROM THREECORNERED
ALFALFA HOPPER ADULTS AND NYMPHS ON SOYBEAN

Abstract

A greenhouse study was conducted to compare the injury and damage caused by adults and nymphs of threecornered alfalfa hopper, *Spissistilus festinus* (Say). A pest density of 3 adults and or 3 nymphs of threecornered alfalfa hopper were infested on potted soybean plants at growth stages from R3 through R6 for one week. Adults caused more leaf petiole girdling than nymphs and they preferred to feed around the middle of the soybean plant. The nymphs preferred lower parts of the main stem as their feeding site. The adult behavior was similar to the observations made in soybean fields. Overall mean yield varied significantly with the adult and nymph treatment when compared with the yield from control plants. Significant yield reductions compared to the control were observed at growth stage R4 by adults and nymphs. No differences in yield were observed between adults and nymphs.

Introduction

Stem or petiole girdles are the most evident form of injury caused by threecornered alfalfa hopper, *Spissistilus festinus* (Say), in soybean, *Glycine max* (L.) Merrill. A girdle is formed when a threecornered alfalfa hopper adult or nymph creates a

series of punctures around a stem or petiole which later becomes necrotic and swollen. Girdles formed due to the feeding either by adults or nymphs have a similar appearance. Girdles are visible as a slightly thickened green ring on the stem or leaf petiole soon after it is formed. It takes a few days to a week for the girdle to develop into a dark necrotic ring. Studies comparing injuries and damage to soybean by adults and nymphs of threecornered alfalfa hopper are limited. The interest in this pest has increased recently due to changes in soybean cultivation systems and a report that this pest is injurious in crops other than soybean (Rahman et al. 2007). The profitability of soybean cultivation in Mississippi has increased significantly in recent years due to higher yields and better prices (USDA-NASS 2009). A study by Spurgeon and Mueller (1992) on nymphs investigated plant part associations of various instars and their interaction with different soybean structures such as main stems, branches and leaf petioles as the plant matures. However, the focus was the impact of soybean on nymphs and not the impact of the nymphs on soybean. Sparks and Boethel (1987a) compared late-season damage by adults and nymphs and reported that nymphs caused up to 46% of total yield reduction. They controlled the second generation of threecornered alfalfa hopper in the field by applying insecticides at various stages of nymph development and compared the yield from sprayed and unsprayed plots so that damage by adults and nymphs could be separated. Little attention was given to soybean phenology as they had to wait for a natural population of nymphs to reach the different stadia. Considering the significant role of nymphs in reducing yield, they suggested modifying the sampling method such that current treatment threshold would account for damage by nymphs as well. Other published research on this pest has addressed basic biology such as instar duration,

oviposition behavior, and life history studies in relation to soybean (Meisch and Randolph 1965, Mitchell and Newsom 1984a). Other experiments have investigated effective sampling methods for adults and nymphs (Sparks and Boethel 1987b, Spurgeon and Mueller 1991). The effects of main stem girdling on soybean yield were investigated extensively (Caviness and Miner 1962, Mueller and Dumas 1975, Mueller and Jones 1983, Tugwell et al. 1972). The differences in interaction of adults and nymphs to the soybean during reproductive stages are not well addressed.

The location of injury by adults and nymphs is known to change with soybean plant development. As the woodiness increases due to growth they tend to move towards less woody structures like lateral branches and leaf petioles (Bailey et al. 1970, Mitchell and Newsom 1984b, Rice and Drees 1985, Spurgeon and Mueller 1992). Because late-season infestations are more injurious than that of vegetative stages (Sparks and Newsom 1984, Sparks and Boethel 1987a, Sparks et al. 1987, Spurgeon and Mueller 1992), understanding the differences in adult and nymph behavior on soybean during reproductive stages of soybean growth is important. The objective of this study was to compare injuries caused by adults and nymphs of threecornered alfalfa hopper on soybean plants during reproductive stages from pod initiation (R3) through full seed (R6) and the impact of these injuries on soybean yield.

