# ESTABLISHMENT OF THREECORNERED ALFALFA HOPPER (*SPISSISTILUS FESTINUS*) AS A PEST OF PEANUT (*ARACHIS HYPOGAEA*)

by

BRENDAN ALLEN BEYER

(Under the Direction of Mark R. Abney)

## ABSTRACT

Field and greenhouse experiments were conducted to determine the economic injury level of the threecornered alfalfa hopper, *Spissistilus festinus*, (Say), using early and late season infestations, and increasing levels of nymphal infestation rates in cages to isolate the plants from outside *S. festinus* populations. Significant yield and plant biomass losses in both field and greenhouse settings were recorded only when using damage as a metric. Greenhouse observations noted a significant preference of feeding based on instar and plant maturity. A second experiment was implemented to establish the foundation for a comprehensive sampling program of *S. festinus* in peanut using the sweep net and beat sheet as relative samples of adults and nymphs, respectively, to compare to absolute samples of nymphs and the progression of damage. Sampling indicates the possibility of using sweep nets and beat sheets to estimate the true population and damage based on the time of season.

INDEX WORDS: *Spissistilus festinus*, *Arachis hypogaea*, threecornered alfalfa hopper, peanut, economic injury level, sampling

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# DEDICATION

To my mother, Andrea R. Beyer and step-father, James L. Williamson; siblings: Colleen, Kerrie, and Dylan; grandparents: Nora and Walter Dunderville; my friends, specifically Walker Evans; and finally Julie Jones, without whom I would have been lost.

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# CHAPTER 1

# INTRODUCTION AND LITERATURE REVIEW

## Introduction

The threecornered alfalfa hopper, *Spissistilus festinus* (Say) (Hemiptera: Membracidae), was first described in 1831 as *Membracis festina*. In 1869, the species was moved to a new genus, *Stictocephala*, and later placed in the genus *Spissistilus* (Caldwell, 1949; Meisch, 1964). Since its discovery, it has been identified as a serious economic pest of alfalfa (Osborn, 1911; Wildermuth, 1915) and soybean (Sparks & Boethel 1987; Tugwell et al. 1972) and has been observed on many other plants. In the past, *S. festinus* and the damage it produces have been recorded on peanut, though little information is available on the economic impact of the insect on this crop. Increasing numbers of *S. festinus* in peanut in the last decade led to a rise in concern among peanut growers. The intention of this project was to ascertain the pest status of *S. festinus* on peanut. The first objective of this study, described in Chapter 2, was to estimate the economic injury level of *S. festinus* by measuring dry weight vegetative and reproductive biomass as a response to damage. Peanut plants were caged in the field and greenhouse with differing rates of nymphs per plant to evaluate the impact of feeding. The second objective, described in Chapter 3, was to learn more about the population dynamics of *S. festinus* as they relate to peanut, and to determine the most efficient sampling method. Chapter 4 provides summaries of the results from each study, and provides suggestions for further research.

# Literature Review

## Distribution

Wildermuth (1915) reported sightings of *Spissistilus festinus* ranging from Ottawa, Canada to Mexico; this distribution was refined by Caldwell (1949). His description of the different species of *Spissistilus* using the genitalia revealed that the original reported distribution was a result of misidentification of *Spissistilus* species. The upper limits of the distribution are closer to the Midwest United States as opposed to Canada. In the United States, *S. festinus* is prevalent in the southeast, where the abundance of preferred host plants such as soybean and peanut provides suitable habitat for breeding and development (Moellenbeck et al. 1993; Deitz & Wallace, 2012).

## Host Range

*Spissistilus festinus'* host range consists of many plant species in a number of families. It was first identified in personal communications between Oemler and the University of Georgia as a potential pest of tomato (1888). Cockerell (1889) first reported *S. festinus* as a pest on alfalfa. The reported host range of *S. festinus* includes alfalfa, cowpeas, clover, various trees, shrubs, grasses, herbs, sugarcane, potato, cotton, and field peas (Wildermuth, 1915; Van Zwaluvenburg, 1926; Swezey, 1937). Plant species of Fabaceae have been shown to be better reproductive and developmental hosts, and the insect is considered an economic pest of alfalfa, soybean (Caviness & Miner 1962; Johnson et al. 1988; Mitchell & Newsom, 1984a; Sparks & Boethel, 1987; Sparks & Newsom, 1984; Tugwell et al. 1972) and peanut (King et al. 1961; Todd et al. 1979; Andersen et al. 2002; Rahman et al. 2007). In Louisiana, overwintering adults have been observed on pine; in spring, adults were found on vetch and clover, before moving into newly emerged soybeans (Newsom et al. 1983).

## Description and Behavior of *S. festinus*

The threecornered alfalfa hopper adult is light green and about 6 to 7 mm long, with an elongated pronotum that extends to the tip of the abdomen (Wildermuth, 1915). *Spissistilus festinus* receives its common name from its pronotum; when observed from the front, it possesses three corners, one at each "shoulder" and one at the apex of the pronotum. Adult males are readily distinguishable from females by a red tint on the dorsal surface of the male's pronotum, marginally smaller body size, and lack of an ovipositor (Wildermuth, 1915).

Eggs range in size from 0.9-1.3 mm long. They are white in color and oblong in shape. The larger end of the egg is covered in papillae, which are thought to secure the egg within the plant tissue (Wildermuth, 1915). Eggs are inserted under the epidermis in a slit created by the ovipositor. Oviposition behavior has been shown to vary depending on the host species and the maturity of that species. For example, in soybean, oviposition occurs near the base of the main stem early in the season (Wildermuth, 1915), and occurs in softer tissues such as terminals and nodes as the season progresses (Mitchell & Newsom, 1984a; Rice & Dress, 1985). The number of eggs laid in each slit varies between different plant species. In soybeans, approximately 6 eggs are laid per slit, but in alfalfa 1-2 eggs were found per slit (Wildermuth, 1915; Jordan, 1952). These slits are also known to be harmful to the plant, as the plant tissues are damaged by heavy oviposition (Meisch & Randolph, 1965).

*Spissistilus festinus* undergoes hemimetabolous development. It progresses through 4-6 nymphal instars before emerging as an adult. The number of nymphal instars varies depending on nutrition and weather conditions; 5 total instars is reported the most common (Wildermuth, 1915; Moore & Mueller, 1976; Deitz & Wallace, 2012). The first and second instars are 1.6 mm and 2.1 mm in length, respectively; they are pale green or straw colored, with a series of dorsal spine-like protrusions. At each successive nymphal stage, the spines grow and develop divergent, lateral spurs that occur along the length of each spine. Wing pads and pronounced development of the pronotum appear in the third nymphal stage; third instar nymphs are a darker yellow-brown color with green markings and are approximately 2.9 mm in length. Fourth and fifth instars are similar in appearance, and grow progressively greener with pronounced wing pads, dorsal spikes, and pronotum.

Third through fifth instars are much more excitable and mobile than the first and second instars (Wildermuth, 1915). When disturbed, nymphs will sometimes produce a globule of liquid from the abdomen as a defense mechanism and quickly move to the opposite side of the stem. Adults will also attempt to conceal themselves in a similar manner, though are more likely to fly away when disturbed (Wildermuth, 1915). Adults generally fly within 33 centimeters above the soil or just above the plant canopy (Johnson & Mueller, 1989; Johnson & Mueller, 1990).

## Life History

*Spissistilus festinus* can have multiple generations per year, depending on weather conditions and availability of host plants (Wildermuth, 1915; Mitchell & Newsom, 1984b). Both adults and eggs will enter diapause during winter, though diapause initiation is variable in different climates. In mild winters, the adults have been reported to forego diapause and continuing reproduction (Wildermuth, 1915). A nascent adult female reaches sexual maturity in 7-14 days, after which she will mate and lay eggs soon after (Jordan, 1952; Meisch, 1964; Meisch & Randolph, 1965). Males reportedly die soon after copulation, but females live for an average of 38.6 days post-copulation (Mitchell & Newsom, 1984b). Wildermuth (1915) reported that populations generally consist of more males than females. Mitchell and Newsom (1984b) and Newsom et al. (1983) documented that sex ratios vary throughout the season. An egg-laying female can be found with an average of 21 to 30 eggs in her ovaries at any one time, and can produce up to 220 offspring over her lifetime (Mitchell & Newsom, 1984b).

The embryo's development lasts from 6 to 27 days, with an average of 16.5 days from oviposition to eclosion (Meisch & Randolph, 1965). The first three instars are completed in 3 to 5 days each, depending on temperature, humidity, and nutrition. The fourth and fifth instars last 4 to 8 days each (Wildermuth, 1915; Jordan, 1952; Meisch & Randolph, 1965; Spurgeon & Mack, 1990). Total nymphal development time has been shown to vary with temperature. Wildermuth observed that nymphal development required 69 and 32 days when mean temperatures were 16°C and 30°C, respectively. Other reports estimate development time to be 18 to 24 days at temperatures of 32°C and 26.6°C and 75-80% RH (Jordan, 1952; Meisch, 1964; Meisch & Randolph, 1965; Spurgeon & Mack, 1990).

## Direct and Indirect Injury

*Spissistilus festinus* is a phloem feeder with piercing-sucking mouthparts and two distinct feeding behaviors. The first behavior involves sporadic probing and consumption of phloem sap and amino acids (Andersen, 2002). The other behavior involves the formation of a continuous series of lateral punctures around the circumference of a stem (Wildermuth, 1915). Commonly referred to as a girdle, the aforementioned ring of punctures often results in a gall-like growth in the area surrounding the feeding site (Wildermuth, 1915). Smith (1933) found that after the insect feeds insoluble salivary sheaths are left in the plant tissue. Mitchell and Newsom (1984a) discovered that it is these sheaths that disorganize and disrupt the vascular bundles of the phloem, as well as introduce cellular hyperplasia (Johnson et al. 1988). It has been shown in multiple studies across different plant species that the third through fifth instars (as well as adults) are capable of creating this girdle (Meisch & Randolph, 1965; Moore & Mueller, 1976; Mitchell & Newsom, 1984a; Andersen et al. 2002).

Girdling interrupts the flow of nutrients in the phloem, and causes an accumulation of photosynthates in the area above the girdle (Osborn, 1911; Wildermuth, 1915; Mitchell & Newsom, 1984a; Andersen et al. 2002). Andersen (2002) reported that many of the amino acids that increase in concentration above the stem girdle are likely the result of plant responses to feeding and are not essential to insect development. Nevertheless, concentrations of amino acids required for *S. festinus* development are also elevated by the girdling process (Andersen, 2002). On peanut and other host plants infested, nymphs gather within 5mm above the girdle and feed for up to 7 days (Moellenbeck & Quisenberry, 1991; Andersen et al. 2002). New girdles are formed above existing girdles, and nymphs will relocate to continue feeding (Moellenbeck & Quisenberry, 1991; Andersen et al. 2002). In alfalfa and soybean, heavy girdling reduces forage quality due to lowered levels of carbohydrates and amino acids, as well as increased levels of detergent fibers (Wilson & Quisenberry, 1987; Moellenbeck et al. 1991).

Nutrient loss is not the only consequence of girdling. Girdles on the main stem can impact the structural stability of the plant, leaving the girdled stem susceptible to lodging from wind and/or mechanical disturbance (Sparks & Boethel, 1987). In crops such as peanut, which has more prostrate growth and multiple branches, stand loss due to lodging is not a serious concern. Petiole feeding and subsequent leaf drop in soybean also reduced yield, especially in the late season when *S. festinus* numbers were high (Sparks & Newsom, 1984).

*Spissistilus festinus* damage has been linked to an increase in the likelihood of disease complexes in soybean. Herzog et al. (1975) observed increased incidence of blight caused by *Sclerotium rolfsii* (Sacc.) infection in girdled versus non-girdled stems. While *S. festinus* did not actively transmit the disease, the presence of girdles close to the soil where *S. rolfsii* occurred significantly increases frequency of infection. *Sclerotium rolfsii* is one of the most damaging peanut diseases. It can cause up to 12% yield loss in Georgia, where losses and accompanying treatment costs associated with *S. rolfsii* are estimated to be $41 million (Kemerait, 2010-13). No work has been published on the interaction of *S. festinu*s infestation and *S. rolfsii* incidence in peanut, but it should be noted that *S. rolfsii* incidence in soybean increases with mechanical damage. Therefore, *S. festinus* infestation on peanut could increase occurrence of *S. rolfsii* (Herzog et al. 1975; Russin et al. 1986). A study of soybean with symptoms of stem canker, *Diaporthe phaseolorum* (Cke. & Ell.), found that girdle presence resulted in larger cankers and reduced yields (Russin et al. 1986). Russin et al. (1987) found that girdle presence on soybean did not increase infection rate of pod and stem blight, *Phompsis sojae* and *Colletotrichum truncatum* (Schw.), but did increase symptom severity of both diseases and reduced yields.

### As a Pest on Alfalfa

The threecornered alfalfa hopper was first described as a pest of alfalfa, *Medicago sativa* (L.) in 1899 (Cockerell, 1899). Alfalfa is a perennial forage/hay crop that can be harvested multiple times a year, but *S. festinus* feeding girdles can cause significant loss in quality of the new growth (Wilson & Quisenberry, 1987). Heavy infestations and high stem girdle counts can require reseeding of entire fields due to the persistent nature of the girdles (Graham, 1938).

*Spissistilus festinus* has multiple and overlapping generations annually on alfalfa, with two population peaks occurring late June to early July and late August through September in

Louisiana (Farlow et al. 1981; Moellenbeck et al. 1993). The latter peak is often much greater than the former, and as a result, the amount of damage to the new growth can be considerable (Wilson & Quisenberry, 1987). Nymphs in a greenhouse study did not have any measurable effect on “Florida 77” alfalfa at 1 and 3 nymphs per plant (Wilson & Quisenberry, 1987). However, when the rates increased to 6 nymphs per plant, protein content decreased, fiber density increased, and root carbohydrate levels decreased (Moellenbeck & Quisenberry, 1991). While overall dry weight was not affected, the quality of harvested hay was reduced. It is possible that the regrowth capability of the plants was affected due to reduced root carbohydrate concentrations (Moellenbeck & Quisenberry, 1991).

Currently, *Spissistilus festinus* is not considered a major pest of alfalfa. The insect is either not mentioned (Undersander et al. 2011;Whitworth et al. 2015) or is described as a minor, occasional pest (Summers et al. 2007) in alfalfa pest management handbooks. Recent pest management handbooks for Tennessee, Georgia, and Alabama suggest that insecticides may be needed if adults/nymphs are present on 10% of seedlings/young plants (up to 10-12 inches tall) or if 10% of lateral stems are being killed from damage (Flanders and Everest, 2014; Horton, 2015; SICR, 2015). For older plants, an economic threshold of 2 adults or nymphs per sweep with a 0.38m sweep net has been published (SICR, 2015). In Tennessee however, a study showed that a 25% reduction in stand count did not cause any economic impact (Bates et al. 2005). Growers can increase seeding rates to mitigate stand loss, and remove weedy borders around fields to eliminate overwintering sites (Bates et al. 2005).

### As a Pest on Soybean

*Spissistilus festinus* has long been considered a pest of soybean. It was first detected on soybean in the early 1900’s, but significant damage was first reported in 1957 (Caviness &

Miner, 1962). It was initially thought that the yield loss resulted from early season girdling of the main stem (V1- V5 growth stages). The damage to the main stem caused large swathes of soybean plants to lodge. However, Caviness and Miner (1962) found evidence that lodging had minimal effects on yield; artificially reduced stands had the highest loss in yield when stand loss occurred after bloom and minimal yield loss when damage occurred two weeks prior to bloom. It should be noted that lodging simulations in this study were homogenous throughout the plots, allowing maximum compensation from adjacent plants. Bailey (1975) found significant reductions in yield when *S. festinus* adults were caged on soybeans; however, seeding rate was not reported. Likewise, the distribution of lodged plants was not described.

The number of generations of *S. festinus* in soybean varies. Mueller (1980) found three overlapping generations, whereas Mitchell and Newsom (1984b) found only two. When observing effects of the different generations, Sparks and Newsom (1984) found that early season damage (when there is the highest danger of lodging from girdles on the main stem) did not significantly impact yield. Mueller and Jones (1983) showed that only when 70% or more of plants had a main stem girdle was any yield response recorded. The lack of yield response at lower feeding levels was attributed to compensation and high seeding rates (12 seed per 0.33m row). No difference in yield between individual girdled and non-girdled plants was observed, indicating that lodging is a cause of yield reduction when girdling rates are high. In at least one study, late season damage, during the second in-field generation of *S. festinus*, resulted in significant reductions in yield (Sparks & Boethel, 1987). This result was most likely related to *S. festinus* feeding on the succulent petioles, peduncles, and pedicels after the soybean's R1 stage (Mitchell & Newsom, 1984a). Though nutrient flow on a girdled petiole resumes after 10 days

(Spurgeon & Mueller, 1993), the damage may be enough to decrease production of new seedpods, thereby reducing yield (Sparks & Newsom, 1984).

The relatively recent shift from traditional timings of soybean planting to early season planting dates resulted in greater yield, while simultaneously reducing late season pest damage (Heatherly, 1999). Earlier planting dates may have affected many of the previously established economic thresholds for pests of soybean, including *S. festinus*. There are no recently published or validated thresholds for *S. festinus* in early-planted soybean. With traditional soybean planting practices, Tugwell et al. (1972) found significant differences in injury levels between insecticide treated plots and the non-treated check, but no effect on yield. However, Bailey (1975) found significant reduction in yield at a rate of one hopper per plant when plants were 1.5-2.0 inches tall. Mueller and Dumas (1987) revealed that the timing and quantity of main stem girdling had a significant impact on individual plant yield. Nevertheless, Caviness and Miner (1962) showed that soybeans have incredible compensatory power.

The first economic threshold was established by Sparks and Newsom (1984), who determined that one adult per sweep at soybean pod set until leaf yellowing is enough to cause economic damage and should be treated. This threshold was based on the hypothesis that late season damage consisting of mostly petiole girdling is the cause of significant yield loss. Sparks and Newsom (1984) also hypothesized that when the threshold for adults is met, the nymphs have already done a substantial amount of damage. Sparks and Boethel (1987) found that yield reductions occurred at 60% of the aforementioned threshold, and also introduced the hypothesis that it is more pod feeding and less petiole girdling that causes yield loss. With the introduction of early season soybean production, *S. festinus* populations are higher in the early season (Bauer et al. 2000; McPherson et al. 2001). Pulakkatu-thodi (2010) and Ramsey (2015) were unable to observe any yield effects in reproductive soybeans as a result of adult numbers at 3 times the established threshold of 1 adult per sweep. It should be noted that only adults were utilized in both of these studies, and as such, the full impact of *S. festinus* in contemporary soybean production models is as of yet undetermined.

## As a Pest on Peanut

*Spissistilus festinus* has been observed feeding on peanut for the past half-century, though there have been no intensive investigations of its economic impact (King et al. 1961; Todd et al. 1979). In Georgia and surrounding states, growers now see *S. festinus* in great abundance in peanut fields, and there are concerns about possible yield loss associated with feeding. Though there have been estimates of the economic impact of *S. festinus* in peanut ranging from $2 million in yield loss (Todd et al. 2000) to no impact other than treatment costs (Brown & Adams, 2005), no science-based economic injury level has been determined. *Spissistilus festinus* has been shown to cause significant damage- characterized by number of girdles- in a few peanut cultivars, including runner-type GA Green and Virginia-types AT VC2, GA-HI-O/L, Virugard, Wilson, and Phillips (Rahman et al. 2007). In 2014, 83% of Georgia growers planting Foundation, Registered, or Certified seed chose the runner-type cultivar Georgia 06G; no data related to susceptibility of Georgia 06G to *S. festinus* exist (Monfort, 2014).

Two nymphal *S. festinus* population peaks are observed in peanut annually. The first generation of nymphs appears in late June to early August after an initial appearance of adults, and the second generation develops during late August into early September (Rahman et al. 2007). The majority of girdling occurs three weeks after the initial increase in number of nymphs (Rahman et al., 2007); this coincides with the approximate development time from eclosion to fourth instar (Meisch, 1964; Meisch & Randolph, 1965; Spurgeon & Mack, 1990).

The fourth instar is thought to be the most responsible for girdles (Moore & Mueller, 1976; Mueller & Johnson, 1988).

While *Spissitilus festinus* is capable of girdling peanut, no published reports correlate peanut damage with yield loss. Andersen et al. (2002) reported reduced biomass, nitrogen content, and carbon content in girdled peanut stems in northern Florida, but this effect was not consistent over years of the study. Rahman et al. (2007) found no measurable effect on yield from girdling alone with damage rates up to 6 girdles per plant. It is important to know at what point *S. festinus* damage impacts peanut yield. Growers may be treating for *S. festinus* unnecessarily, while unleashing the litany of problems that ensues from a broad-spectrum insecticide application, like flaring secondary pests, non-target effects on natural enemies and pollinators, and the monetary costs associated with the application itself (Ware, 1980; Adams, 2005).

The economic injury level for *S. festinus* in peanut is unknown, and there are no valid science-based economic thresholds. However, at least one set of anecdotal thresholds has been reported, where 1 adult per 6 feet of row at 75 days prior to digging or 1 adult or nymph per 3 feet of row 25-75 days prior to digging warrants treatment (Brown & Adams, 2006). Preliminary studies conducted recently at the University of Georgia suggest that these thresholds overestimate the economic impact of *S. festinus* and trigger insecticide applications at population levels that are not yield limiting.

## Sampling *S. festinus* in Crops

To prevent early season damage resulting from main stem girdles in soybean, the critical sampling period is when the plants are around the V3 stage (Spurgeon & Mueller, 1992). To avert late season damage in soybean, sampling for *S. festinus* populations should occur before V12 growth stage, when flowers are just setting (Fehr et al. 1971; Spurgeon & Mueller, 1992).

In peanut, Rahman et al. (2007) recommends monitoring for nymph and girdle presence beginning in the first two to three weeks of July. Damage increased in the third week of July, after consistent weekly increases in nymph numbers, indicating that the first few weeks of July are the most optimal for scouting nymphs (Rahman et al. 2007). An increase in adult numbers coincided with a sudden rise in girdle counts, but the increase in insect abundance was observed just a week before damage levels rose. Using adults as a direct, numerical indicator of injury may result in an appropriate diagnosis, but only after the damage has been done (Rahman et al. 2007). Because the correlation between the number of adults and nymphs in the field is unknown, using adults as a predictor of damage could over or under estimate the impact of *S. festinus*.

In soybean (Sparks & Boethel, 1987) and peanut (Rahman et al. 2007), *S. festinus* females are randomly distributed throughout the field, therefore samples of plant injury can be taken from 10 meters into the field to adequately estimate whole field injury levels. The beat sheet method is not ideal for sampling the younger, smaller nymphs in soybean, as their small mass reduces the chance of dislodging (Spurgeon & Mueller, 1991). Whole plant observations provide a more accurate assessment of nymph populations (Spurgeon & Mueller, 1991a), but it is impractical for IPM because of the time required for each sample. Adult sampling can be achieved with a sweep net in soybean (Kogan & Herzog, 1980) and peanut (Rahman et al. 2007). Adults can also be monitored in soybean with yellow sticky traps (Johnson & Mueller, 1988) placed at 33 cm above ground or at canopy level (Johnson & Mueller, 1989); however, on soybean, trap counts did not always correlate with numbers from sweep samples (Johnson &

Mueller, 1988, Johnson & Mueller, 1990). There is no data on the effectiveness of yellow sticky traps in peanut.

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# CHAPTER 2

# ESTABLISHMENT OF ECONOMIC INJURY LEVEL FOR *SPISSISTILUS FESTINUS* (SAY) (HEMIPTERA: MEMBRACIDAE) IN PEANUT (*ARACHIS HYPOGAEA* (L.))

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## Abstract

Threecornered alfalfa hopper, *Spissistilus festinus* (Say) (Hemiptera: Membracidae), has long been an economic pest of soybean and alfalfa and has recently become common in peanut. *Spissistilus festinus* feeds by forming girdles, caused by a series of lateral punctures around the stem. These girdles can result in galls that form a pool of photosynthates where *S. festinus* aggregate to feed. *Spissistilus festinus* is currently being treated as a pest in peanut, yet there are no economic thresholds for this insect in this crop. The purpose of this study was to generate data needed to establish an economic injury level of *S. festinus* nymphs in peanut. *Spissistilus festinus* was caged on peanut and the relationship between feeding damage and plant biomass was examined. Insect density did not affect yield, however there was a significant negative relationship between damage and vegetative and reproductive biomass of peanut. Plant and insect phenology affected feeding location in greenhouse studies. Early instars fed on leaf petioles and moved to stems as they matured. On plants older than 120 days, there was more feeding on the stems regardless of developmental stage.

**Keywords:** *Spissistilus festinus,* threecornered alfalfa hopper, *Arachis hypogaea*, peanut, economic injury level, integrated pest management

## Introduction

Threecornered alfalfa hopper, *Spissistilus festinus,* (Hemiptera: Membracidae), has been observed in peanut (King, 1961; Todd et al. 1979), though it was seldom regarded as an economic pest. In 2000, estimates of yield loss attributed to *S. festinus* in peanut totaled 2 million dollars in Georgia (Todd, 2000). In 2005, the only losses attributed to *S. festinus* were those associated with treatment costs, and these were estimated to be up to $468,000 (Brown & Adams, 2006). In recent years, *S. festinus* abundance in peanut has caused concern among peanut farmers in the southeastern United States. Many acres of peanut are treated annually for *S. festinus*, but there is no published effect on yield.

*Spissistilus festinus* is a phloem-feeder, and its damage is relatively easy to quantify. The insect creates a series of lateral punctures around the circumference of a stem or petiole, with every puncture leaving behind an insoluble salivary sheath (Smith, 1933). The ring of salivary sheaths results in the formation of a girdle, which is a very distinct ring of necrotic tissue that circles the stem/petiole. The large numbers of adult *S. festinus* that are often seen in peanut fields distress farmers, but it is the late instar nymphs that cause the majority of girdle damage (Meisch & Randolph, 1965; Moore & Mueller, 1976; Mitchell & Newsom, 1984a; Andersen et al. 2002). Girdle formation requires up to 24 hours. Nymphs are more sessile and more likely to girdle a plant compared to adults that are more predisposed to flight (Wildermuth, 1915; Jordan 1952; Meisch & Randolph, 1965).

*Spissistilus festinus* overwinters in the adult stage and has multiple generations per year (Wildermuth, 1915; Mitchell & Newsom, 1984b). Two distinct peaks of nymphal populations occur in peanut, and though the timing of the first peak varies, it is often late June to early August when the first generation of nymphs appears (Rahman et al. 2007). Early feeding damage by first generation nymphs in soybean can cause yield losses due to lodging. It has been reported that second generation nymphs feeding on leaf petioles can have a larger negative impact on yield because of excessive leaf drop before pod maturity (Caviness & Miner 1962; Sparks & Newsom 1984; Sparks & Boethel 1987). Due to it's growth habit, peanut is largely unaffected by lodging. Unlike soybean, peanut has many prostrate growth habits and the fruit mature in the ground. It is unknown whether there is a difference in impact between early season and late season damage, though anecdotal reports suggest late season damage does not affect yield in peanut (Brown, 2006).

While girdling does not typically kill the stem, it affects the nutrient profile by forcing photosynthates causing them to pool in the disrupted phloem tissue (Andersen et al. 2002). Phloem disruption is not permanent, but *S. festinus* can girdle the same stem/petiole multiple times over the course of the season (Wildermuth, 1915, Andersen et al. 2002). Girdling not only affects nutrient flow, but it also can affect the structural integrity of the stem/petiole. We do not know if this is an issue in peanut, but it has been shown to reduce yield in soybean (Caviness & Miner, 1962).

It is unknown what level of *S. festinus* damage can impact yield. The objective of this study was to develop an economic injury level for *Spissitilus festinus* in peanut.

## Methods and Materials

Studies to evaluate the impact of *S. festinus* feeding on peanut were conducted in the field and greenhouse over two years at the Tifton and Athens Campuses of the University of Georgia.

### ***Spissistilus festinus*** **Nymphs**

Collecting adults were collected multiple times throughout the season from peanut and alfalfa fields using a 38cm diameter sweep net. Adults were placed in BugDorm® cages located in a rearing room maintained at 70%+ RH and 26-28°C as per Meisch and Randolph (1965). Rhizombium-inoculated soybeans grown in 10cm pots grown in an incubator as well as loose organic green bean pods from Wal-Mart (Wal-Mart Stores, Bentonville, AR), were placed in the cages as a food source and oviposition substrate for adults. Prior to being placed in a cage, soybeans were grown in an incubator at 28°C and 14:10. First instars appeared on soybean plants and green bean pods 1-2 weeks after adults were introduced to the cage. First and second instar nymphs were used for all experiments in this study. Prior to being placed in the field, nymphs were removed from cages using a size 2, horsehair soft-bristle paintbrush; the number of nymphs placed in each vial corresponded to infestation treatment rate.

**Field Trials**

All experiments were conducted at the University of Georgia Tifton Campus Lang-Rigdon (31°31'17.8"N, 83°32'43.6"W) and Ponder (31°30'30.3"N, 83°38'14.9"W) research farms in Tift County, Georgia. The experiments were arranged in a randomized complete block design with 8 treatments (9 in 2015) and 6 replications. Treatments consisted of two infestation dates corresponding with the two generational population peaks observed by Rahman (2007), with four different rates of infestation (0, 3, 15, 30 nymphs per infestation period).

Seedbeds 1.8 m wide were prepared using a 1.83m tractor-mounted rototiller (KMC® Corp. Tifton, GA). A single row of peanut, Georgia-06G, was seeded down the middle of each bed to be used in the study with a single-row push planter (Earthway® Products Inc. Bristol, IN). A border bed was established between each research bed; two rows were seeded to peanut cultivar GA 06-G on each border bed with a tractor mounted vacuum planter (Monosem®, Edwardsville, KS). All plantings were treated with a same-day application of 0.033L/ha Valor® (Valent® © 2010), 0.017L/ha StrongArm® (DOW Agrosciences LLC © 2010), and 2.35L/ha

Sonolan® (DOW Agrosciences LLC © 2014) herbicides. Trials received applications of land plaster ($CaSO_4$) at a rate of 1135kg/ha on 25 June 2014 and 16 June 2015. In 2014, fungicide applications of Headline® (BASF Corporation © 2013) at a rate of 0.675L/ha were made on 9 and 24 July and Provost (Bayer CropScience © 2007) at a rate of 0.8L/ha on 7 August, 19 August, and 2 September. In 2015, fungicide applications of Headline (BASF Corporation © 2013) at a rate of .68L/ha were made on 8 July, 22 July, and 5 August and Convoy (Nichino America, Inc. © 2014) at a rate of 0.95L/ha on 19 August and 2 September.

At peanut emergence, cages (Fig 2.1) were centered over three peanut plants located within a 30-cm section of row; all other plants within a 60-cm radius were removed. Cages were constructed of pressure treated 5.1cm x 10.2cm lumber held together with 7.62cm deck screws to create a 91cm x 91cm square frame. Four 12.7mm holes were drilled into the top of each frame; two 1.52-meter lengths of 12.7mm schedule 80 CPVC pipe were bent into approximate half circles with the ends of each pipe placed into one of the parallel holes in the wood frame to create a hoop structure. 39.05 (7.1 x 5.5) holes per $cm^2$ mesh fiberglass window screen (PHIFER Inc., Tuscaloosa, AL) was placed over the structure and secured at the bottom with wood lattice strips (size) stapled into the wood frame. After placement over the plants, soil was mounded around the base of the cage.

Treatments consisted of populations of 0, 3, 15, 30 nymphs per cage (NPC) placed using a soft-bristle paintbrush near the base of each plant's main stem. There were two infestation periods. The first infestation (Gen 1) was terminated after 30 days, to eliminate all *S. festinus* and prevent Lepidopteran damage by treating the plants in each cage with Besiege® (Lambda-cyhalothrin 9.26% + Chlorantraniliprole 4.63%) at the rate of 5.16mL of per liter for ten seconds using a backpack sprayer with a single 8002XS nozzle at 40 psi. Cages were removed during application and replaced after treatment. The second generation (Gen 2) was infested as previously described; no insecticides were applied to Gen 2 treatments. In 2015, an additional infestation rate of 60 NPC was examined. This treatment was infested between the Gen 1 and Gen 2 infestation periods.

Peanuts were planted on 2 June 2014 at Lang-Rigdon farm. Cages were placed just after cracking on 9 June. On 7 July 2014, *S. festinus* nymphs were observed in adjacent, non-caged peanut plants, and on 9 July, first generation (Gen 1) cages were infested with first and second instar nymphs. All Gen 1 cages were treated with Beseige® on 5 August. On 5 September, second generation (Gen 2) cages were infested with the same infestation rates as Gen 1. All plants within cages were harvested on 6 October at 2,117 cumulative adjusted growing degree-days (aGDD) (calculated using UF PeanutFARM). The optimal cumulative aGDD for GA-06G peanuts is estimated to be 2500. Peanuts were harvested early due to logistical concerns regarding the seasonal timing.

Peanuts were planted on 26 and 27 May 2015 at UGA Ponder and Lang-Rigdon farms respectively. Cages were placed on 8 June. Gen 1 cages at Lang-Rigdon were infested on 1 July with first and second instar nymphs at identical rates as in 2014. On 31 July, Gen 1 cages were treated with Besiege®. On 31 August, Gen 2 cages were infested. All cages at Lang-Rigdon were harvested on 8 October at 2,117 cumulative aGDD.

Cages at the Ponder Farm location were infested with adults on 14 August. The treatments for this experiment consisted of infestation rates: 0, 10, 20 and 30 adults/cage (APC) with a sex ratio of 7:3 (male:female). This ratio was based on observation of the sex ratio of the extant *S. festinus* population at the Ponder farm at the time the cages were infested. Each treatment was replicated 12 times. Six reps were destructively sampled 30 days after infestation

(DAI) on 16 September at 1935 cumulative aGDD. Plants in the cages were removed from the ground intact, placed in 50-gallon plastic bags, and transported to the lab, where each cage sample was examined for the number of plants, total number of stem girdles (later differentiated into main and lateral stem girdles, where the main stem is that from which all other stems branch), leaf petiole girdles, and nymphs number and instar. Remaining cages were sampled on 30 September (44 DAI) at 2079 aGDD.

At harvest, plants from Lang-Rigdon and Ponder were exhumed from the ground intact, and all vegetative and reproductive parts were collected. Plants were examined for stem and leaf petiole girdles, number of pods, and number of plants. In 2015, main stem girdles and lateral stem girdles were recorded separately. Pods and plant material were separated and placed into a drying oven at 60°C until plants were dry; dry weight vegetative and pod biomass was recorded using a bench top laboratory balance (OHAUS CS5000g x 1g). Root biomass was measured separately from vegetative biomass for plants from Lang-Rigdon farm in 2015.

### Greenhouse Study

In 2014 and 2015, peanuts were grown in the greenhouse in Athens (33°55'49.0"N, 83°21'45.3"W) and Tifton (31°28'24.6"N, 83°31'45.3"W), Georgia, respectively. Two seeds of cultivar ‘Georgia-06G’ peanut were planted in 12” plastic pots filled with propagation media (Sungro Propagation Mix). The pots were placed individually in separate BugDorm 2120F insect tents (160 x 160 mesh), and watered twice weekly. Plants were fertilized once with 20 grams of six-month Osmocote (13-13-13) 30 DAP. After emergence the plants were culled to one plant per pot. Cages were monitored once every 6-8 days in 2014 and every 3-5 days in 2015 for the number, instar, and location (stem or petiole) of nymphs, and presence of stem and leaf petiole girdles. Main stem girdles were differentiated in 2015.

In 2014, peanuts were planted on 9 October in a greenhouse located in Athens, Georgia. Temperature ranged from 22-32°C, and photoperiod was 14:10 day:night throughout the experiment. Gen 1 cages were infested on 15 November with 1st and 2nd instar nymphs. Nascent adults were removed when spotted during weekly observations; by December 16 all *S. festinus* had been removed. Gen 2 cages were infested on 12 January 2015. Cages with no visible nymphs on 17 January were reinfested with nymphs. On 12 Feb 2015 all *S. festinus* were removed from Gen 2 cages, and plants were harvested.

In 2015, peanuts were planted on 2 June in greenhouses in Tifton, Georgia. Temperature ranged from 26-32°C, and photoperiod was 14:10 day:night. Generation 1 cages were infested on 6 July. By 17 August, all nymphs had molted into adults, and the adults were removed. On 7 September, Gen 2 cages were infested. Generation 2 cages with no visible nymphs were reinfested on 14 September. Plants were harvested on 13 October.

Girdles were counted at harvest, and the number of plants and pods were recorded. Pods and plant material were separated and placed into a drying oven at 60°C until plants were completely dry. Dry weight pod and vegetative biomass were recorded using a bench top laboratory balance (OHAUS CS5000g x 1g). Root biomass was recorded separately in 2015.

## Statistical Analysis

For all analyses, significance was measured with $\alpha = 0.05$, and SAS Studio 3.4 (SAS Institute Inc., Cary, NC, USA) was used for the following tests. Dry weight biomass data analyzed included total biomass of all plants per cage, peanut pod biomass of all plants per cage, and mean pod weight. Cages with plants possessing heavy incidence of disease (TSWV or white mold) were excluded from analyses.

### *Dry Weight Yield and Vegetative Biomass as Responses to Girdles*

Statistical comparisons for analyzing stem girdles and dry weight yield and biomass readings as response variables were made with PROC GLIMMIX with infestation rate and infestation period as fixed effects, and block replicates as random effects. No data transformations were made, and all response variables were modeled with the negative binomial distribution, which provided the best fit for the data. All means using the negative binomial distribution were back-transformed using the ILINK function. When significant differences were observed ($p<0.05$), means were separated with the LSMEANS procedure and sliced by effect. PROC GLM was utilized for modeling dry weight yield and biomass readings as the response variables and total stem girdles, plant number per cage, infestation period as fixed effects. For comparisons of generation, the 20 nymphs per plant treatment data were excluded from analysis.

### *Greenhouse observations*

PROC GLIMMIX was used to model the progression of stem girdles using week, total number of nymphs in each sample, and infestation period as fixed effects, with block replicate as a random effect. PROC LOGISTIC was used to measure the preference of feeding location of nymphs, with nymph instar as a fixed effect and block replicate as a random effect. When comparing infestation period, the 20 nymphs per plant treatment was excluded from analyses.

## Results

### Field trials

#### *Girdle Damage*

The presence of total stem girdles (Fig. 2.2-2.3) increased with increasing infestation rate (IR) across both infestation periods in both years of nymphal trials (2014: F=8.44; df=3,31;

P=0.0003 and 2015: F=2.88; df=3,38; P=0.0487). There was no relationship between infestation rate and the number of leaf petiole girdles. When comparing the effect of individual treatment IR on total stem girdles, only the 3NPC and 15NPC treatments were significantly different (t=-2.89, df=31, P=0.0333). When comparing IR effects between generations, total stem girdles in Gen 2 controls were much higher than Gen1 (Control: t=-3.21, df=31, P=0.0031) and Gen 1 30NPC girdle counts were higher than Gen 2 (t=2.69, df=31, P=0.0113).

In 2015, there was no effect of IR on main stem girdles (F=1.90, df=3, P=0.1446) or leaf girdles (F=0.55; df=3,43; P=0.6509), the following analyses are of total stem girdles. A significant correlation between total stem girdle formation and IR was only seen in Gen 1 in 2015 (F=3.82; df=3,38; P=0.0174). The 30NPC treatment of Gen 1 resulted in the highest number of total stem girdles between the two generations ($\bar{x}$=10.66, s=9.07), but 60 NPC had the most of all treatments ($\bar{x}$=14.5, s=1.5). There were no significant differences in the number of total stem girdles between individual infestation rates. Only 30NPC showed any significant difference in total stem girdles between Gen 1 and Gen 2 (t=2.86, df=40, P=0.0067). The additional treatment of 60NPC was not included in comparisons between generations.

At the Ponder farm, at 30 DAI, the total number of nymphs moderately correlated (F=11.18; df=1,20; P=0.0034) (R-Square=0.370444) with the number of total stem girdles found. The number of leaf petiole girdles was not affected by IR (F=0.04; df=1,15; P=0.8383) and few main stem girdles were observed. 30APC had the highest mean number of nymphs of all IR (F=8.66; df=3,15; P=0.0008) (Fig. 2.4). In the 44 DAI sample, stem girdle numbers positively correlated with IR (F=6.13; df=3,15; P=0.0062), but did not differ significantly between individual IRs (Fig. 2.5). There was no difference in stem girdle number between any of the IR treatments and the control at 30 days after infestation. Direct comparisons between girdle counts at 30 DAI and 44 DAI showed no differences.

### *Girdle Impact on Dry Weight Vegetative and Yield Biomass*

At the Lang-Rigdon farm in 2015 and at Ponder farm in 2015, number of total stem girdles did not affect dry weight vegetative biomass (Lang-Rigdon 2014: F=0.58; df=1,43; P=0.4516; and Ponder 2015: F=1.12; df=1,23; P=0.3017) (Fig. 2.6-7). At Lang-Rigdon farm in 2015 trials, a weak but significant negative correlation between total stem girdles and dry weight vegetative biomass was observed (F=4.54; df=1, P=0.0385, R-Square=0.101288).

At Lang-Rigdon farm in 2014 (Fig. 2.8) and Ponder farm in 2015 (Fig. 2.9), lateral stem girdles had no effect on total dry weight yield (Lang-Rigdon 2014, F=0.50; df=1,43; P=0.4839; Ponder 2015: F=1.11; df=1,43; P=0.3026). At Lang-Rigdon farm in 2015, yield was negatively impacted by lateral stem girdles (F=4.41; df=1,48; P=0.0413), but the correlation was weak (R-square=0.163439). At Lang-Rigdon farm in 2015, infestation period had an effect on total yield (F=3.90; df=2,48; P=0.0480); yields from Gen 2 were lower than Gen 1. Stem girdles from all fields and years had no effect on the mean dry mass of peanut pods (Lang-Rigdon 2014: F=0.01; df=1,43; P=0.9170; Lang-Rigdon 2015: F=0.00; df=1,48; P=0.9820; and Ponder 2015: F=0.33; df=1,23; P=0.5714). When the data from both years of trials from Lang-Rigdon farm were combined, there was a significant relationship between the total yield and the number of total stem girdles (F=4.19; df=1,89;P=0.0434) and infestation period (F=4.64; df=1,89;P=0.0341) (Fig. 2.10). In 2014, there were two cages with 4 plants, and in 2015, there were two cages with 2 plants and three cages with 4 plants. The cages with differing ($n \neq 3$) number of plants had no effect in the previous model (F=2.01, df=2,89, P=0.1405).

## Greenhouse Cages

### *Girdle Damage*

In 2014, IR had a significant effect on total stem girdles (2014: F=3.34; df=3,64; P=0.0427). In 2015, total stem girdles (F=18.26; df=3,17; P<0.0001), lateral stem girdles (F=10.13; df=3,17; P=0.0005), and main stem girdles (F=7.68; df=3,17; P=0.0007) (Fig. 2.11) were positively correlated with IR, as were leaf girdles (F=9.17; df=3,17; P=0.0003). Generation affected the number of leaf petiole girdles only in 2015; Gen 1 had fewer girdles than Gen 2 (t=-2.11, df=17, P=0.0497).