Materials and Methods

Group IV soybean cultivar AG 4605 was grown in 15 cm diameter pots containing commercial growing media (Promix) initially seeded at 5 seeds per pot and then thinned to 3 healthy plants per pot. The experiment was conducted in a greenhouse

located near the Clay Lyle Entomology building, Mississippi State University, Mississippi State, MS from 7 May to 17 October 2009. Adult threecornered alfalfa hopper used in this experiment had been collected from soybean fields and maintained on caged soybean plants until infestation. The nymphs were the F₁ generation of the field collected adults. Separate sets of soybean plants were maintained for the purpose of rearing nymphs of threecornered alfalfa hopper. These soybean plants, kept inside aluminum framed screen cages, were heavily infested with field collected threecornered alfalfa hoppers to ensure the availability of nymphs during the study, roughly following the method demonstrated by Meisch and Randolph (1965). Adults were added to these cages as collected and additional plants were added to the cage as needed to replace injured plants. Temperature inside the screen cage was not allowed to exceed 32° C which was an optimum temperature for nymph development (Moore and Mueller 1975) and screen cages were kept near the greenhouse cooling pad to ensure sufficient humidity.

Treatments included adults and nymphs with a density of 3 per plant and a control with no threecornered alfalfa hopper. The pots were arranged in completely randomized design. Each pot containing 3 plants was considered as a replication. For R3 stage, there were 5 replications of nymph and adult treatments and 3 replications of the control. From R4 to R6, there were 4 replications of nymph and adult treatments and 3 replications of the control. A nylon screen, with the top end made into a knot, was pulled over the plants in each pot at the time of infestation. After infestation, the bottom end was tucked beneath the pot. Infestation of adult hoppers was done by carefully releasing into the screen. The nymphs were placed on each plant using a camel hair brush. Third to fifth instars were used because they were reported to cause visible injury. Every two days,

survival was checked, and dead insects were replaced. When a nymph molted into an adult, it was replaced with another nymph. The period of infestation was one week and it was done on the following dates: R3 (beginning pod) from 27 July to 3 August, R4 (full pod) from 9 August to 16 August, R5 (beginning seed) from 22 August to 29 August, and R6 (full seed) from 21 September to 28 September. At the end of each infestation the adults and nymphs were removed and plants were allowed to grow without screens. Observations of injuries were recorded soon after the screens were removed. These included counts of stem girdles (SG), leaf petiole girdles (LPG), and the position of girdles with respect to node on the main stem. Because girdles were absent on control plants, comparison of injuries was done between adult and nymph treatments. The girdling injuries by the adults and nymphs on the soybean plant were mapped based on the main stem nodes. The mapping of injuries by the adult threecornered alfalfa hopper in the greenhouse was compared with a similar experiment (Chapter II) conducted in the field using 3 hoppers/plant. When plants reached maturity, pods from each pot were harvested and threshed and their weight was recorded. Statistical analysis was done using PROC GLM and PROC MIXED procedures of SAS 9.2 (SAS Institute 2009).

Results and Discussion

Analysis of Injury

Girdling injuries were absent on the control plants. For meaningful comparison of injuries, only adult or nymph infested plants were considered. The overall mean number of girdles was 5.36/plant in the entire study. Threecornered alfalfa hopper adults produced 5.64 ± 0.32 girdles per plant while nymphs produced 5.20 ± 0.32 girdles over the

period of one week. Overall analysis of injury by adults and nymphs of threecornered alfalfa hopper showed that the mean number of total girdles/plant did not vary significantly as a result of insect stage ($F= 0.90$; $df= 1, 26$; $P= 0.3504$) but varied with growth stage of the soybean plant ($F= 3.91$; $df= 3, 26$; 0.0197). No significant interactions were observed between insect stage and soybean growth stage ($F= 1.62$; $df= 3, 26$; $P= 0.2085$). While the mean number of total girdles did not significantly differ between adult and nymph treatments, a comparison of mean number of leaf petiole girdles (LPG) and stem girdles (SG) showed a significant difference between adults and nymphs (Table 3.1 and Fig. 3.1). Adults caused significantly more LPGs than nymphs during R4, R5 and R6. In contrast, nymphs caused significantly more SGs than adults during R3, R5 and R6 which confirmed their tendency to associate with stem structures. Considering the level of infestation of 3 adults or nymphs per plant, the number of girdles produced by individual insect was ≈ 2 / week. In this study, the maximum number of girdles was 6.99 ± 0.94 /plant (R4) for adults and 6.58 ± 0.38 /plant (R5) for nymphs (Table 3.1). Moore and Mueller (1975) reported that the time required to cause one complete encircling around stem or leaf petiole can be as short as 24 hours for a fourth instar threecornered alfalfa hopper. However, I could not find any reports on the number of girdles which could be produced by either adults or nymphs under a given time frame. Occasionally more than one nymph was observed feeding around the vicinity of the same girdle, so each insect may not cause individual girdles. Both adults and nymphs can randomly puncture the plant to feed rather than creating a more visible girdle. Therefore, girdles, while the only visual sign of threecornered alfalfa hopper feeding, may not be complete indicators of plant stress caused by feeding.

Table 3.1 Least square mean estimate \pm SEM of girdles caused by adults and nymphs of threecornered alfalfa hopper on soybean plants from R3 through R6 growth stages

Maturity	Girdle	Adult	Nymph	F	df	P
R3	LPG	3.46 \pm 0.56	2.20 \pm 0.68	2.47	1, 8	0.1545
	SG	0.93 \pm 0.27	2.13 \pm 0.27	9.29	1, 8	0.0159
	Total	4.39 \pm 0.65	4.33 \pm 0.65	0.00	1, 8	0.9483
R4	LPG	5.41 \pm 0.84	1.99 \pm 0.84	8.15	1, 6	0.0290
	SG	1.58 \pm 0.66	2.83 \pm 0.66	1.76	1, 6	0.2332
	Total	6.99 \pm 0.94	4.82 \pm 0.94	2.61	1, 6	0.1572
R5	LPG	4.25 \pm 0.20	2.66 \pm 0.20	30.94	1, 6	0.0014
	SG	1.83 \pm 0.35	3.92 \pm 0.35	16.94	1, 6	0.0062
	Total	6.08 \pm 0.38	6.58 \pm 0.38	0.86	1, 6	0.3886
R6	LPG	3.83 \pm 0.49	1.88 \pm 0.49	8.26	1, 6	0.0283
	SG	1.25 \pm 0.26	3.24 \pm 0.26	28.01	1, 6	0.0018
	Total	5.08 \pm 0.35	5.08 \pm 0.35	0.00	1, 6	0.9962

The distributions of girdles on soybean show a clear distinction between feeding preferences of threecornered alfalfa hopper adults and nymphs (Fig. 3.2). Nymphs preferred the lower parts of the main stem. In contrast, adults caused most of the injury on upper parts of the main stem. It has previously been reported that nymphs tend to be associated with the woody structures such as main stems or stems of lateral branches (Spurgeon and Mueller 1992). Moore and Mueller (1975) reported that nymphs were more injurious than adults. He observed that only third to fifth instars caused girdles, while initial instars caused random punctures. The late nymphal instars fed on plant sap by making girdles primarily on the main stem and fourth instars were frequently observed on the lower part of the plant. In this study I used third to fifth instars and our observation is in agreement with previous observations on vertical movement of nymphs. The gradual decline in the number of girdles by nymphs from the base towards the center of the plant and their concentration at the basal nodes confirms the behavior of nymphs (Fig. 3.2).

The distribution of girdles on a soybean plant caused by adult hoppers in the greenhouse was comparable to what was observed in a caged field study with the same level of infestation (3 hoppers/plant) (Fig. 3.3). In both studies, most of the girdles were concentrated above the upper half of the soybean plant and their pattern of distribution was similar.