### *Girdle Impact on Dry Weight Vegetative and Yield Biomass*

There was a significant negative correlation between total stem girdles (F=5.54; df=1,26; P=0.0272) (Fig 2.12) and dry weight vegetative biomass, as well as between main stem girdles (F=13.33; df=1,26; P=0.0013) (Fig 2.13) and dry weight vegetative biomass in 2015. Main stem girdles also negatively impacted root biomass (F=6.50; df=1,26; P=0.0176) (Fig 2.14). Leaf petiole girdles had no relationship with dry weight vegetative biomass (2014: F=1.96; df=1,22; P=0.1767; 2015: F=3.81; df=1,26; P=0.0629). There was no direct effect of IR itself on dry weight vegetative biomass for either year (2014: F=1.56; df=3,16; P=0.2382) (2015: F=2.31; df=3,14; P=0.1209). Plants infested at Gen 2 had lower dry weight mass than plants infested at Gen 1 regardless stem girdle number (F=64.55; df=1,26; P=0.0433) in 2015.

Total stem girdles (Fig. 2.15) and main stem girdles (Fig. 2.16) negatively influenced peanut pod biomass, but only in 2015 (total stem girdles: F=5.39; df=1,26; P=0.0290; main stem girdles: F= 7.61; df=1,26; P=0.0109). However, counts of pods were very low in 2015 ($\bar{x}$=1.88, s=2.11) compared to 2014 ($\bar{x}$=11.00, s=8.00). Leaf girdles for both years had no effect on pod biomass (2014: F=0.03; df=1,22; P=0.8737; 2015: F=2.21; df=1,26; P=0.1504). Mean pod weight did not correlate with any damage rating in either year: stem girdles, (2014: F=1.01; df=1,22; P=0.3273 and 2015: F=2.79; df=1,26; P=0.1171) leaf girdles, (2014: F=0.00; df=1,22; P=0.9662 and 2015: F=0.01; df=1,26; P=0.9262) and main stem girdles (2015: F=1.79; df =1,26; P=0.2021). Gen 2 plants had significantly lower mean pod biomass in 2015 regardless of IR (F=6.81; df=1,26; P=0.0154).

*Greenhouse Observations*

In 2014, there was an increase of stem girdles over time (F=3.99; df=3,90; P=0.0102) and number of nymphs observed (F=10.61; df=1,90; P=0.0016). Generation had no effect on stem girdle counts over time (F=0.00; df=1,90; P=0.9682). Location of nymphs varied by instar ($X^2$ (3, N=93)=21.5018, P<0.0001) and generation ($X^2$ (1, N=93)=8.5645, P=0.0034). Leaf girdle numbers did not change over time, but were greater in Gen 1 (F=5.40; df=1,22; P=0.0298).

In 2015, stem girdles increased as the number of nymphs increased (F=40.38; df=1,247; P<0.0001) and at each sample date over time (F=30.64; df =10,247; P<0.0001). Generation had no effect on stem girdles over time (F=0.71; df=1,247; P=0.4070). Main stem girdle counts were too low to be analyzed separately, and were combined with lateral stem girdles. Leaf petiole girdles increased as the number of nymphs increased (F=13.24; df=1,247; P=0.0003) and over time (F=7.73; df=10,247; P<0.0001).

Location of nymphs in 2014 differed as the nymphs matured ($X^2$ (3, N=90)=21.5018, p<0.0001). As nymphs matured, they transitioned from leaf petioles to stems (Fig. 2.17). From these observations, there was a 92% probability of first instar nymphs being located on a leaf petiole (8% on stems); the probability of fifth instars being on a leaf petiole was only 9%. Between the two infestation periods Gen 1 and Gen 2, the likelihood of nymphs being found on stem girdles was much higher in Gen 2.

Nymphs in 2015 are also more abundant on leaf petioles during early instars ($X^2$ (8, N=457)=29.7560, P=0.0002), and infestation period ($X^2$ (2, N=457)=7.1140, P=0.0285) influenced the observed location of nymphs (Fig 2.18). The probability of first instars feeding on the leaf petiole was 64%, with the remaining 36% found on the lateral stems and none on the main stem. Fifth instars were found 19% of the time on the leaf petiole, 18% on the main stem, and 63% on lateral branches. Gen 2 had a similar transition to lateral stems, though the proportion on the main stem remained the same throughout the instars. Between infestation periods, the probability of finding nymphs being located on leaf petioles was significantly lower in Gen 2 than in Gen 1 (z=2.65; SEM=0.2698; P=0.0080).

## Discussion

### Damage and IR

Increasing infestation rates of *Spissistilus festinus* on peanut resulted in a higher number of stem girdles, though this was only observed in Gen 1, which occurs in the early part of the season. Previous work on *S. festinus* on the peanut cultivar 'Georgia Green' documented up to six girdles per plant with no measurable pod yield response; however, the study did not report the location of the girdle (Rahman et al. 2007). The highest mean number of total stem girdles in either year of the field study was 18.5 in the cages with the 30APC treatment (about 5.33 stem girdles per plant), and IR did not affect yield of GA-06G peanuts in any field or greenhouse trial.

In the field, Gen 2 nymphs showed little capacity to create girdles. Observations made in the greenhouse experiments may offer an explanation for this result. The Gen 2 treatment in both years of the greenhouse experiment had to be reinfested with nymphs. In 2015, Gen 2 treatments had to be reinfested multiple times before ≥70% of the intended population would survive. We hypothesize that the low survival of early instar nymphs in Gen 2 treatments is due to tougher stems and petioles found on older plants. Once the population in the greenhouse cages was established, similar girdle counts for each IR were found in generations 1 and 2.

The aforementioned observations may be used to explain the results from nymphal cages in the field, where infesting with Gen 2 nymphs alone resulted in weak counts of stem girdles in both years. For comparison, the adults used at Ponder farm trials were added to cages only 15 days prior to the Lang-Rigdon farm trials' Gen 2 nymphs. Given that the average time of eclosion is 16.5 days after oviposition (Meisch & Randolph, 1965), nymphs from these cages would be on a similar timetable as those at Lang-Rigdon farm. Destructive sampling 30 days after infestation showed that up to 54 nymphs ($\bar{x}$=28.0) were present in the highest IR of 30 adults per cage, with up to 33 total stem girdles ($\bar{x}$=18.3). This indicates that it is possible for nymphs to survive (and most likely form girdles) in the later part of the season. The main difference between adding adults and adding nymphs alone is that the adults in the cage are likely girdling and feeding. Nymphs of all instars capitalize on girdles, feeding and aggregating above the nutrient dam (Moellenbeck & Quisenberry, 1991; Andersen et al. 2002), suggesting that younger instars later in the season may rely on older nymphs or adults for formation of girdles and the subsequent nutrient concentration.

Main stem girdles in the field were only found in Gen 1 nymph treatments. Results of the greenhouse experiment were similar, though there were more main stem girdles in total. This is likely a result of either tissue maturation, as the main stem is the most developed part of the peanut plant at the time of infestation, or lower nymph survival suggested previously. Numbers of main stem girdles were too few for a valid analysis of effect, which can be attributed to the timing of infestation and overall plant health. By the time Gen 2 infestations were made, the base of the main stem -where nymphs were often found in the field (Rahman et al. 2007)- was more developed and stouter than the relatively fresh and succulent lateral stems.

Mean numbers of leaf petiole girdles were largely inconsistent between IR and generations for all trials. This result may be explained through greenhouse observations. Petioles that were girdled often displayed necrotic tissue above and below the girdle, and these leaves were easily dislodged from the plant at the node. As nymphs developed, feeding sites changed from petioles to stems, and subsequent girdled petiole counts remained stable. Eventually, the number of petiole girdles decreased as the petiole tissue died around the girdle and through mechanical stimulation or plant-induced abscission. This is similar to what has been reported with girdled leaf petioles in soybean (Sparks & Newsom, 1984).

Girdle counts in the greenhouse in 2015 were much higher than those in 2014. Location effects could be responsible for this difference; greenhouse facilities used in 2014 had better lighting conditions, leading to apparently healthier and stronger plants. It is well established that *S. festinus*, prefers softer tissues (Mitchell & Newsom, 1984b; Rice & Drees, 1985; Andersen et al. 2002; Rahman et al. 2007), and plants grown in less than optimal lighting can have reduced health, stem thickness, and strength (Shirley, 1929). If the plants were weaker due to poor lighting conditions, it is likely that more girdles would be made not only because it is easier, but also because with a less substantive food source, more girdles would needed to compensate for low nutritional content. Girdles on healthy plants will become callused after about 7 days (Moellenbeck & Quisenberry, 1991; Andersen et al. 2002), but is unknown whether a girdle on an unhealthy plant will callus faster or slower. The possibility exists that *S. festinus* may have more impact on plants under less-than-optimal growth conditions.

## Girdle Effects on Vegetative Biomass and Pod Biomass

### Field Trials

Based on the results presented here indicate that *S. festinus* has the capability of reducing peanut pod yield and thus should be a considered a potential pest. In 2015, there was a high amount of variation in girdles and vegetative and pod biomass, but stem girdles resulting from nymphal infestations had a negative impact on both vegetative biomass and pod biomass. However, biomass did not differ by IR. IR only reflects the number of nymphs initially added, and it was shown in the greenhouse trials that nymph mortality could be high. Counting nymphs in field cages was not feasible. Quantifying nymph densities within the cages would have been impractical without destructively sampling the cages. For this reason, stem girdles were best suited to evaluate the impact of nymphs on yield.

The $R^2$ values of the regression of stem girdles on dry weight vegetative and pod biomasses were low, indicating that other factors contribute more significantly to variation in yield. The cages were not designed to be insect (specifically thrips) proof; the main purpose of the cage was to contain *S. festinus* nymphs and prevent infestation by extant adults. Some feeding girdles were found in insect-free control cages; this likely occurred as a result of rodent damage that allowed *S. festinus* adults access to the cages. The only other observable effect of this permeability was Tomato Spotted Wilt in less than five cages; these cages with diseased plants were excluded from analysis.

Monitoring nymph populations within cages during the season would provide a better measure of impact of a specific population density on damage and yield. Greenhouse observations and the destructive sampling at Ponder were conducted in an attempt to quantify number of nymphs within cages. Nevertheless, the relationship between nymph number and girdles cannot be examined based on the data collected. The number of girdles formed by *Spissistilus festinus* can be quite variable, as the 30 DAI samples had marginally higher counts of girdles than the 44 DAI samples.

### *Greenhouse Trials*

We found a significant effect in 2015 of total stem girdles and main stem girdles on dry weight vegetative biomass and pod yield. The effect on yield was muted somewhat by the low number of pods found in all cages, most likely a result of lighting concerns mentioned previously. It was impossible to differentiate the effect of main stem girdles or lateral stem girdles. A reduction in biomass and pod yield was only seen with a very high number of stem girdles. Levels of damage in the field trials never exceeded 37 stem girdles in a cage, or 12.33 stem girdles per plant.

Leaf petiole girdles did not affect yield, though accumulation of leaf petiole girdles could not be accurately measured due to abscission of leaves from girdled petioles. It is possible that *S. festinus* can cause enough defoliation to affect yield as has been reported in soybean (Sparks & Boethel, 1987). However, our greenhouse observations suggest that older nymphs move away from leaf petioles as the season progresses; this is different than what is observed in soybean (Mitchell & Newsom, 1984b; Spurgeon & Mueller, 1993). Nevertheless, peanut can sustain a great deal of defoliation with no measurable impact.

## **Generational Effect on Biomass and Yield**

All treatments with nymphs assessed the effect of only one generation at any time, and the cumulative effect of damage caused by multiple generations is yet to be determined. Experiments from the field and greenhouse demonstrated that damage or feeding occurring later in the season has more impact on dry weight vegetative biomass and yield than early season damage. This effect is possibly a result of the plant focus shifting from vegetative growth to fruit production later in the season, as flowering begins about 40 days into the season. Even though more girdles were formed by the first generation of nymphs, damage occurring later may impact pod production. This may be explained by the fact fact that the girdle's effects on translocation are not permanent. Phloem activity resumes 7-11 days after girdle formation (Andersen et al. 2002). So that plants damaged only in the early season may not suffer yield loss.

**Economic Injury Level**

A preliminary EIL was established using stem girdles and yield from Lang-Rigdon trials of both years combined using the following formula as per Higley and Pedigo (1996):

$$\frac{Insecticide\ cost}{crop\ value * Yield\ loss\ per\ girdle * expected\ control}$$

Where the cost of the insecticide (calculated from an average treatment cost/acre of a typical pyrethroid) and application costs are totaled in the numerator (Smith & Smith, 2015). Crop value was obstained from 28 October 2015 report from USDA (Soto, 2015). Girdle counts were used in the equation instead of nymph numbers because nymph numbers (IR) could not be related to yield. Expected control was calculated by using treatment effects of lambda-cyhalothrin on girdle counts (Rahman et al. 2007) in Georgia Green, a peanut variety that had similar girdle damage to what was observed in Georgia-06G in the current study. The final formula resulted in:

$$\frac{\$3.29\ per\ application\ per\ acre}{\$424.51\ per\ ton * 0.0000018\ ton\ loss\ per\ girdle\ per\ 0.31m\ row * 50\%\ girdle\ control} = 4429 \text{ girdles/0.31m of row}$$

The highest number of girdles found per treatment was 34 girdles per 0.31m of row, or 11.33 girdles per plant. These data combined with the results of our equation suggest that *S. festinus* is not an economically important pest of peanut.

Nevertheless, the possibility remains that yield loss may occur due to potential interactions of *S. festinus* with disease. It is known that *S. festinus* damage can influence *S. rolfsii* severity in soybean (Herzog et al. 1975; Russin et al. 1986). Plants infected with white mold in one cage had the one of the highest numbers of girdles of all trials. Additional studies are needed to study this potential interaction.

## Conclusions

These data suggest that while stem girdles formed by *S. festinus* are capable of lowering yield, they are not capable of reducing yield enough to warrant treatment. The EIL we calculated indicates that thousands of stem girdles are required to justify the expense of treatment for *S. festinus*; the numbers of stem girdles seen in the field never exceeded 12 per plant. Because this study was conducted entirely with caged plants, the results cannot be fully extrapolated to field situations. Sample sizes were relatively small, and variation in yield was between cages with similar numbers of girdles was high.

The single most important limitation to this study was the inability to quantify the number of nymphs and damage in real-time. Nevertheless, the relationships between girdles and yield and vegetative biomass are valid and suggest that *S. festinus* is not an economic pest of peanut. Future experiments involving *S. festinus* could examine the cumulative effect of girdling over multiple generations.

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**Figure 2.1:** The cage used in the field trials at Lang-Rigdon and Ponder farms.

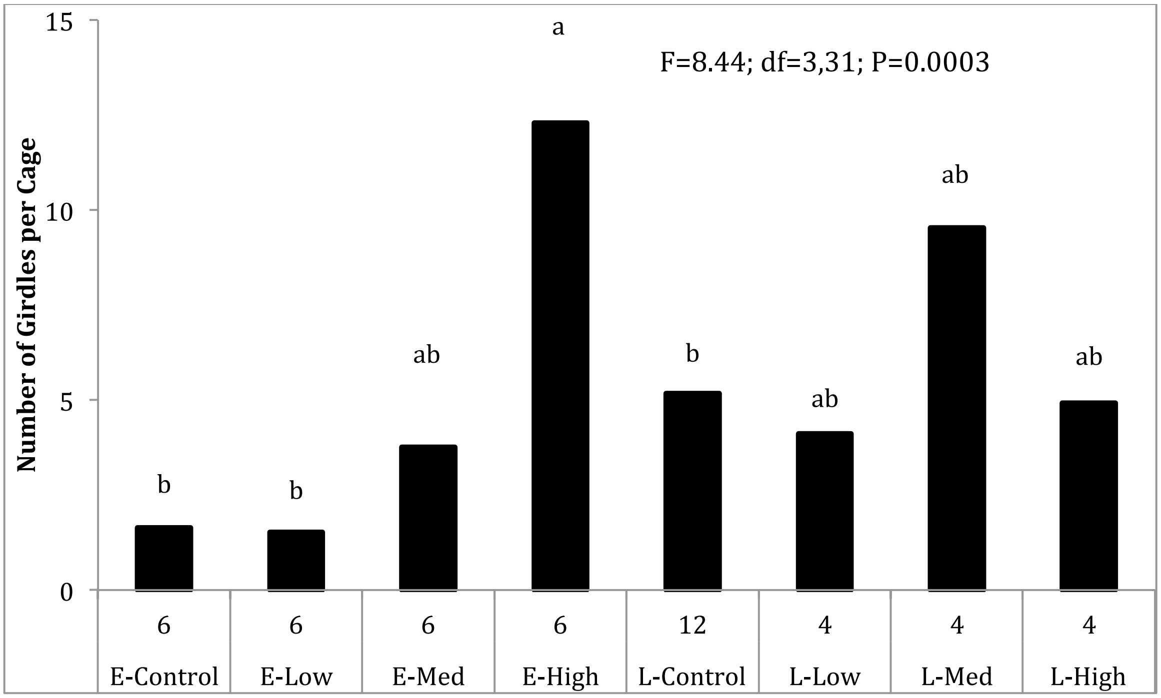

**Figure 2.2**: Mean ± Standard error of the mean (SEM) for *Spissistilus festinus* stem girdles in each treatment of NPC (nymphs per cage) ordered by infestation period for Lang-Rigdon farm in 2014. Different letters above bars indicates significant differences in girdle counts among treatments and generations (LSMEANS, P<0.05).

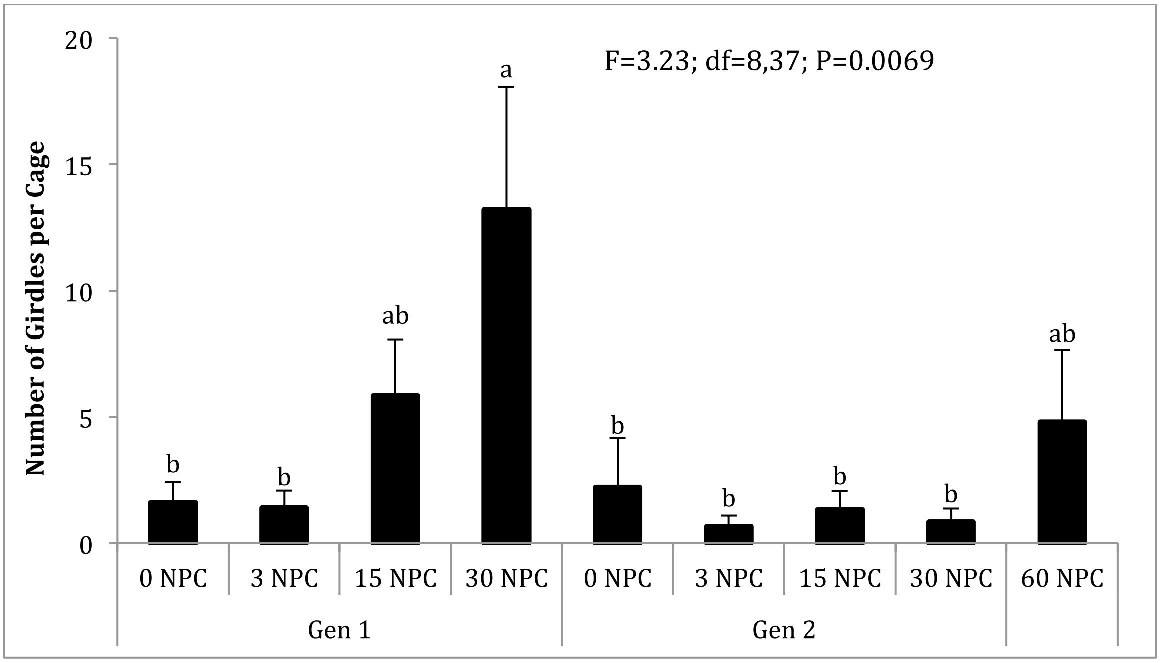

**Figure 2.3:** Mean ± SEM for *Spissistilus festinus* stem girdles in each treatment of NPC (nymphs per cage) ordered by infestation period for Lang-Rigdon farm in 2015. 20 NPP has no generation as the infestation was made in between Gen 1 and Gen 2. Different letters above bars of the same girdle type indicates significant differences between IR (LSMEANS, P<0.05).

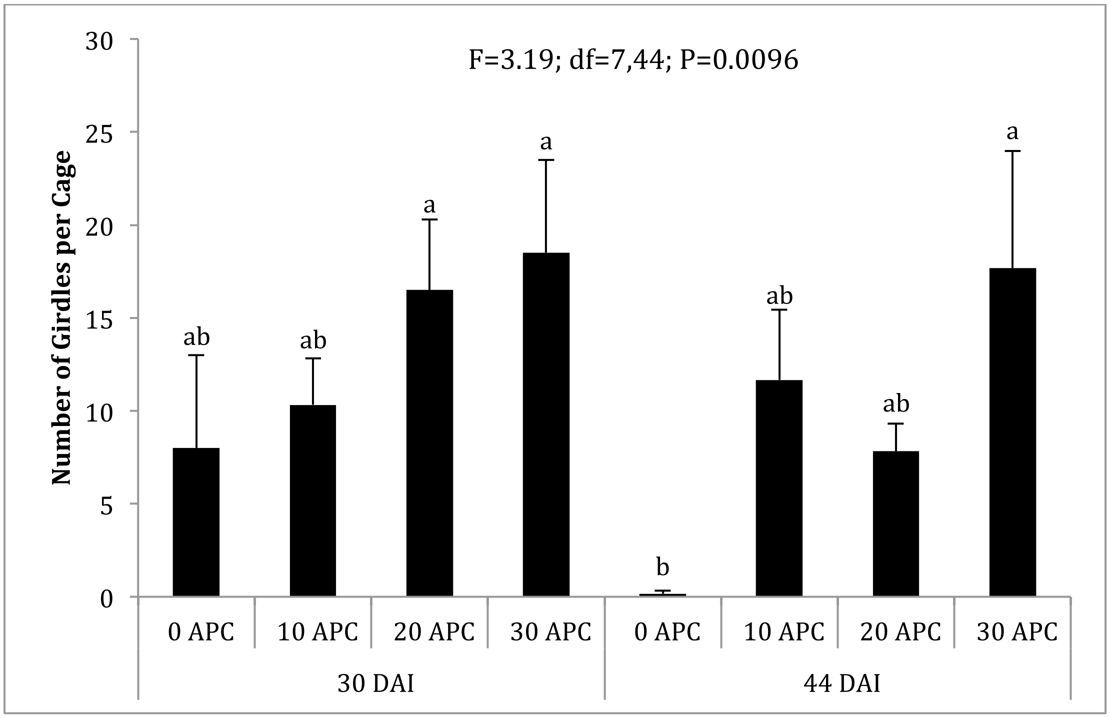

**Figure 2.4:** Mean ± SEM for *Spissistilus festinus* stem girdles in each treatment of APC (adults per cage) from different DAI (days after infestation) for Ponder farm in 2015. Different letters above bars indicates significant differences of girdle counts among treatments and generations (LSMEANS, P<0.05).

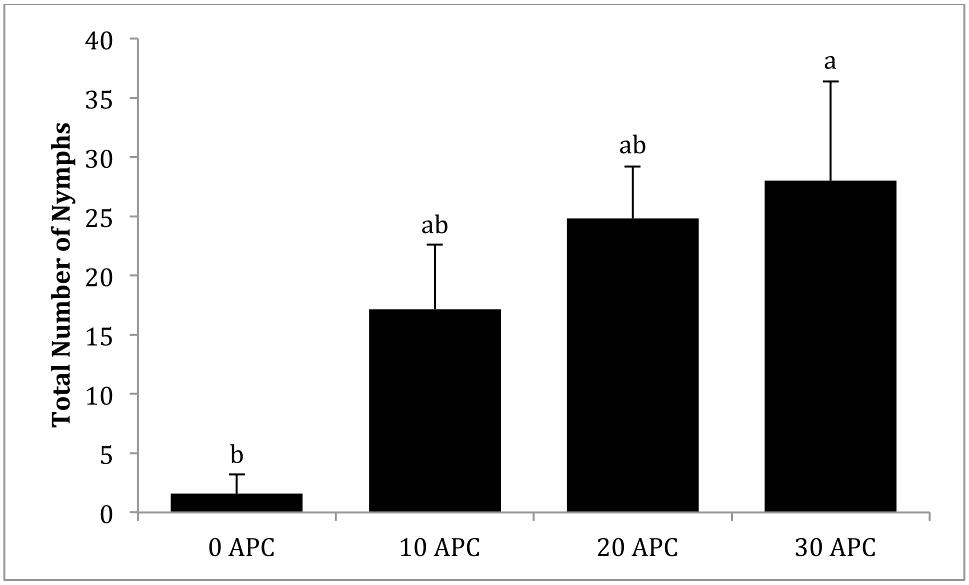

**Figure 2.5:** Mean ± SEM for 30 DAI (days after infestation) total nymph counts ordered by APC (adults per cage) for field cages at Ponder farm 2015. Different letters above bars indicates significant differences of nymph counts (LSMEANS, $P<0.05$).

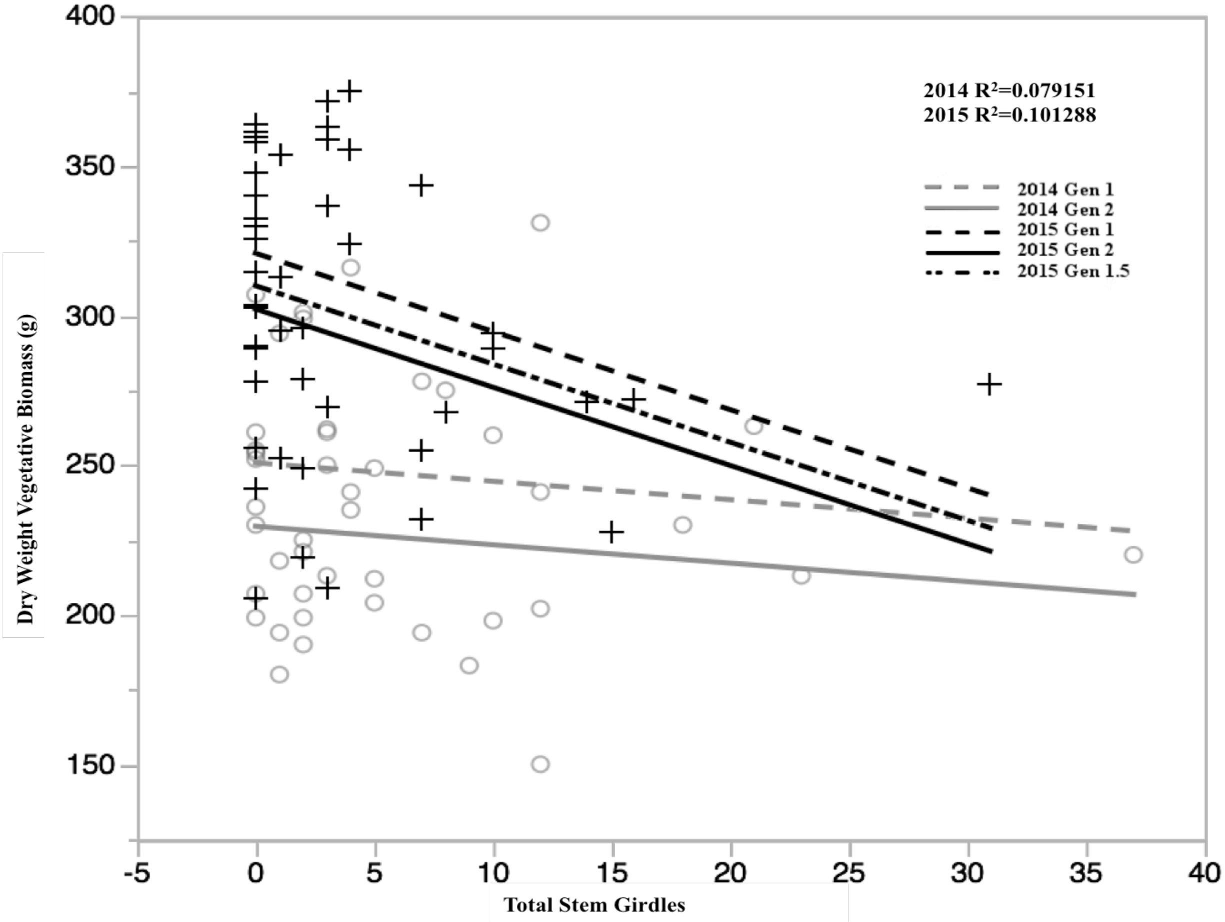

**Figure 2.6:** Relationship between total numbers of stem girdles per cage and the dry weight vegetative biomass of peanut for each year of Lang-Ridgon farm cage trials.

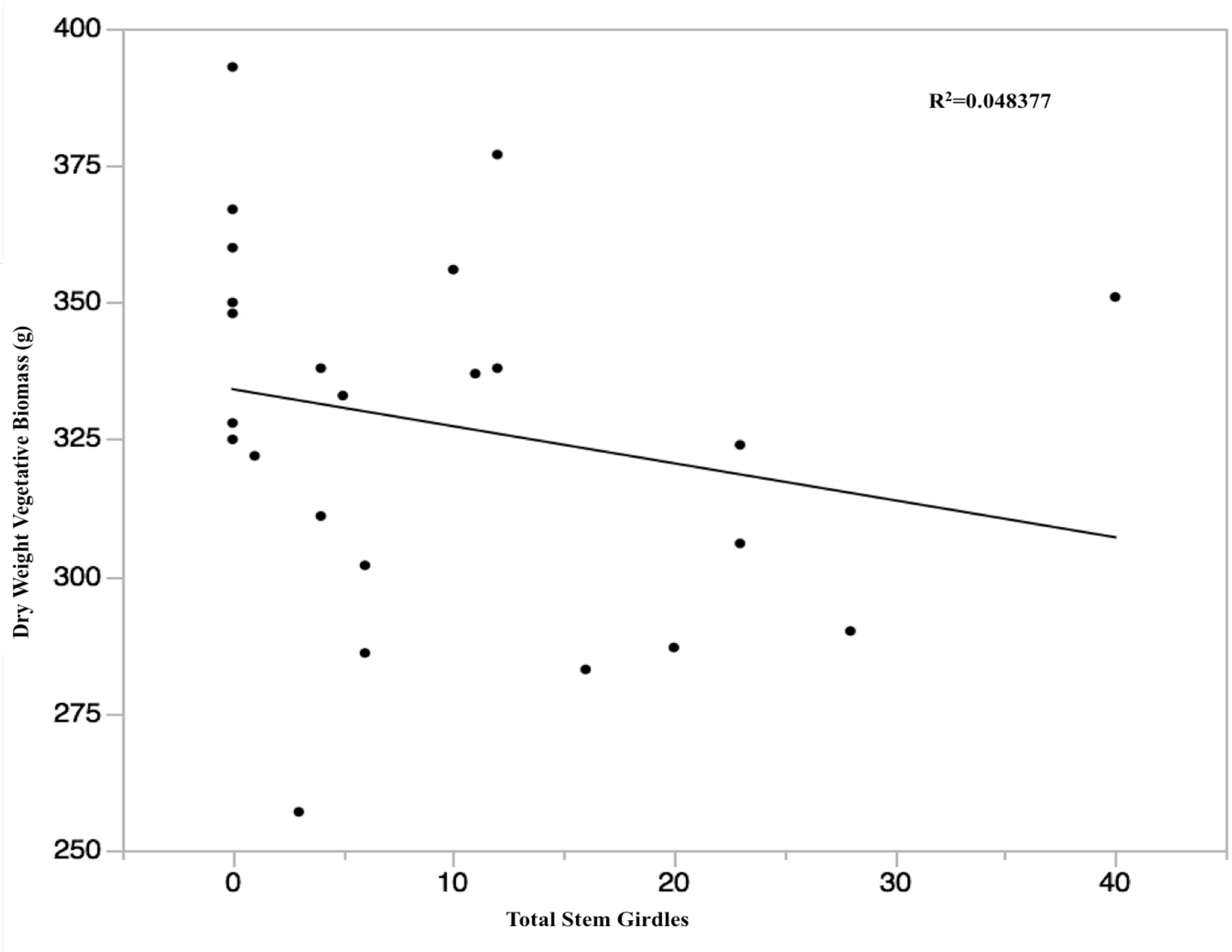

**Figure 2.7** Relationship between total numbers of stem girdles per cage and the dry weight vegetative biomass of peanut for Ponder farm cage trials.

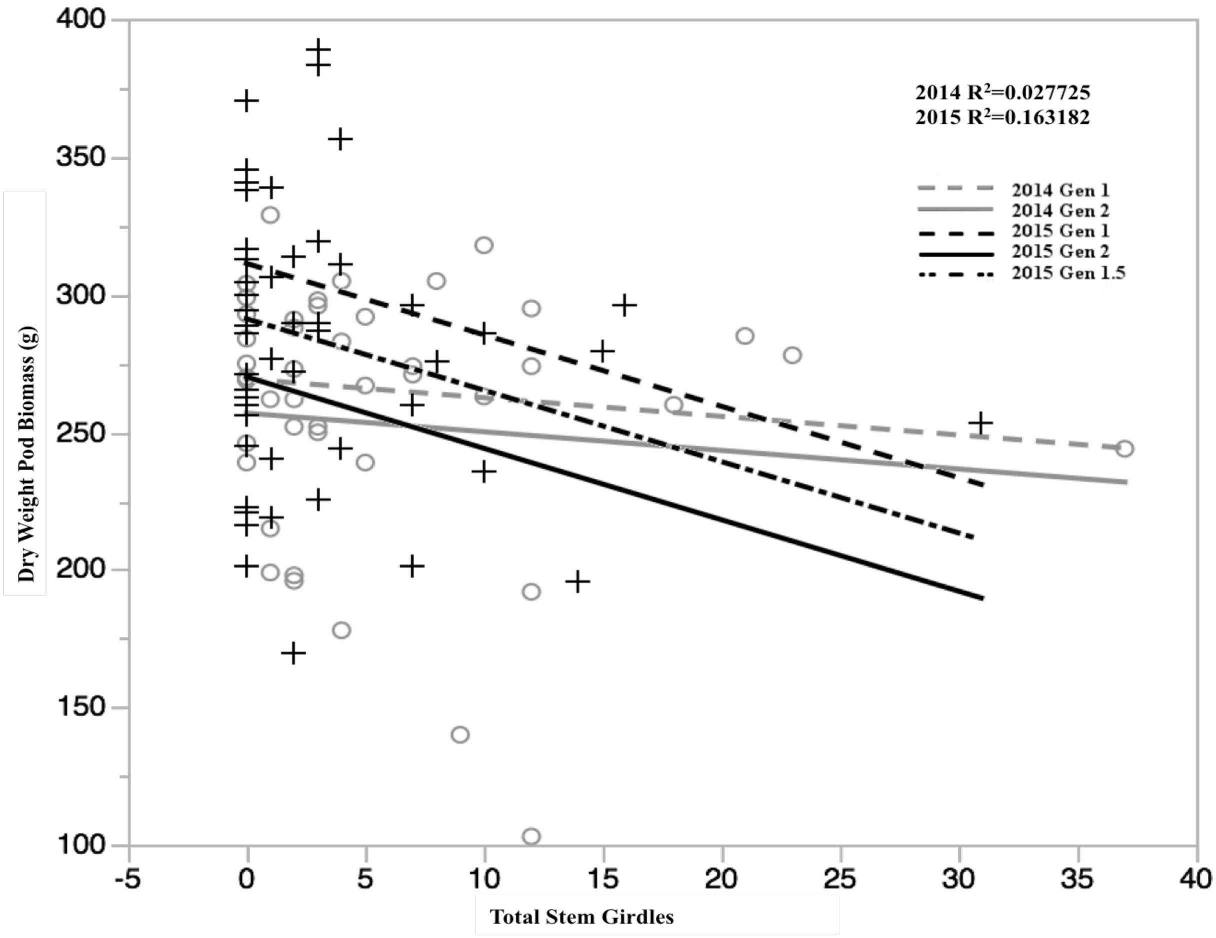

**Figure 2.8:** Relationship between total numbers of stem girdles per cage and the dry weight yield biomass of peanut for each year of Lang-Ridgon farm cage trials.

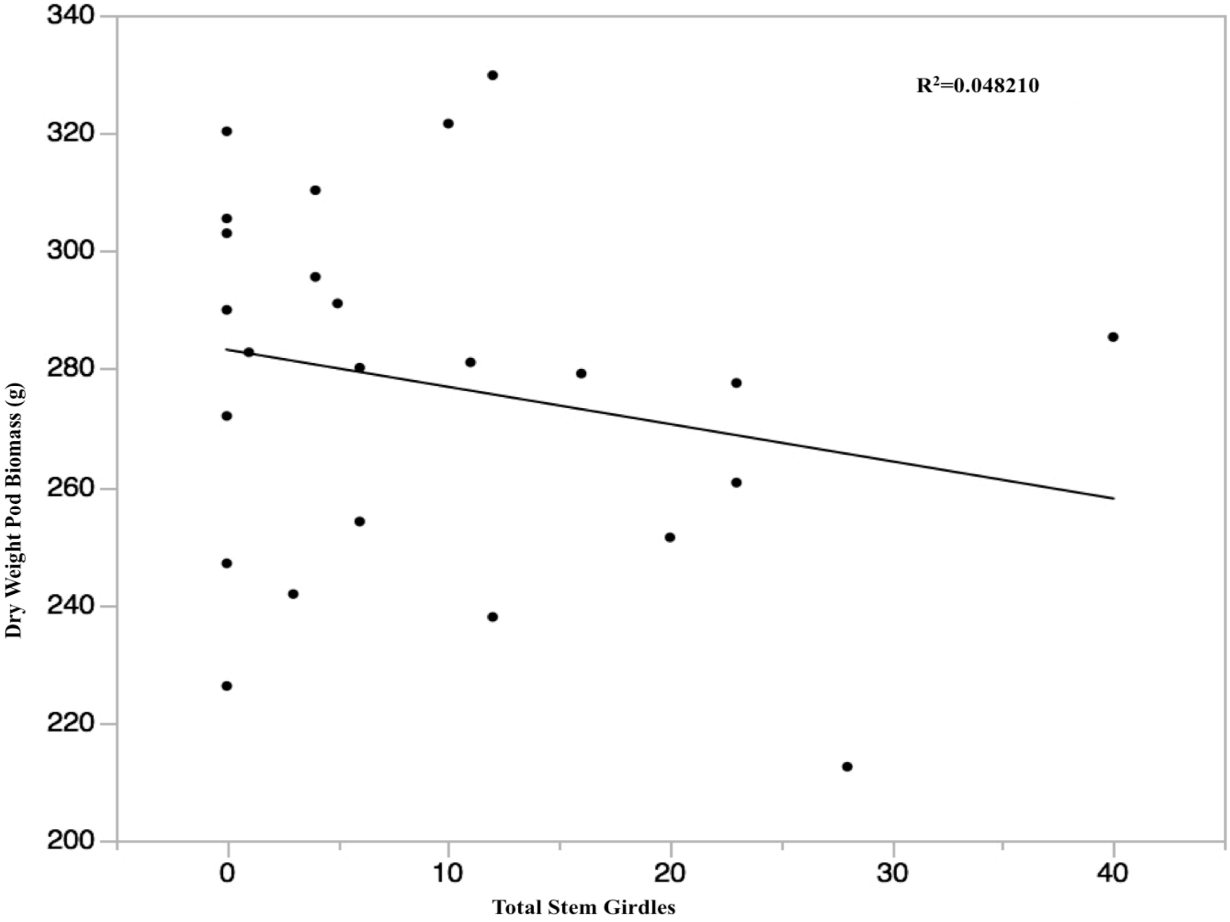

**Figure 2.9:** Relationship between total numbers of stem girdles per cage and the dry weight yield biomass of peanut for Ponder farm cage trials.

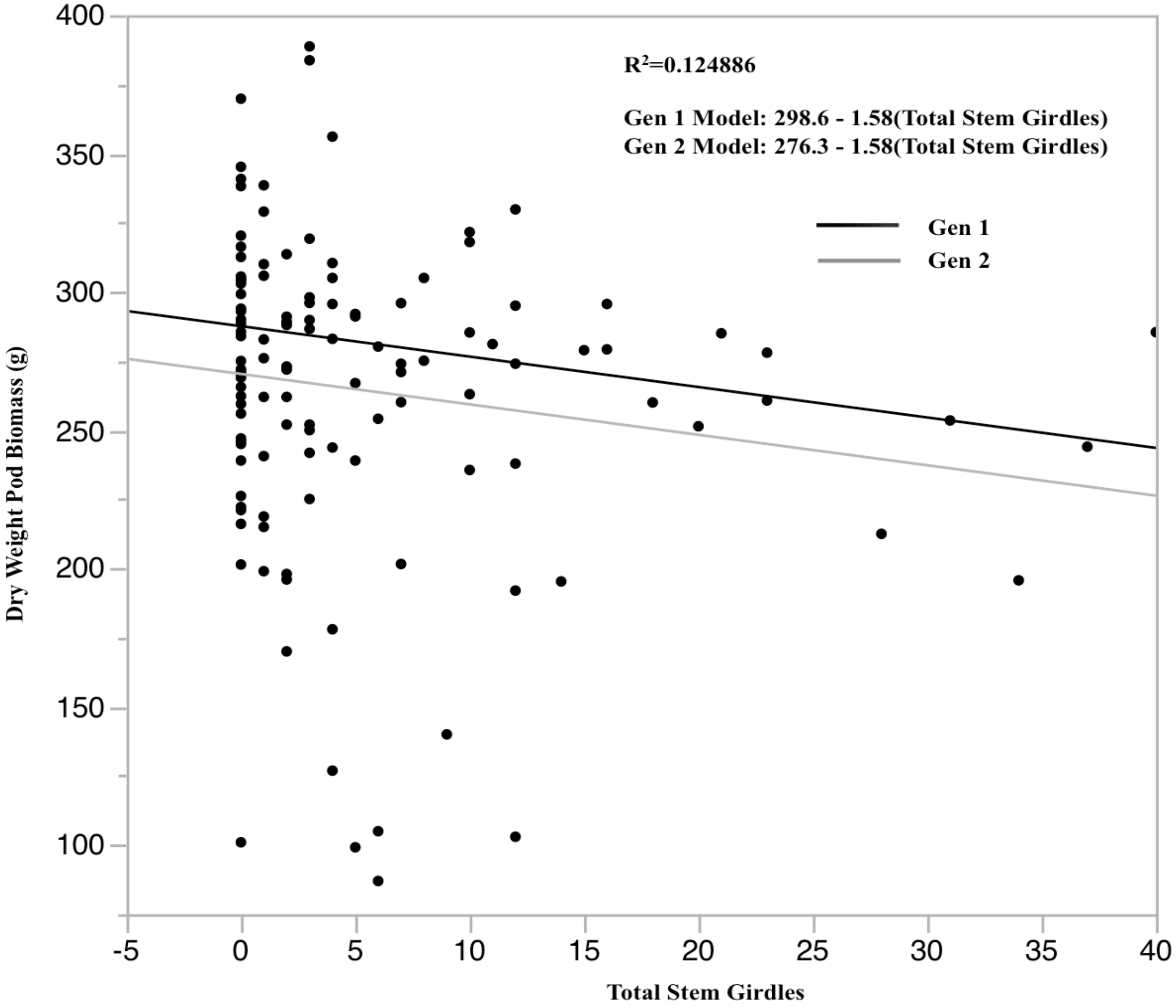

**Figure 2.10:** Relationship between total numbers of stem girdles per cage and the dry weight yield biomass of peanut for both years of Lang-Ridgon farm cage trials combined.