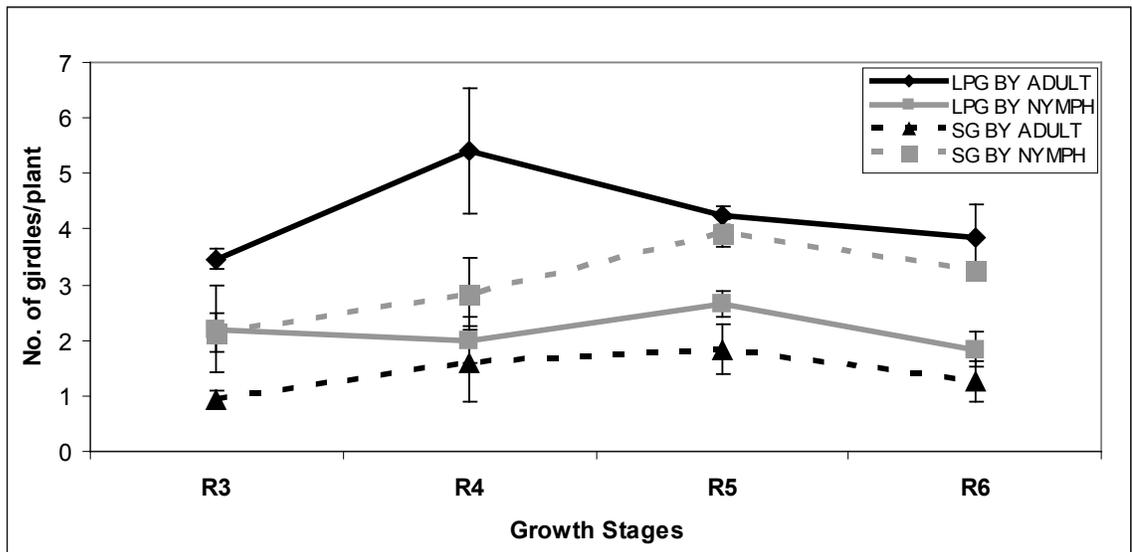


Figure 3.1 Mean number of leaf petiole girdles (LPG) and stem girdles (SG) \pm SEM caused by adults and nymphs of threecornered alfalfa hopper on soybean plants at growth stages R3 through R6