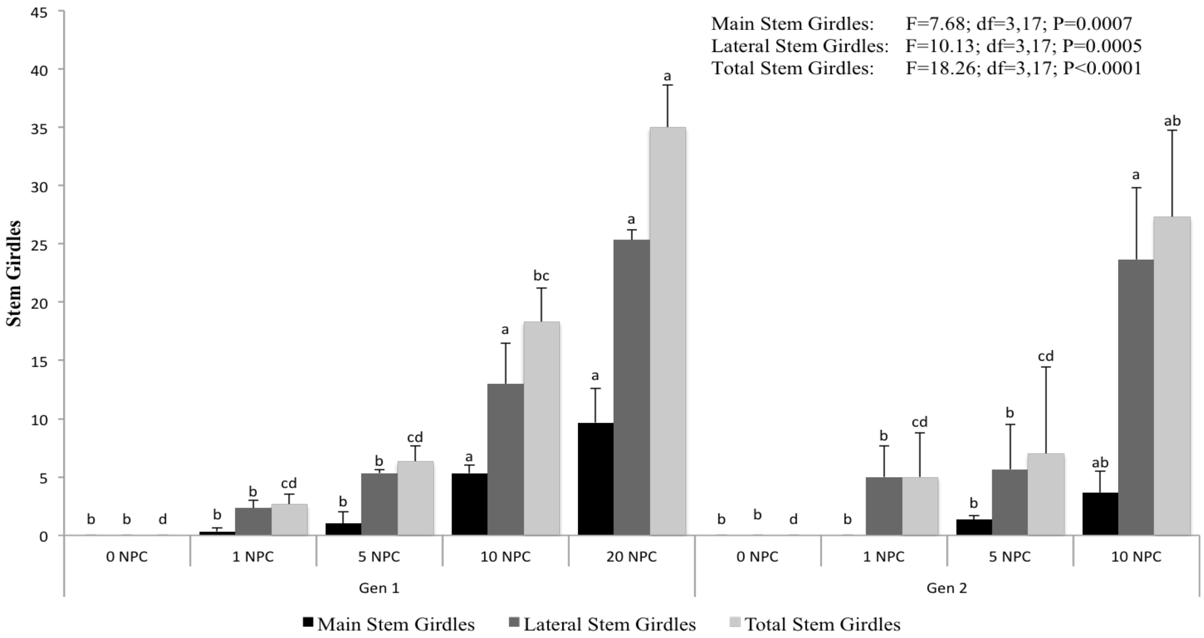

**Figure 2.11:** Mean ± SEM for Greenhouse 2015 stem girdles in each treatment ordered by generation. 20 NPC was included only in Gen 1. Different letters above bars of the same girdle type indicates significant differences between IR across infestation period (LSMEANS, P<0.05).

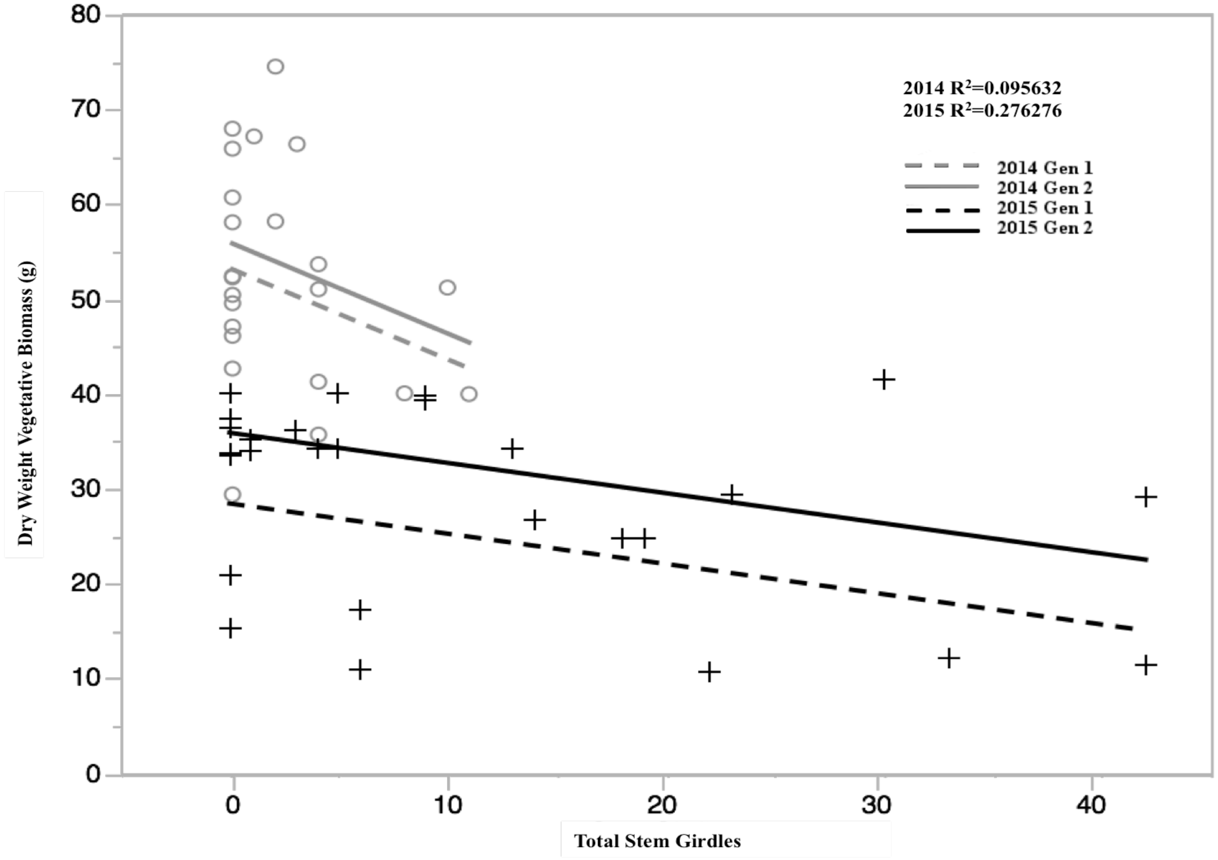

**Figure 2.12:** Relationship between total numbers of stem girdles per cage on the dry weight vegetative biomass of peanut for each year of Greenhouse cage trials.

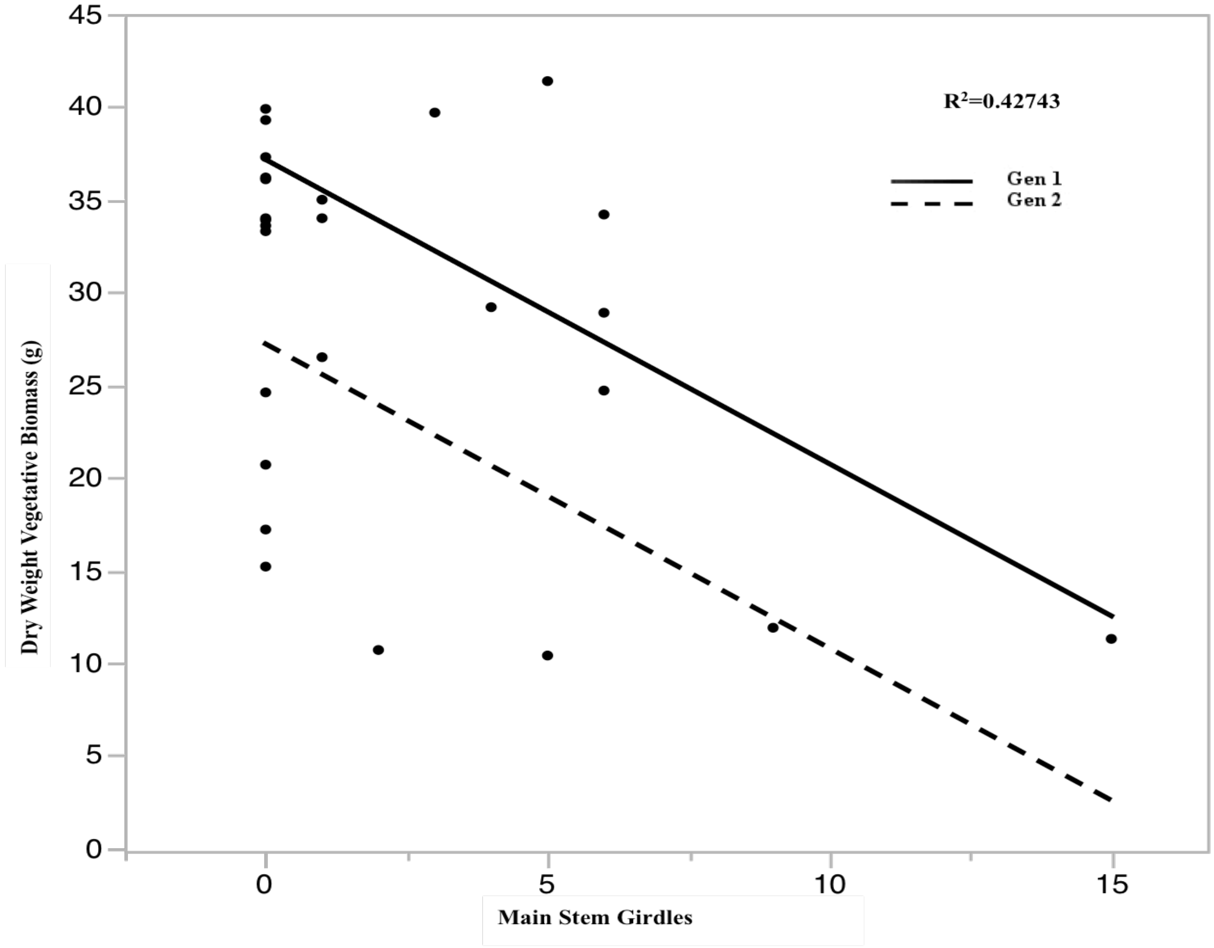

**Figure 2.13:** Relationship between main stem girdles per cage on the dry weight vegetative biomass of peanut for the 2015 Greenhouse cage trials.

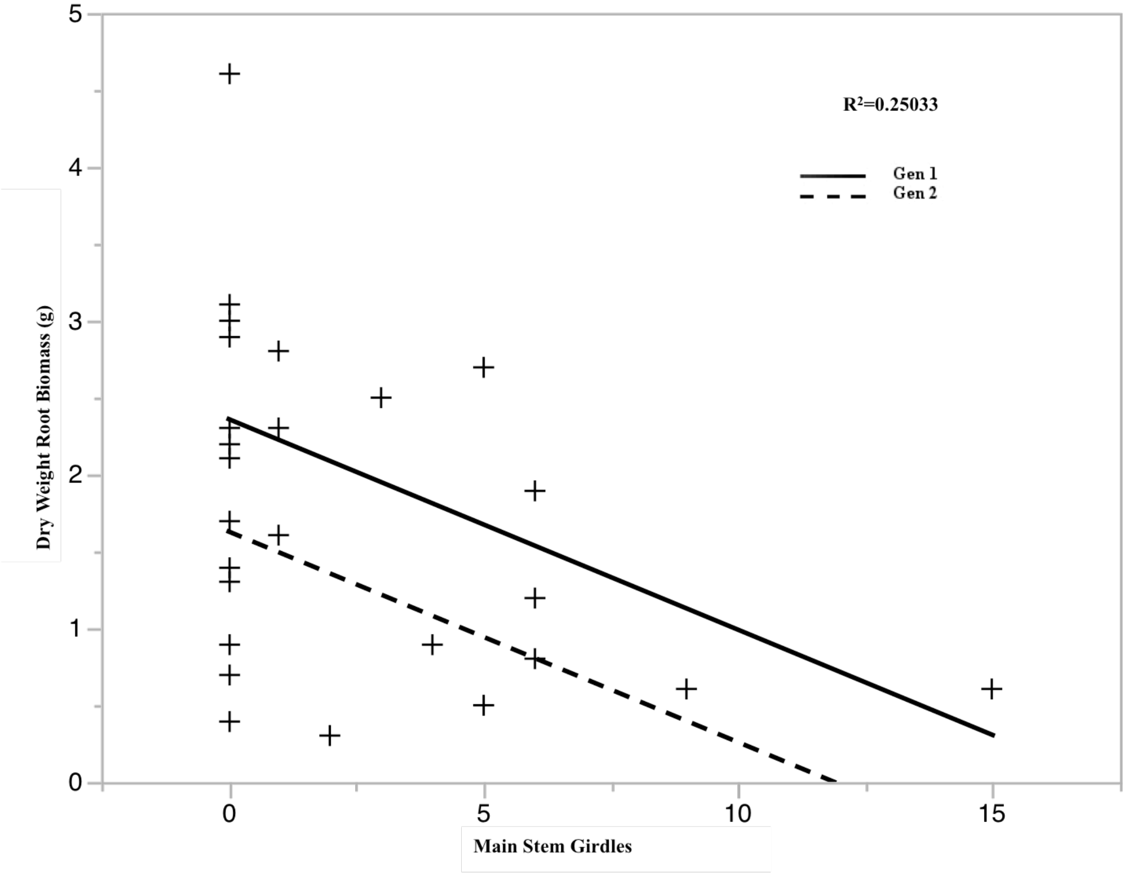

**Figure 2.14:** Relationship between total numbers of stem girdles per cage on the dry weight root biomass of peanut for each year of Greenhouse cage trials.

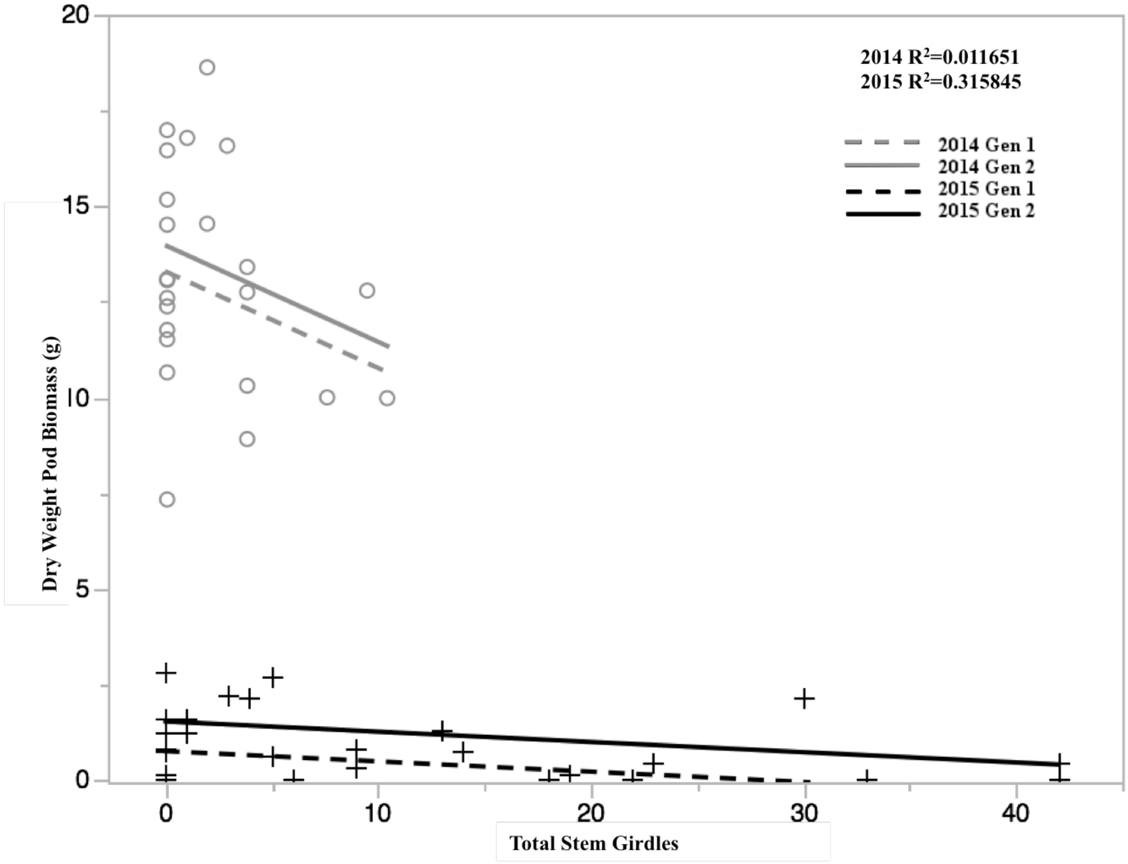

**Figure 2.15:** Relationship between total numbers of stem girdles per cage on the dry weight yield biomass of peanut for each year of Greenhouse cage trials.

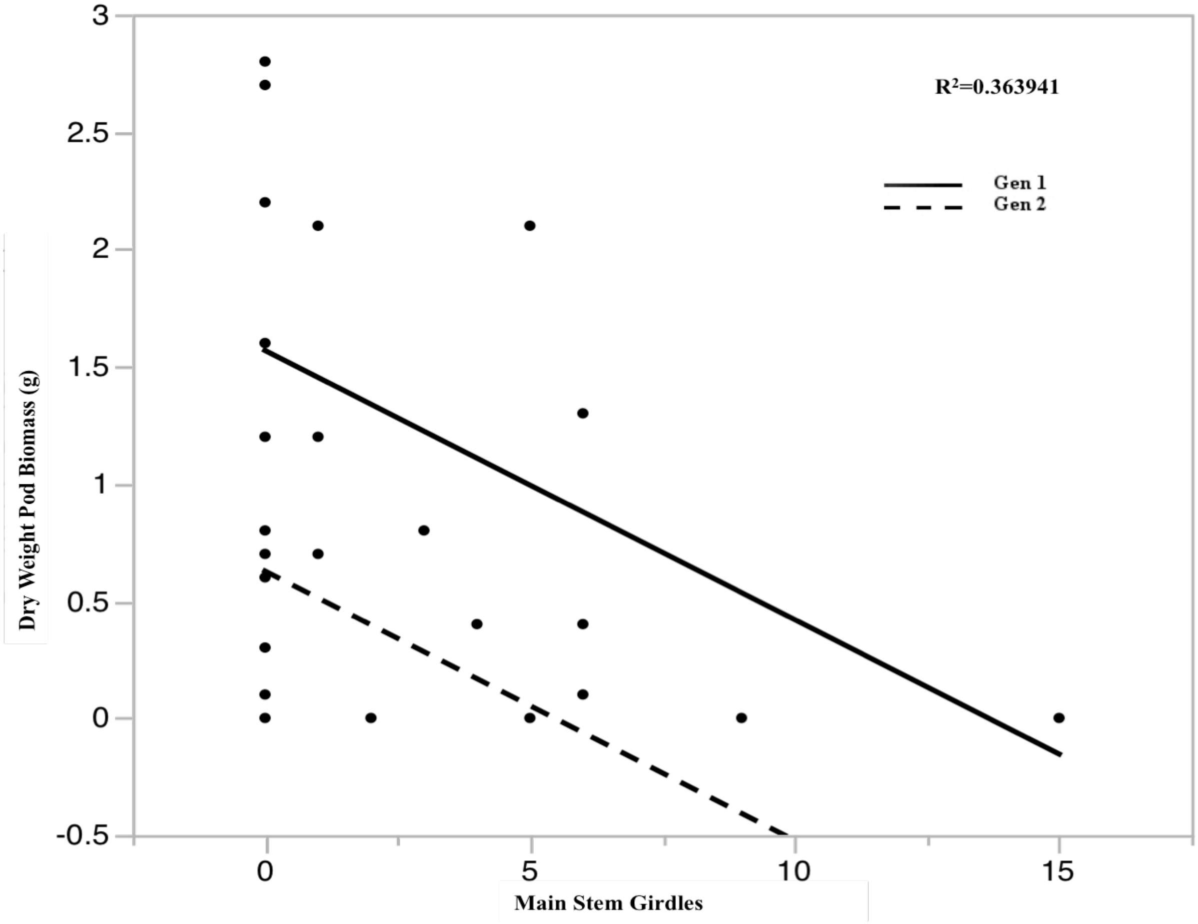

**Figure 2.16:** Relationship between main stem girdles per cage on the dry weight yield biomass of peanut for each year of Greenhouse cage trials.

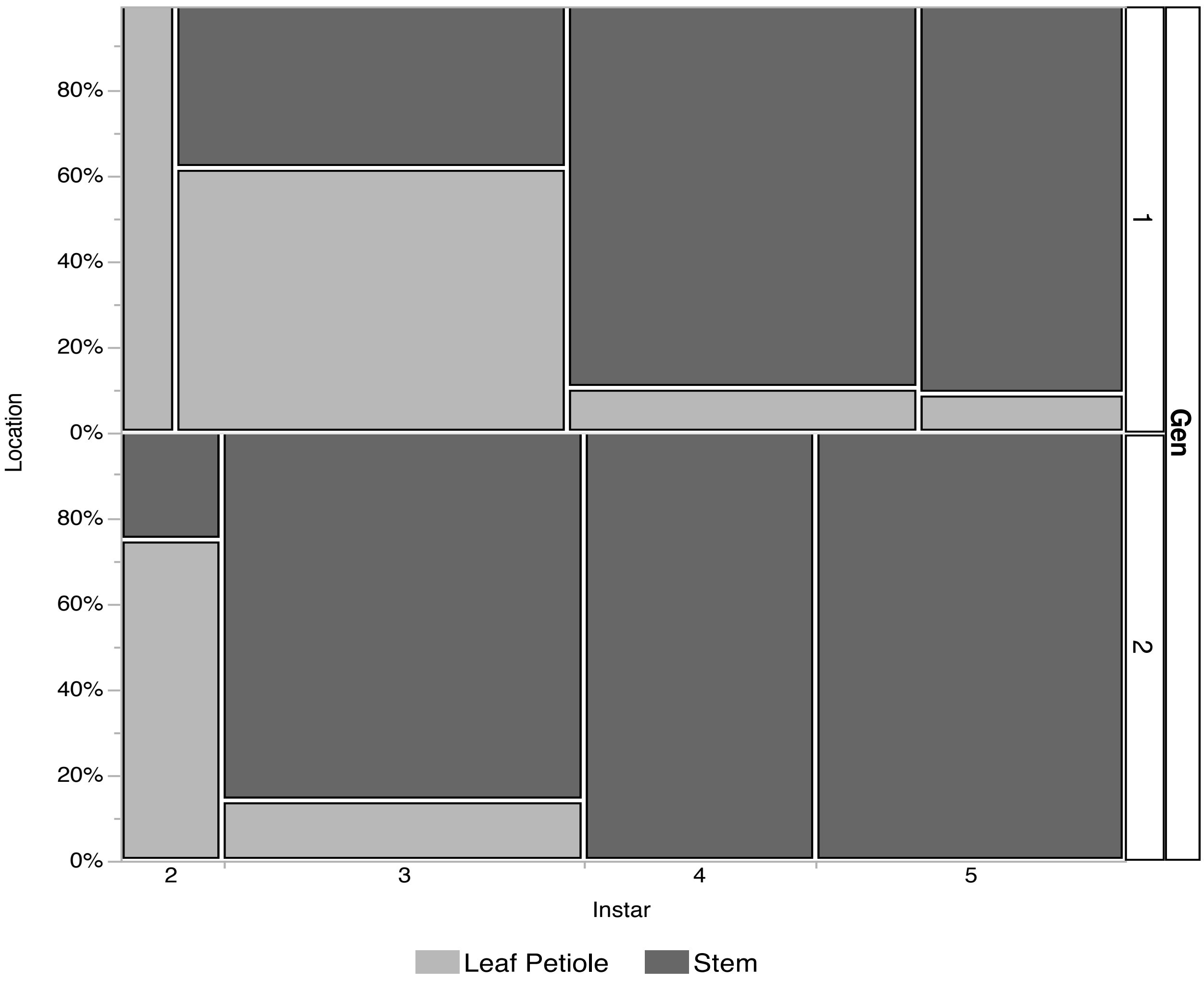

**Figure 2.17:** Mosaic plot showing the progression of location preference as instar increases, separated by generations for 2014 Greenhouse trials. Wider boxes indicate higher N observed. Instars 2 and 3 were more likely to be found on leaf petioles in Gen 1, and in Gen 2, only the 2$^{nd}$ instar was more likely to be found on leaf petioles than on stem girdles.

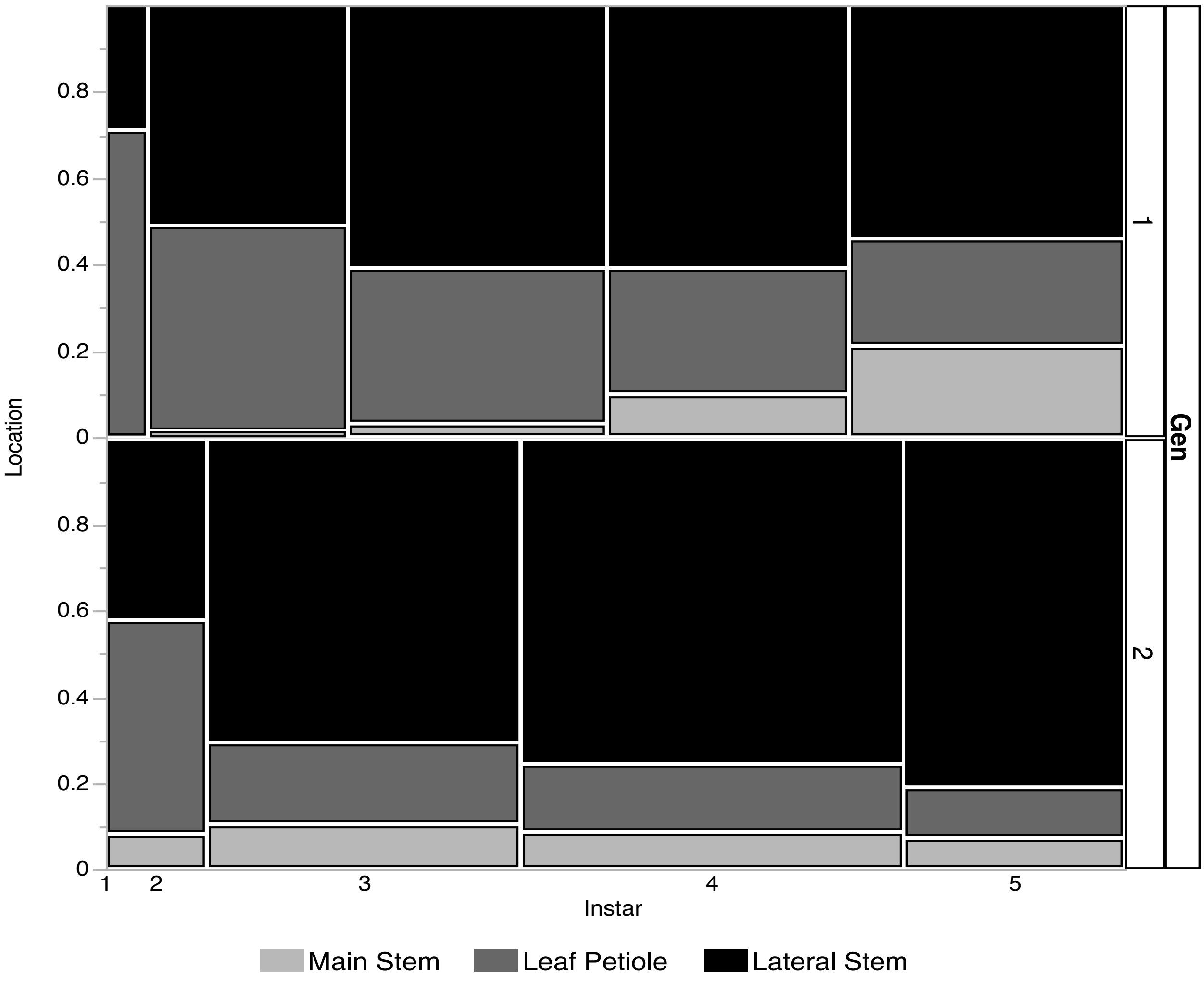

**Figure 2.18:** Mosaic plot showing the progression of location preference as instar increases, separated by generations for 2014 Greenhouse trials. Wider boxes indicate higher N observed. The location preference of nymphs transitions from leaf petioles to total stems in Gens 1 and 2. Nymphs prefer the main stem more as instar progresses in Gen 1, but no changes occur in main stem preference for Gen 2.

# CHAPTER 3

# SEASONAL PHENOLOGY OF *SPISSITILUS FESTINUS (SAY)* IN PEANUT AND A COMPARISON OF SAMPLING METHODS FOR INTEGRATED PEST MANAGEMENT

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## Abstract

Monitoring, or scouting, pest populations is one of the most crucial aspects of Integrated Pest Management (IPM) programs. Threecornered alfalfa hopper, *Spissistilus festinus* (Say), is considered a pest of peanut, though no economic injury level has been published. Likewise, a sampling program has yet to be developed for this insect in peanut. This study was designed to compare the utility of two sampling methods, sweep net and beat sheet, for use in an IPM program. Results of these sampling methods were compared to an absolute method that counted nymphs and girdles on whole plants. The adult counts from sweep net samples and nymph counts from beat sheet samples were representative of the true (absolute) nymphal population. However, only the absolute sampling method could be used to relate girdle number and nymph population. Yellow sticky traps were also installed in peanut fields to passively collect and indicate when adult numbers were high. Results indicate that sticky trap captures cannot be used to predict the number of in-field adults, but are useful to indicate presence in the field.

**Keywords:** *Spissistilus festinus*, sampling methods, seasonal phenology

## Introduction

The threecornered alfalfa hopper, *Spissistilus festinus* (Say) (Hemiptera: Membracidae), has long been considered a potential economic pest of peanut, *Arachis hypogaea* (L.) (King, 1961). In 2000, the estimated loss attributed to *S. festinus* in Georgia was $2 million (Todd, 2000). In 2006, the cost of controlling *S. festinus* was reported to be almost a half million dollars (Adams, 2006). No formal survey of *S. festinus* damage in Georgia has been conducted since 2006. Nevertheless, *S. festinus* has been attributed key pest status; a key pest is defined here one for which the first insecticide treatments are made in a field (Hollis, 2010). *Spissitilus festinus* is present annually in almost all peanut fields in Georgia, and growers commonly use insecticides to control populations. While the cost of treatment with Pyrethroids is minor (the most common form of treatment for *S. festinus*), the broad-spectrum insecticide kills beneficial insects and can flare secondary pests.

The damage caused by *S. festinus* is most visible in the form of girdles (Wildermuth, 1915). These girdles are formed by repeated stylet punctures around the circumference of the stem. These punctures leave behind insoluble salivary sheaths (Smith, 1933), which disrupt phloem tissues and interrupt the flow of nutrients in the phloem, creating a nutrient sink of photosynthates (Andersen et al. 2002). Third to fifth instar nymphs and adult *S. festinus* are capable of forming girdles, though the fourth instar have been reported to produce the most girdles (Moore 1976, Johnson, 1988). The girdles create a nutrient dam, and nymphs feed above the girdles to exploit the nutrient accumulation (Andersen et al. 2002).

The most efficient sampling method *S. festinus* depends on which life stage is targeted. *Spissistilus festinus* adults are visible in the field, as they disperse readily by flying following plant disturbance (Wildermuth, 1915). Sweep nets are effective for monitoring adults

(Kharboutli & Mack, 1993); yellow sticky traps have been shown to attract *S. festinus* adults in alfalfa and soybean when placed above the canopy (Johnson & Mueller, 1989; Johnson & Mueller 1990). Drop cloths, also known as beat sheets, are not very effective for sampling adults (Kharboutli & Mack, 1993). Nymphs are difficult to see on the plant due to small size, yellow-green color, and cryptic behaviors such as moving to the other side of the branch when disturbed (Wildermuth, 1915). Scouting for nymphs in peanut with a beat sheet is difficult, as early instar nymphs can be difficult to knock off the plant due to their small mass and aggregation at lower portions of the plant (Rahman et al. 2007). Whole plant examination for scouting purposes is too time consuming to be practical in an IPM program; it also requires good eyesight and concentration, which can lead to inconsistency in results.

An understanding of an insect's seasonal phenology can inform decisions regarding sampling initiation and frequency. S*pissistilus festinus* has multiple generations per year (Wildermuth, 1915; Mitchell & Newsom, 1984b) and has been reported to overwinter in pine stands (Newsom et al. 1983). In South Carolina peanut, two generations of *S. festinus* occur, one in mid-summer and one in late summer to early fall (Rahman et al. 2007).

Economic thresholds (ET) have been developed for *S. festinus* in soybean, *Glycine max* L. (Sparks & Newsom, 1984; Sparks & Boethel, 1987) and alfalfa, *Medicago sativa* (Wilson & Quisenberry 1987, Moellenbeck & Quisenberry, 1991), but no science-based economic injury level has been established in peanut. Research is currently focusing on determining EILs for *S. festinus* in peanut and establishing ETs (Beyer, 2015). Implementation of a successful Integrated Pest Management (IPM) strategy relies on a valid ET as well as a practical and accurate sampling protocol.

There is currently a need for a greater understanding of the population dynamics of *S. festinus* in peanut; likewise, there is a need for an accurate sampling method for the different life stages of this insect. The first objective of this study was to determine a practical sampling method that could be used in an IPM program. The second objective was to monitor seasonal phenology and damage progression of *S. festinus* adults and nymphs in peanut.

## Methods

### Field Sampling

Studies to compare sampling methods and monitor seasonal phenology of *S. festinus* were performed in peanut fields on University of Georgia and USDA research farms. Seed beds 1.8m wide were prepared with a tractor mounted 1.83m Rototiller (KMC Corp. Tifton, GA), with two rows of Georgia-06G peanuts seeded at a rate of 6 seed per foot using a tractor mounted vacuum planter (Monosem®, Edwardsville, KS). All plantings were treated with a same-day application of 0.033L/ha Valor® (Valent® © 2010), 0.017L/ha StrongArm® (DOW Agrosciences LLC © 2010), and 2.35L/ha Sonolan® (DOW Agrosciences LLC © 2014) herbicides. Fields received applications of land plaster ($CaSO_4$) at a rate of 1135kg/ha on 25 June 2014 and 17 June 2015. In 2014, fungicide applications of Headline® (BASF Corporation © 2013) at a rate of 0.675L/ha were made on 9 and 24 July and Provost (Bayer CropScience © 2007) at a rate of 0.8L/ha on 7 August, 19 August, and 2 September. In 2015, fungicide applications of Headline (BASF Corporation © 2013) at a rate of 0.68L/ha were made on 8 July, 22 July, and 5 August and Convoy (Nichino America, Inc. © 2014) at a rate of 0.95L/ha on 19 August and 2 September.

In 2014, two field measuring 6,188m$^2$ at University of Georgia Tifton Campus Lang-Rigdon Research Farm (31°31'17.8"N, 83°32'43.6"W) in Tifton, Georgia were used for sampling. The first early-planted field was planted on 7 May; the second late-planted field was planted on 2 June. Twenty different sample locations in each field were randomly selected on each sample date. Each sample location consisted of two rows 9.14m in length of peanut to execute sampling procedures. All sample locations were at least 5m from the field border. At each sample location, a sweep net sample consisting of 20 single row cross sweeps (Kogan & Herzog, 1980) of adults was taken with a 38.1cm diameter sweep net. One beat sheet sample was also taken at each sample location from an area not previously swept. Beat sheet samples were collected using a 0.92m tan nylon-weave sheet with two 1.22m wooden dowels sewn parallel to each other on opposite side of the sheet to provide structure. The edge of the beat sheet was carefully placed underneath one side of peanut plants on one row. The plants were bent over the beat sheet and vigorously shaken to dislodge the nymphs. The number and development stage of nymphs were recorded. Nymphs on the fabric were shaken back into the sample area. In each field on each sample date whole plant samples were collected along with the sweep net and beat sheet samples at 5 of the 20 locations. The locations for whole plant samples were chosen at random. A 0.92m section of plants was removed from the ground by cutting the taproot off with plant shears. Plants were submerged and agitated for 10 seconds in a 100:1 water-soap solution (Dawn Ultra; P&G, Cincinnati, OH) using; the material washed off the plants was then successively sieved through size 3, 20, and 40 meshes (6350, 841, and 420 microns). The 3 and 20 mesh sieves were examined for nymphs, where number and developmental stage were recorded. The plants were then examined for stem and leaf petiole girdles. Sampling began on 18 July, and was repeated every 6-8 days between the hours of 1300 – 1600 until plants were harvested on 18 September.

In 2015, one location at USDA Belflower Farm (31°30'22.5"N, 83°33'36.2"W) in Tifton Georgia was used for sampling. Peanuts were planted in a field measuring 44x61m (2684m$^2$) on 2 June. The field was divided into a 5x4 grid, each square of the grid was 6 rows (5.49m) of peanut wide and 9.14m long. Sweep sampling in each grid square was performed on the left row of the center 2 rows, and each sample consisted of 20 single row cross sweeps (Kogan & Herzog, 1980) of the peanut plants. The number and sex of each adult captured were recorded. One .91m beat sheet sample was taken per plot from the right row of the center 2 rows; the number and developmental stage of nymphs were recorded. Black beat sheets made from nylon fabric were used. In 2015, whole plant samples consisted of the removal of plants from 30.5cm sections of row from the right-side bed of the plot. Plants were placed in 113.45-liter trash bags; 20 30.5cm sections of plant were collected per sampling date. Plants were taken to the laboratory where they were individually examined for main stem, lateral stem, and leaf petiole girdles, as well as developmental stage and number of nymphs. Soil remaining in the bag was left to dry and was subsequently sieved and examined under 2x magnification for dislodged nymphs. Samples were collected every 6-8 days between the hours 1300 – 1600, when weather conditions were acceptable. Sampling began on June 17, and concluded on 30 September.

### Use of Yellow Sticky Traps to Monitor *S. festinus* Adults in Peanut Fields

The utility of yellow sticky traps for monitoring *S. festinus* populations was evaluated in peanut fields in 2014 and 2015. Yellow sticky traps (Great Lakes IPM, Vestaburg, MI) of size 22.9x35.6cm were placed in 8 commercial peanut fields in 2014 and 5 commercial peanut fields in 2015 in Tift County, GA. Traps were placed just above peanut canopy or at 33cm above the soil, whichever was higher. Traps were placed at distances of 0, 30.5, 61.0, and 121.9 meters into the field. Traps were oriented to face the field border at 0m, irrespective of the direction of the rows. At each trap location at 30.5, 61.0, and 121.9 meters, adults in peanuts were sampled with 5 sets of 20 single row cross sweeps (Kogan & Herzog, 1980) with a 38.1cm diameter sweep net. Trap locations at 0m were sampled with sweep nets in 2015. Sweep net sampling occurred perpendicular to the orientation of the traps, so that the sampling area for each trap did not overlap. Yellow sticky traps were removed prior to sweep net sampling on each sampling date. After sampling, traps were replaced with cleaned traps that had the adhesive (Tangle-Trap, Tanglefoot® Sticky-Coating) reapplied. Traps were cleaned by removal of all adhesive material, and traps that were damaged or discolored were replaced. The number of adults per trap was recorded. In 2015, the sex of each adult was recorded for both trap counts and sweep net samples.

## Data Analysis

Due to changes in sampling procedures, analysis between sampling methods in 2014 and 2015 varied slightly.

### *Comparison of Sampling Methods*

Comparisons of planting date, sample date, and insect numbers in 2014 were performed using PROC GENMOD with a Poisson distribution. In 2015, date and insect count were analyzed using PROC GLIMMIX. Comparisons of different sampling methods were done with GENMOD and GLIMMIX in 2014 and 2015, respectively; both analyses required the negative binomial distribution to account for overdispersion. Whenever means were found to be significantly different ($P<0.05$), they were separated using LSMEANS procedures and reported. Differences in girdle appearance was analyzed with PROC ANOVA, and means were separated with Tukey's HSD. Taylor's Power Law, $s^2 = a\bar{x}^b$ was used to measure the distribution of *S. festinus* counts in all three sampling methods. In the equation, $a$ and $b$ are the intercept and slope of the regression of log variance on log mean, respectively (Taylor, 1961). Data from both fields in 2014 were pooled, but data across years were not combined due to changes in research methods. Sample means on each sample date were used for Taylor's Power Law and Relative Variation analyses, and when a given instar was not present, that date was excluded. Adult count data collected from traps and sweep net samples adjacent to traps were analyzed with PROC CORR using Spearman's Rank Correlation Coefficient.

## Results

### Phenology of *S. festinus*

In 2014, a total of 1205 adults and 382 nymphs (260 from beat sheet, 122 from whole plant) were recorded from two different fields. In 2015, only one field was sampled; 471 adults and 662 nymphs (133 from beat sheet, 529 from whole plant) were found.

The two fields in 2014 were planted a month apart, giving some insight into effect of planting date on phenology of *S. festinus*. Girdles in the early-planted field (Fig 3.1A) were more abundant than in the late-planted field; however, at the end of the season the number of girdles in each field did not differ.

Two peaks of adult activity were observed in the early-planted field. The first occurred in late July and the second in early September. The number of adults gradually increased over the season in the late-planted field (Fig. 3.1B). Nymphs from whole plant samples in the late-planted field peaked twice, once at the end of July and again at the end of August. This was also seen in the early-planted field, though sampling began in the middle of the first peak of nymphs. Adults in the early-planted field peaked after a peak of whole plant nymphs from two weeks before. Nymph counts from beat sheets in the early field showed little response to the number of nymphs from the whole plant samples, but in the late field there were weak beat sheet nymph increases when whole plant nymphs increase.

In 2015 (Fig. 3.2), sampling began much earlier to facilitate a better understanding of the population dynamics of *S. festinus* in the early season. Stem girdles began to appear in late July, and steadily increased until early September. The number of adults increased as stem girdles increased, but adult numbers plateaued by the third week of August. By the end of September, adult numbers increased sharply. The first females (Fig 3.3) appeared in mid July, after which nymph numbers began to rise. The number of nymphs from whole plant samples peaked twice: once in mid-August and again in mid-September. No distinct peaks in nymphal activity were visible from beat sheet samples. By the end of September, adult numbers from sweep samples increased sharply, while the number of nymphs in whole plant samples decreased. Sex ratios of adults that were skewed towards males during the season approached 1:1 by the end of September.

The number of adult *S. festinus* captured on yellow sticky traps and sweep samples varied between years. 2599 and 1810 adults were found on traps and sweep samples respectively in 2014, compared to 582 adults (523 male, 29 female) trapped and 1284 adults (849 male, 398 female) swept in 2015. In both years (Fig. 3.4-5), the number of adults found on traps was highest at the end of August, though there was a smaller peak at the beginning of August. Number of adults collected in sweep samples at trap locations followed a similar trend. More adults were collected in sweep samples than on traps in 2015. By late September 2015, the number of adults collected in sweep samples increased sharply. Adults in 2014 also increased in Septmber, though not as dramatically. In 2014 and 2015, adult numbers decreased at the end of September.

**Comparisons between Adult and Nymphal Populations**

Sample data collected from UGA research farms were analyzed separately by year because of differences in sampling methodology between years.

Data from 2014 showed that adults were more common in early-planted ($\bar{x}$=2.97) peanuts than late-planted ($\bar{x}$=2.55) peanuts ($X^2$ (1, N=400)=6.88, P=0.0087) throughout the season. Nymphs from beat sheets correlated well with adults during the season, but only in the early-planted peanuts (Fig. 3.1B) ($X^2$ (1, N=20)=6.88, P=0.0087). Adult counts increased two weeks after nymphs from beat sheets increased ($X^2$ (1, N=18)=5.40, P=0.0202), regardless of field.

Nymph counts from the beat sheets in 2015 were directly related to whole plant sample counts (F=21.62; df=1,277; P>0.0001). The number of adults collected in sweep nets on a given sample date was strongly correlated with the nymphs collected on beat sheets the previous week (F=4.99; df=1,265; P=0.0263) and with whole plant nymphs on the same date (F=6.38; df=1,265; P=0.0121) and the previous week (F=11.53; df=1,265; P=0.0008).