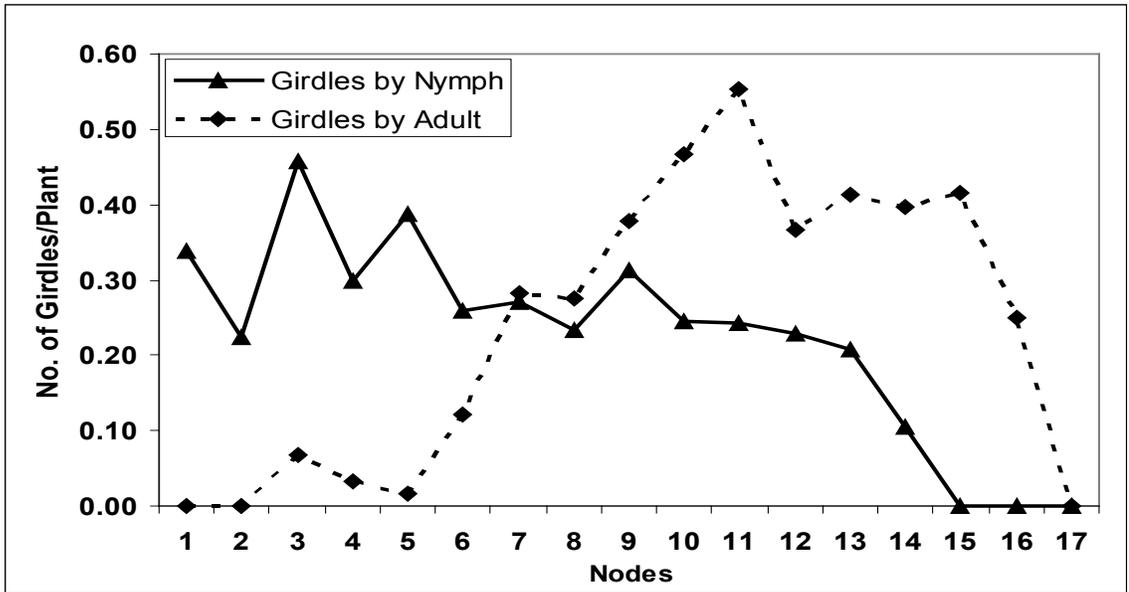


Figure 3.2 Distributions of girdles by plant node caused by adults and nymphs of threecornered alfalfa hopper on soybean plant. The data are averaged among infestations during growth stages R3 through R6.

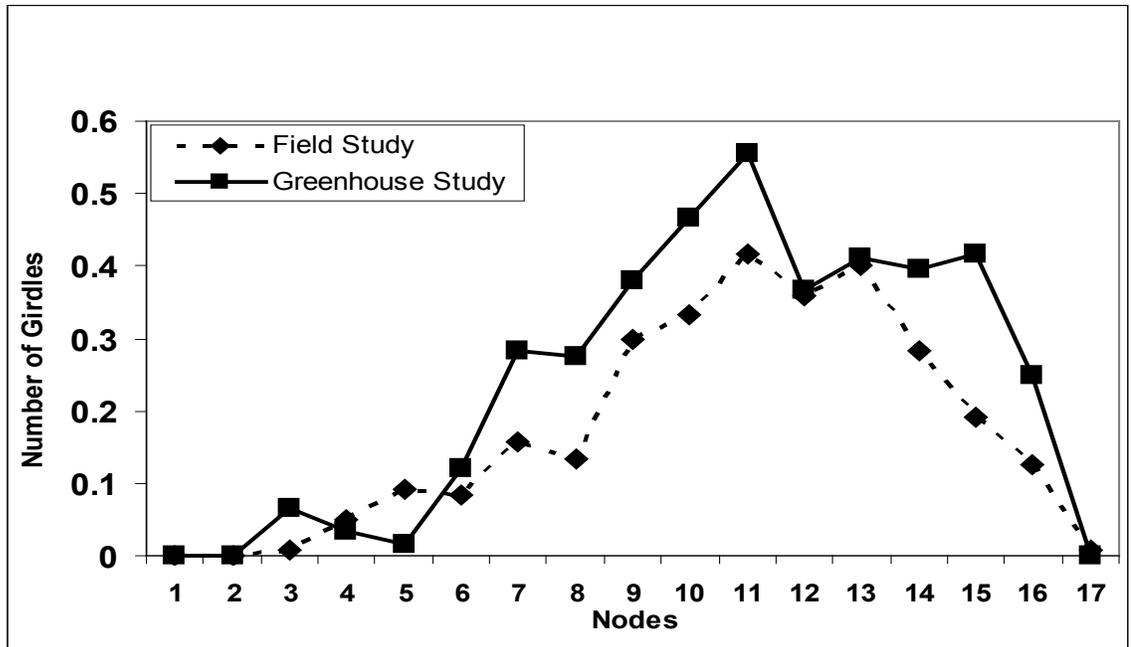


Figure 3.3 Comparison of girdling injuries by plant node caused by adult threecornered alfalfa hopper in the greenhouse and in the field when soybeans were infested with 3 threecornered alfalfa hoppers/plant. The data are averaged among infestations during growth stages R3 through R6.