More nymphs were found in 2015 using the whole plant methods than in 2014 using the washing sieving methods. Significantly more nymphs per whole plant sample than per beat sheet sample were found in 2015 (t=-3.06; df=130; P=0.0027). Beat sheet samples collected more first and fifth instars than all other instars in 2014 (Fig. 3.6) and more first instars than fourth in 2015 (Fig. 3.7). No differences were seen in nymphal instar counts in whole plant samples of either year.

In 2014 and 2015, the distribution parameters of Taylor's Power Law were inconsistent (Table 3.1-2), possibly due to small sample size, limited sampling locations, and lower insect numbers overall in 2015. Relative variation was lower for whole plant samples than beat sheets in both years (Table 3.3-4). Values of RV<25 was not uncommon for individual nymphal instar or total number of nymphs in whole plant samples from both years; beat sheet samples with a RV <25 were relatively scarce, except for total nymphs. Sweep samples had the greatest precision of any sampling method with consistently lower RV ranges from 7.4-20.3 in 2014 and 12.3-48.7 in 2015.

### Relationship of Girdle Injury to Insect Number

In 2014, stem girdles (Fig. 3.8) (F=3.12; df=8,72; P=0.0044) and leaf girdles (Fig 3.9) (F=4.24; df=8,72; P=0.0003) appeared earlier in the season within the early-planted field, though by the end of the season, counts equalized. Stem girdles increased one week ($X^2$ (1, N=18)=5.08, P=0.0242) and two weeks ($X^2$ (1, N=18)=3.93, P=0.0473) after an increase of nymphs from beat sheet samples. Stem girdles increased at the same time ($X^2$ (1, N=400)=28.18, p<0.0001) and two weeks after ($X^2$ (1, N=400)=18.34, p<0.0001) adult counts increased. Leaf girdles increased two weeks after whole plant nymphs increased ($X^2$ (1, N=14)=7.22, P=0.0072).

Of all sample methods in 2015, only whole plant nymphs had any direct relationship with total stem girdles (F=9.19; df=1,265; P=0.0027) and lateral stem girdles (F=8.48; df=1,265; P=0.0039). There were no other relationships in any combination of damage to nymphs or adults collected.

### Passive Yellow Traps as Predictors of In-field Adults

Yellow sticky traps provide a good indication of adult presence in peanut fields. However, the Spearman's Rank Correlation rho coefficients are quite low (2014: $\rho(123)=0.27406$, P=0.0022; 2015: $\rho(449)=0.13292$, P=0.0048) when attempting to predict the number of adults in the field based on the number of adults found on the traps.

## Discussion

### Seasonal Phenology

*Spissistilus festinus* numbers were greater in 2014 than in 2015 in all commercial and research farm locations. Sampling in 2014 started 18 July. The initial invasion of *S. festinus* adults in the early-planted field in 2014 was not observed because sampling began after migration started. In the late-planted field of 2014 and in 2015, adult numbers began to rise in late July; this pattern is similar to observations report by Rahman et al. (2007) in South Carolina. Initial infestation numbers were quite low (1-2 adults total per sample date until 29 July). Low numbers from sweep samples were possibly a result of *S. festinus*' preference to oviposit at the base of younger plants (Wildermuth, 1915; Meisch & Randolph, 1965) where the sweep net is less likely to capture females.

Male to female ratios of *S. festinus* vary during the season, with equal ratios overwintering in pine (Mitchell & Newsom 1984b). During the initial movement to vetch and clover in the early spring, there is a 3:7 male to female ratio (Newsom et al. 1983). The male-female ratios we observed in peanut from sweep samples were higher (2:1) from late July until the end of September, when the adult numbers rose and the ratio equalized to 1:1. This male-biased ratio is possibly the result of a sampling bias, as males are more prone to flight than females (Johnson & Mueller, 1990). Females may be less likely to be collected in sweep nets during oviposition when they are located low in the plant canopy. The transition to an equal ratio of males and females at the end of the season is similar to what Mitchell and Newsom (1984b) observed in soybean, where sex ratios balanced at 1:1 during the migration of *S. festinus* from soybean to pine for overwintering in Louisiana.

Thought the number of adults migrating into peanut fields might be low, each female has the capability to lay up to 6-7 eggs per day (Mitchell & Newsom 1984b; Rice & Drees, 1985), and the number of nymphs in 2015 rose rapidly within two weeks of the initial invasion of adults. The lag time of approximately 2-3 weeks between the first observation of adults and the appearance of nymphs matches the reported average egg development time of 13-16 days (Meisch & Randolph, 1965). Adults are relatively the easiest stage to sample, but these data suggest that adults are likely not the best indicator of damage. Many of the adults seen in the data collected arise from first generation of nymphs that perform the majority of damage to the plants. Therefore, adult numbers are not the best measure upon which to base an economic injury level.

In a similar finding to what Rahman et al. (2007), two nymph population peaks were observed in peanut; one occurred in late June and another in late August. These data suggest that number of nymphs can be used to forecast the appearance of girdles and adults, as nymphs in whole plant samples preceded the appearance of girdles, though not significantly. In 2014, nymphs from beat sheet samples were significantly related to girdles found one to two weeks after sampling. In both years, adult population increases were preceded by increases of nymphs a week before. The brief period between the appearance of nymphs and girdle formation means that nymphs should be monitored weekly. Yellow sticky traps are likely not reliable for use in a sampling program. Trap captures could not be used to predict numbers in the field. Traps do indicate the presence or absence of adults in the field, but adult presence is easily detected by walking in the field.

Stem girdle counts continued to increase until the beginning of September, where mean counts fluctuated from 25 to 30 stem girdles per 0.32m row until harvest. As relatively little "new" damage is formed during this time and a girdled stem's phloem resumes normal translocation activities of photosynthates after 7-11 days in peanut (Andersen et al. 2002), it can be expected that treating for *S. festinus* to deter girdles during this time would be largely ineffective. The ideal treatment timing to prevent stem girdles would target the first generation of nymphs, which was observed to occur in the last two weeks of July.

Unexpectedly, the number of stem girdles observed in this study did not increase continuously, even though nymph numbers remained high. A potential explanation for the lack of a continuous increase is that girdling and feeding has moved from stems to leaf petioles. Mitchell and Newsom observed this phenomenon in soybean (1984a). It is also possible that feeding moved to the gynophore, or the "peg" which connects the peanut pods to the plant; this type of feeding was not recorded during this study. Stem girdles are the most dependable metric of assessing *S. festinus* damage, but because the appearance of new girdles seems to stagnate in the later part of the season, stem girdles may not be the best metric to measure *S. festinus* impact.

There were higher adult counts and damage in early-planted peanuts than in late planted peanuts, despite the fact that the two fields sampled were within 10 meters of each other. By the end of the season, adult and girdle counts reached similar levels in both fields, indicating that planting date may have an effect early in *S. festinus* presence and damage but this effect can no longer be seen in the season. It is unknown whether very early season damage has a different effect than damage later in the season, but in soybean, the major concern of early season damage is lodging from main stem girdles (Mueller & Dumas, 1975; Mueller & Jones, 1983); lodging is an issue of relatively little importance in peanut due to the plant's growth habit (Andersen et al, 2002).

## Comparisons of sampling methods

The high variation in the girdle counts over time in 2014 is most likely due to relatively low efficiency of nymph recovery through the plant washing method. The reason for such small sample sizes is the sheer volume of plant material that has to be sifted for each sample of 0.92m (12-16 plants per sample). The change from plant-washing only five samples per field of 0.92m of row to plant-bagging 20 samples of 0.31m of row significantly reduced variability between samples. The initial idea of using a detergent wash for the plants was to separate the nymphs from the plant, and sieve the nymphs out of solution for identification under a microscope. It is likely that plant washing was ineffective at removing nymphs from the plant material, and the combination soil and nymphs made microscope identification difficult. By bagging entire plants, nymphs were much more likely to survive, making detection of the nymphs much easier.

The difference between plant washing and bagging was also apparent in the detection of girdles, as smaller girdles were easier to see with relatively dry plants. However, as expected of an absolute sample, even the process of bagging, plant examinations, and then sifting through soil required about a single man-hour for each sample. Beat sheets are a much more time-efficient sampling approach requiring only a few minutes per sample. Although the accuracy of the sample suffers somewhat, these data indicate that using a beat sheet sample for estimation of the true nymph population is acceptable. The entire plant is not effectively sampled using the beat sheet method; areas that might be missed are those near the base of the plant where nymphs were often found to aggregate in greenhouse experiments (Beyer, unpub.). While the base of the plant could be examined for the presence of nymphs, the small size, cryptic coloration, and avoidance behaviors of *S. festinus* combined with the many stems and leaves of the peanut plant make such a search impractical for routine IPM scouting.

The effect of sample size can affect the results of analyses with Taylor's Power Law. The size of the sample area of these studies was relatively small. Spurgeon and Mueller (1991a) found that in soybean, *S. festinus* nymphs had a mostly clumped distribution when sampling in 0.8 hectare plots, though the 0.2 hectare quadrants that made up each plot had results that suggested a random distribution. The data presented here were not designed for analysis of Taylor's Power Law, and sample areas were much smaller at $16m^2$. The majority of the different nymphal instars had random distributions, with some instars having a regular distribution.

The size of the sample area may also affect Relative Variation (RV) estimates of sampling methods. RV estimates the reliability or precision of a population estimate, and not necessarily the precision of the sampling method. RV values are considered sufficient for a sampling program when RV values are less than 25, though an RV value less than 10 is preferred (Southwood, 1978). In this study, few beat sheet samples resulted in RV values <25. Likewise, few whole plant samples result in RV values <25. The expected distribution of *S. festinus* nymphs in soybean is clumped (Spurgeon & Mueller, 1991) and a high RV value is not unexpected, as the odds of getting consistent data are smaller when populations are not consistent over the sample area. However, the RV values for sweep samples in peanut in this study were consistently low; at least 78% of samples possessed RV values <25.

## Conclusions

The sweep net was a reliable and efficient method for sampling *S. festinus* adults in peanut in this study. The lag time between adult increases and girdle damage increases are too short to facilitate insecticide treatment that would prevent damage. Due to the inability of beat sheet samples to consistently and accurately predict nymphal population, many samples would be needed to ensure detection of the first generation nymphs. In spite of shortcomings, beat sheet sampling method provides the best method to detect and treat damaging populations before damage occurs.

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**Table 3.1:** Parameters of Taylor's power law for the beat sheet, whole plant, and sweep sampling methods in 2014.

| Method[1] | Instar | n | a | b(±SE) | P(b=1) | $r^2$ | Distribution Type |
|---|---|---|---|---|---|---|---|
| Beat Sheet | 1 | 10 | 0.9587 | 1.3723±0.1001 | 0.0059 | 0.9591 | Clumped |
| | 2 | 9 | 1.7246 | 1.6874±0.2645 | 0.0355 | 0.8533 | Clumped |
| | 3 | 9 | 0.4122 | 1.1476±0.1012 | 0.1881 | 0.9484 | Random |
| | 4 | 8 | 0.2196 | 1.0556±0.1756 | 0.7626 | 0.8572 | Random |
| | 5 | 9 | 0.3491 | 1.1606±0.1282 | 0.2503 | 0.9213 | Random |
| | Total | 10 | -0.0394 | 1.4390±0.3106 | 0.1952 | 0.7285 | Random |
| Whole Plant | 1 | 8 | -1.0894 | 0.5834±0.1018 | 0.0064 | 0.8455 | Regular |
| | 2 | 7 | -0.8574 | 0.6679±0.1317 | 0.0531 | 0.8372 | Random |
| | 3 | 8 | -1.4047 | 0.4792±0.1368 | 0.0089 | 0.6718 | Regular |
| | 4 | 5 | -1.3257 | 0.5937±0.1657 | 0.0915 | 0.8106 | Random |
| | 5 | 6 | -1.8644 | 0.2591±0.1105 | 0.0790 | 0.5788 | Random |
| | Total | 9 | 0.1270 | 0.3335±0.1086 | 0.0005 | 0.5742 | Clumped |
| Sweep | Total | 10 | 0.1753 | 1.1722±0.3494 | 0.6354 | 0.5845 | Random |

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[1] Beat sheets and whole plant samples refer to nymphal instars and total number of nymphs. Sweep samples refer to adults.

**Table 3.2:** Parameters of Taylor's power law for the beat sheet, whole plant, and sweep sampling methods 2015.

| Method[1] | Instar | n | a | b(±SE) | P(b=1) | $r^2$ | Distribution Type |
|---|---|---|---|---|---|---|---|
| Beat Sheet | 1 | 8 | -0.0701 | 0.9753±0.0620 | 0.7040 | 0.9764 | Random |
| | 2 | 10 | 0.0174 | 1.0206±0.0379 | 0.6010 | 0.9891 | Random |
| | 3 | 9 | -0.4509 | 0.8323±0.0241 | 0.0002 | 0.9942 | Regular |
| | 4 | 7 | -0.2720 | 0.9083±0.0074 | <0.0001 | 0.9997 | Regular |
| | 5 | 8 | 0.1347 | 1.0290±0.2000 | 0.8895 | 0.8152 | Random |
| | Total | 14 | 0.0261 | 0.8660±0.0668 | 0.0681 | 0.9333 | Random |
| Whole Plant | 1 | 10 | 1.3040 | 1.7278±0.2417 | 0.0168 | 0.8647 | Clumped |
| | 2 | 11 | 0.1625 | 1.0431±0.0869 | 0.6323 | 0.9412 | Random |
| | 3 | 11 | 0.0662 | 0.9502±0.1659 | 0.7710 | 0.7847 | Random |
| | 4 | 10 | 0.1118 | 1.0432±0.0557 | 0.4605 | 0.9777 | Random |
| | 5 | 11 | 0.2610 | 0.9971±0.1394 | 0.9836 | 0.8503 | Random |
| | Total | 14 | 0.1446 | 1.8398±0.4963 | 0.1164 | 0.5339 | Random |
| Sweep | Male | 14 | 0.0064 | 1.0970±0.1523 | 0.5369 | 0.8122 | Random |
| | Female | 14 | 0.1385 | 0.5143±0.0744 | <0.0001 | 0.7990 | Regular |
| | Total | 14 | 0.4209 | 0.7357±0.2179 | 0.2485 | 0.4445 | Random |

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[1] Beat sheets and whole plant samples refer to nymphal instars and total number of nymphs. Sweep samples refer to adults.

**Table 3.3:** Relative Variation (RV) values for the beat sheet, whole plant, and sweep sampling methods in 2014.

| Method[1] | Instar | n | RV range | no.RV< 25 |
|---|---|---|---|---|
| Beat Sheet | 1 | 10 | 32.0-98.7 | 0 |
| | 2 | 9 | 48.0-64.2 | 0 |
| | 3 | 9 | 42.4-100 | 0 |
| | 4 | 8 | 42.4-100 | 0 |
| | 5 | 9 | 12.1-100 | 1 |
| | Total | 10 | 15.6-76.8 | 7 |
| Whole Plant | 1 | 8 | 12.7-100 | 5 |
| | 2 | 7 | 17.3-100 | 2 |
| | 3 | 8 | 15.9-100 | 2 |
| | 4 | 5 | 17.3-100 | 1 |
| | 5 | 5 | 12.1-100 | 4 |
| | Total | 9 | 8.2-100 | 7 |
| Sweep | Total | 10 | 7.4-20.3 | 10 |

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[1] Beat sheets and whole plant samples refer to nymphal instars and total number of nymphs. Sweep samples refer to adults.

**Table 3.3:** Relative Variation (RV) values for the beat sheet, whole plant, and sweep sampling methods in 2015.

| Method[1] | Instar | n | RV range | no.RV< 25 |
|---|---|---|---|---|
| Beat Sheet | 1 | 8 | 30.8-100 | 0 |
| | 2 | 10 | 42.6-100 | 0 |
| | 3 | 9 | 35.0-100 | 0 |
| | 4 | 7 | 54.6-100 | 0 |
| | 5 | 8 | 49.2-100 | 0 |
| | Total | 12 | 18.2-100 | 4 |
| Whole Plant | 1 | 10 | 35.8-70.3 | 0 |
| | 2 | 11 | 23.5-100 | 1 |
| | 3 | 11 | 20.9-58.5 | 4 |
| | 4 | 10 | 22.9-100 | 1 |
| | 5 | 11 | 18.2-100 | 2 |
| | Total | 12 | 14.3-100 | 8 |
| Adults | Female | 12 | 10.2-33.7 | 10 |
| | Male | 14 | 14.1-36.1 | 10 |
| | Total | 14 | 12.3-48.4 | 11 |

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[1] Beat sheets and whole plant samples refer to nymphal instars and total number of nymphs. Sweep samples refer to adults.

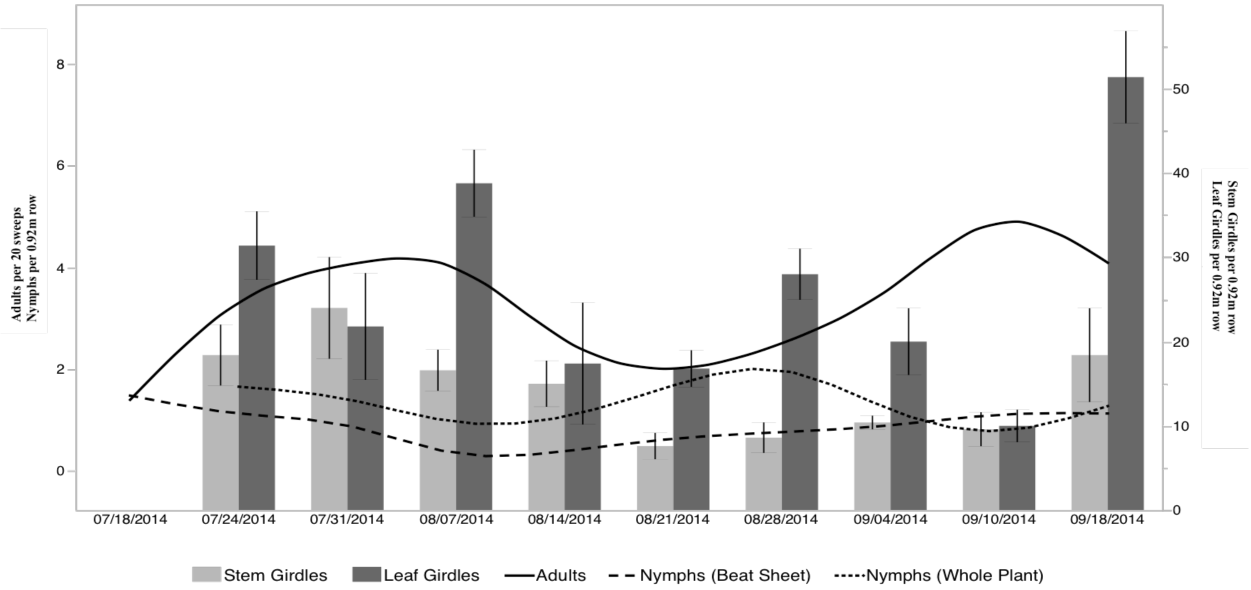

**Figure 3.1A:** Change over time of *S. festinus* adult and nymph means, in context of the means ± SEM of stem and leaf girdles in the "Early" field at Lang-Rigdon farm 2014.

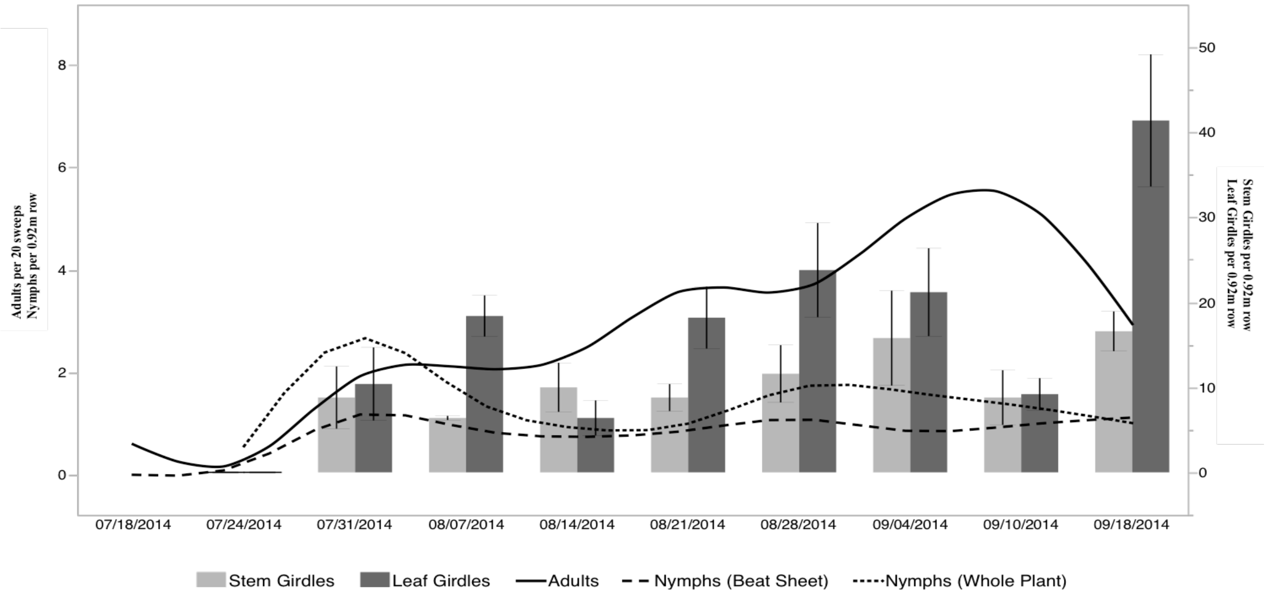

**Figure 3.1B:** Change over time of *S. festinus* adult and nymph means, in context of the means ± SEM of stem and leaf girdles in the "Late" field at Lang-Rigdon farm 2014.