Analysis of Yield

Overall, the maturity of the soybean when infested and the insect stage were significant factors of yield (Soybean maturity: $F= 3.42$; $df= 3, 34$; $P= 0.0280$, Insect stage: $F= 5.33$; $df= 2, 34$; $P= 0.009$). Out of four maturity stages, insect stage was a significant factor of yield only during R4 ($F= 4.76$; $df= 2, 8$; $P=0.0435$). However, least square mean comparison revealed that yield did not vary between adults and nymphs (Adult : 24.73 ± 1.51 , Nymph: 25.07 ± 1.51 , Control: 31.20 ± 1.74). Other stages did not show any significant reduction in yield due the treatments (R3: $F=1.02$; $df= 2, 10$; $P= 0.394$., R5: $F=1.64$, $df= 2, 8$; $P= 0.253$., R6: $F= 2.26$; $df= 2, 8$; $P= 0.166$) (Fig. 3.4). These yield results indicate the potential ability of both adults and nymphs to affect yield.

However, to make any conclusive remark on effect of adults and nymphs on yield, data should be obtained from field studies with larger plots.

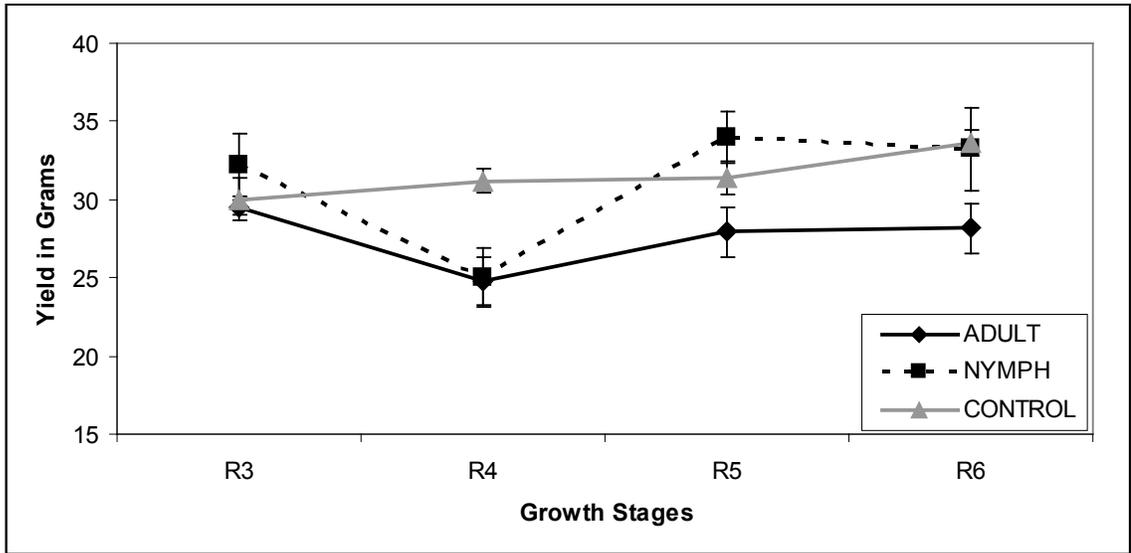


Figure 3.4 Mean yield (grams) \pm SEM from infestations of three adults and nymphs of threecornered alfalfa hopper and a control at R3 through R6 growth stages

Of the total girdles caused by adults in the greenhouse throughout all the maturity stages, only 24.8% were main stem girdles, while the rest were leaf petiole girdles. When a girdle is produced on a leaf petiole, the leaf doesn't succumb to the injury immediately. The leaf follows a more or less similar course of a healthy leaf and is retained on the plant for several weeks. Anatomical studies (Mitchell and Newsom 1984b) and radiotracer studies using ^{14}C labeled glucose (Hicks et al. 1984) on girdles have shown that petiole girdling disrupts the vascular bundles and blocks movement of assimilates in the phloem. However, it is likely that such a disruption is partial and the leaf continues to pass some photosynthates to the plant and may have an effect of partial

defoliation. Soybean plants can withstand up to 20% defoliation during bloom, pod formation and pod filling stages (Catchot 2010). Given these facts coupled with the current study, certain previous observations merit further discussion. One such observation is that yield reduction due to adult hoppers was observed when the population densities of adults reached only 60% of the current threshold of one threecornered alfalfa hopper/plant (Sparks and Boethel 1987a). Such a scenario is possible when nymphs played a major role and adult sampling did not account for the damage by nymphs. Significant yield reductions were not observed in the field study described in the chapter II, using threecornered alfalfa hopper adults. Any efforts to modify the existing threshold based on such reports would be more meaningful when the role of nymphs is better understood. According to Moore and Mueller (1975), nymphs in general are more injurious than adults, primarily due to the fact that they form girdles on the main stem which leads to lodging of the plant before harvest. However, the current experiment examined the role of nymphs in reducing yield through physiological stress. All the plants in this study which had main stem girdles at their base survived until harvest. Earlier studies suggest that both adults and nymphs can feed on pedicels and pods. Pod shedding as a result of girdling on pedicels has been suggested as one of the possible causes for yield reduction. However, pod shedding due to girdling of pedicel was not observed in the greenhouse. A better understanding on the amount of injury either in the form of stem girdles or leaf girdles required to create physiological stress on soybean plants that result in yield loss would be beneficial.

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CHAPTER 4

CONCLUSIONS

Threecornered alfalfa hopper has been considered as a pest of soybean for the last 40 years. A casual observer may not readily identify the problems caused by this pest due to its small size and the inconspicuous nature of injuries or girdles caused. However, considerable awareness was made by several researchers during the 1970's and 1980's and a treatment threshold was developed after considering their potential ability to cause yield reduction when present in high densities. The current research was initiated primarily due to the changes in soybean cultivation systems in the Mid-South in general and Mississippi in particular. The early soybean production system is preferred in Mississippi over the conventional, late planting system due to factors such as high yield and low pressure from late season defoliators. Recent reports suggested that this new system might have potentially altered the seasonal occurrence and abundance of threecornered alfalfa hopper. Published studies on the effect of this pest under the new system of soybean cultivation were lacking.

Building on previous research, the current experiment focused on determining the critical reproductive stages of soybean for threecornered alfalfa hopper damage. The effects of different pest densities of threecornered alfalfa hopper adults were evaluated in the field and a comparison of the nymphs and adults was evaluated in the greenhouse

during growth stages from R3 to R7. I studied the behavior of the adults and nymphs based on the number of girdles and their position in relation to plant nodes. Threecornered alfalfa hopper adults behaved similarly in the field and in the greenhouse. They made girdles on leaf petioles in the upper half of the plants. The concentration of leaf girdles were less on the youngest leaves at the apex. The nymphs behaved differently compared to the adults in terms of the sites of injury. They preferred the basal nodes of the main stem. Significant yield responses were not observed when soybean was treated with different levels of adults in the field. Seed weight was found to vary with the threecornered alfalfa hopper adults at R6, but the observation was not consistent in other stages. Deterioration in seed quality was observed at one growth stage at one location. The adults and nymphs of threecornered alfalfa hopper caused significant yield reduction at R4 growth stage in the greenhouse study. Because earlier reports suggested yield reduction at the field level, a longer period of infestation might be needed to observe a yield response. The role of nymphs at the field level was not addressed, but based on our greenhouse research, studies to better understand the importance of nymphal damage to soybean are highly encouraged. In general, the growth stage of the soybean did not have observable impacts on yield or quality as understood from these trials.

During the field collection of threecornered alfalfa hoppers, I observed that their number occasionally exceeded the current threshold of one threecornered alfalfa hopper/sweep in farmers' fields of early planted soybean. They were abundantly observed in the field during late reproductive stages such as R5 and R6. Because soybeans were planted in April, the number of threecornered alfalfa hoppers were low during early vegetative stages. In conventional soybean, lodging due to the main stem

girdling is more common because the population develops on alternate hosts and infests soybean when they emerge. It may be possible that the early soybean production system avoids early season main stem girdling due to the lack of sufficient pest pressure.