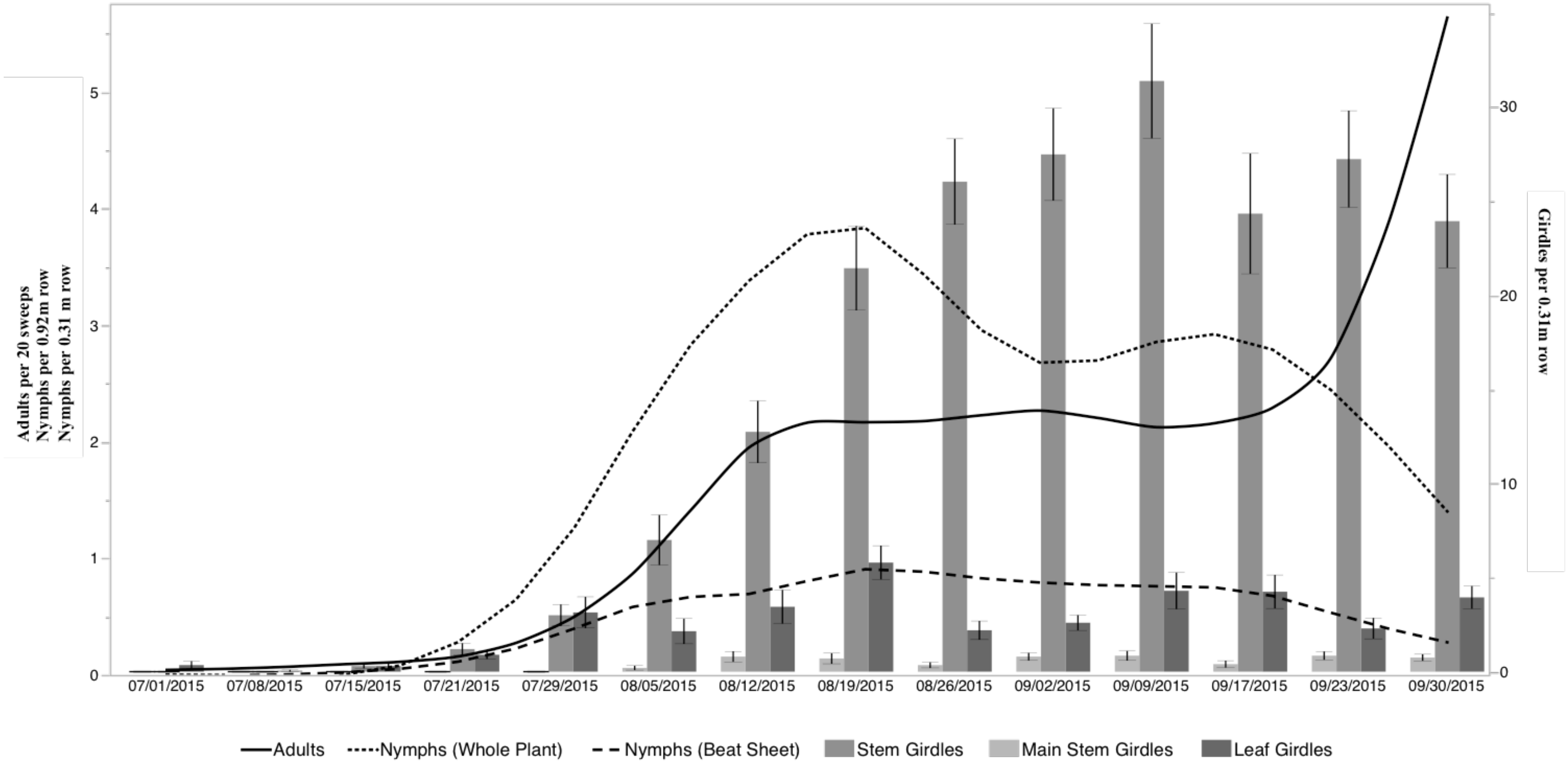

**Figure 3.2:** Change over time of mean *S. festinus* adults and nymphs in context of the means ± SEM of lateral stem, main stem, and leaf girdles from Lang-Rigdon field in 2015.

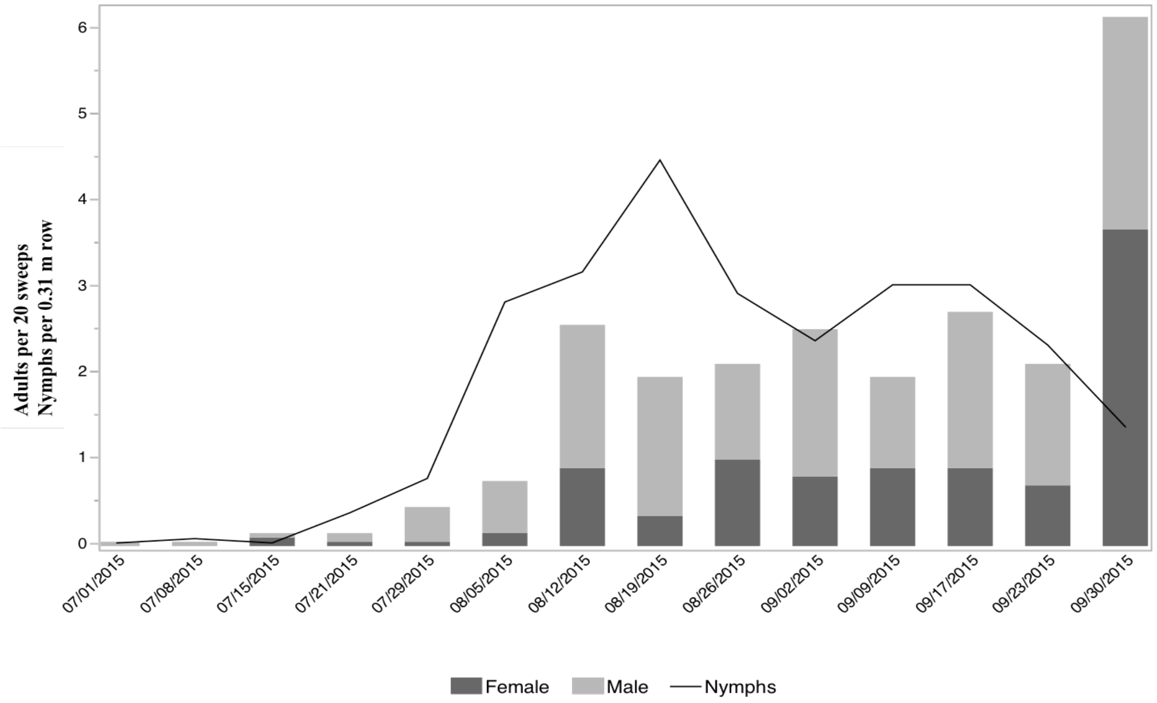

**Figure 3.3:** Comparisons of male and female *S. festinus* means over time, with nymphs from whole plant samples superimposed.

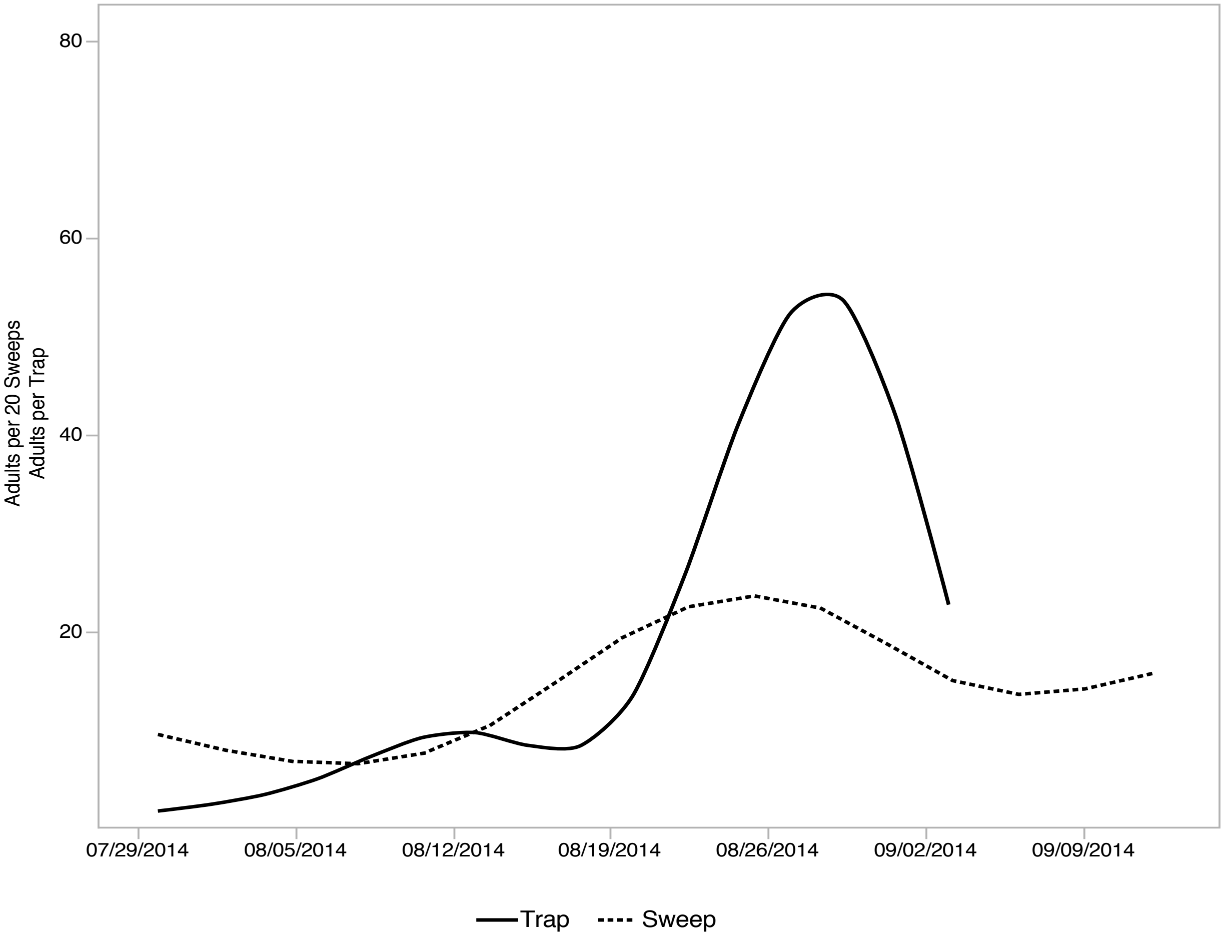

**Figure 3.4:** Population flux of the mean number of *S. festinus* adults from sweep samples and yellow sticky traps from peanut fields in Tift County, GA in 2014.

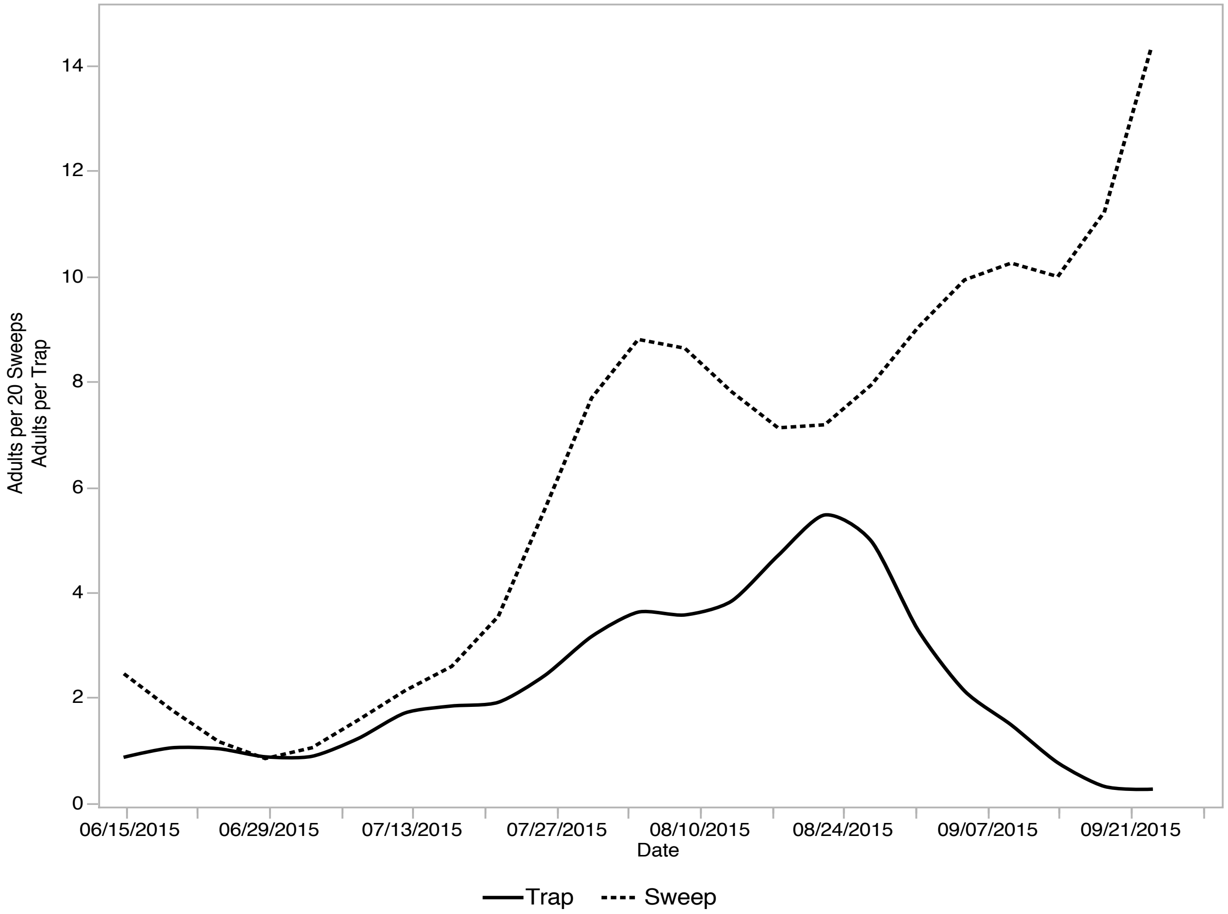

**Figure 3.5:** Population flux of the mean number of *S. festinus* adults from sweep samples and yellow sticky traps from peanut fields in Tift County, GA in 2014.

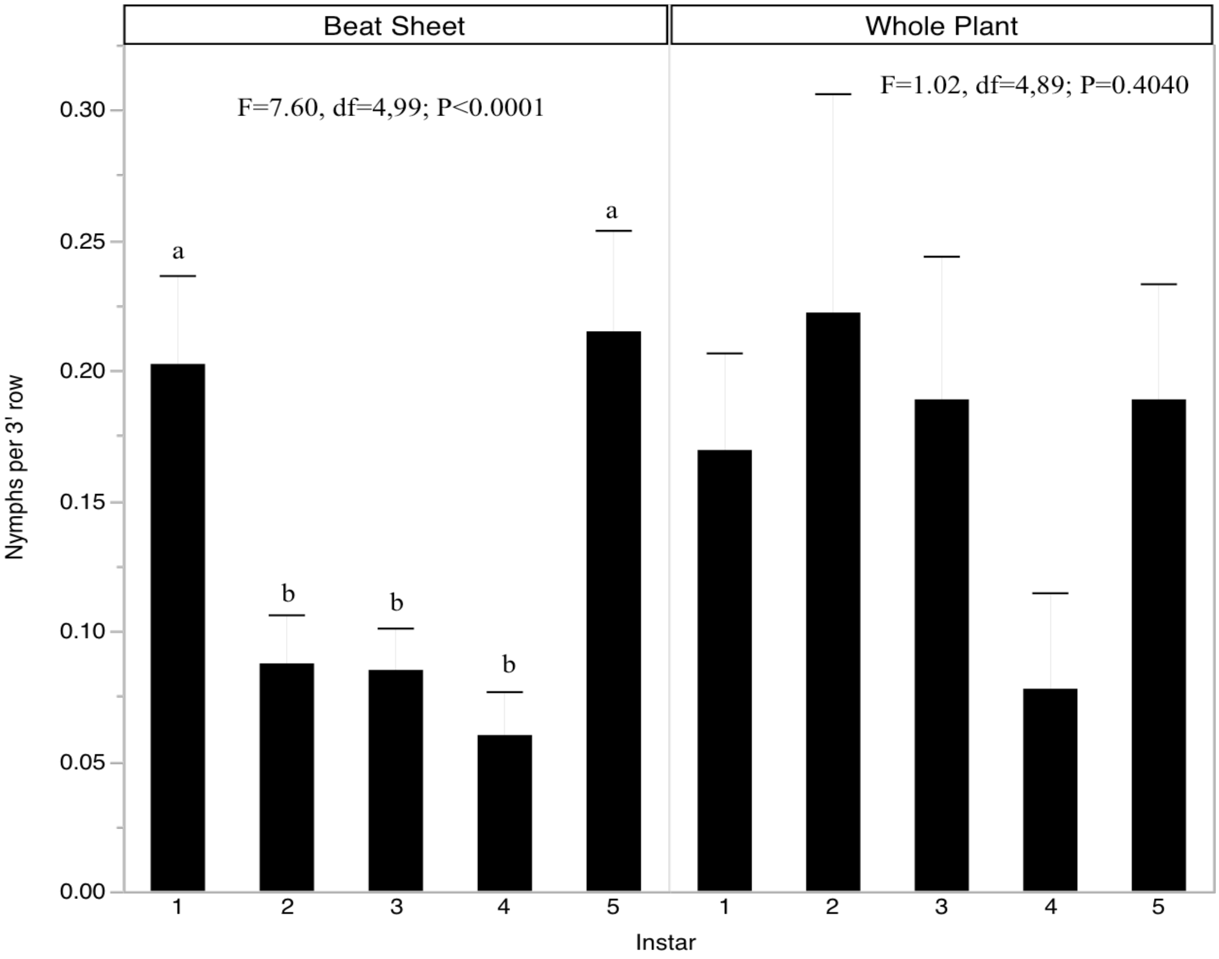

**Figure 3.6:** Means ± SEM of nymphal instars found per sampling method. Means within sampling method with different letters are significantly different from the others.

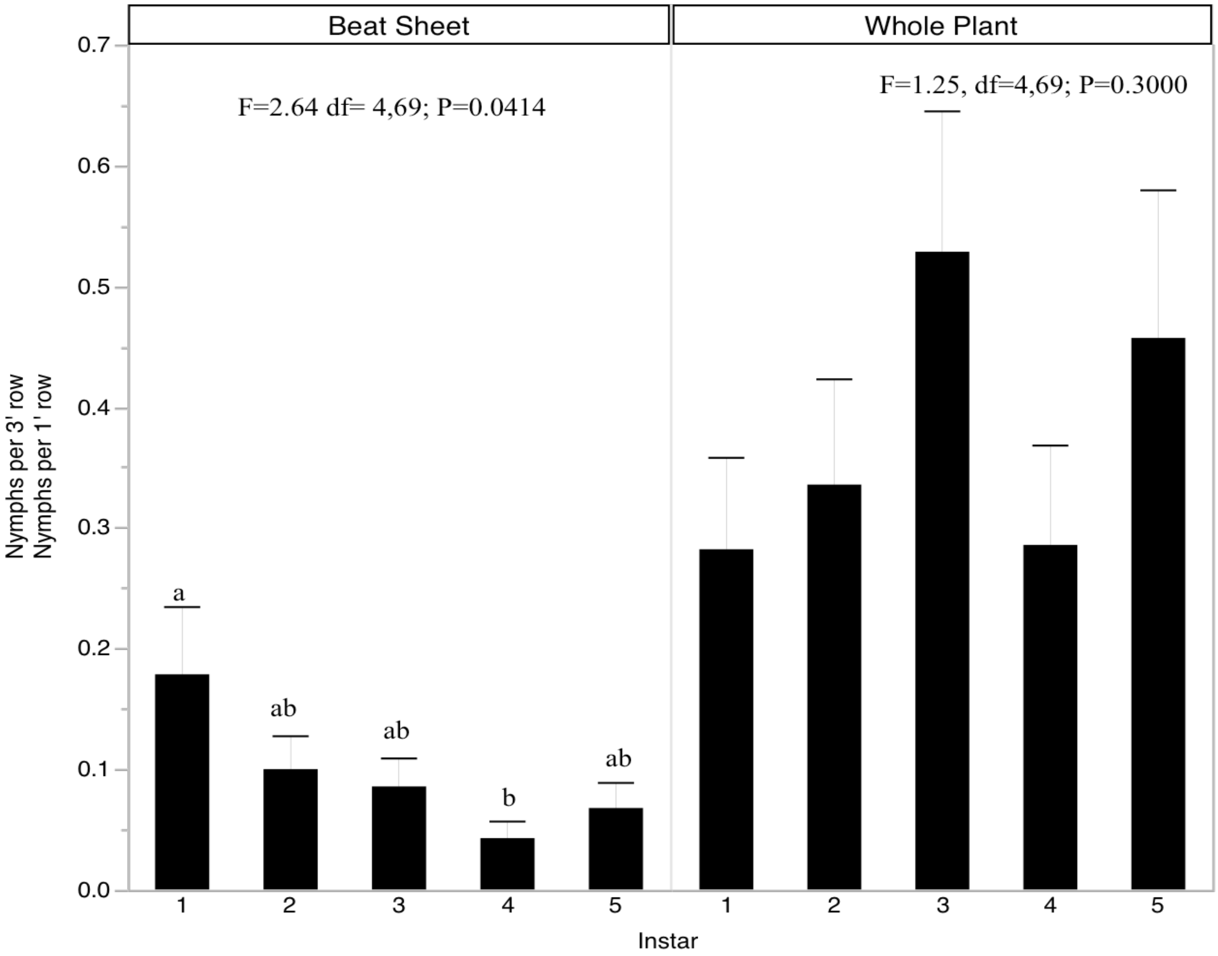

**Figure 3.7:** Means ± SEM of nymphal instars found per sampling method. Means within sampling method with different letters are significantly different from the others.

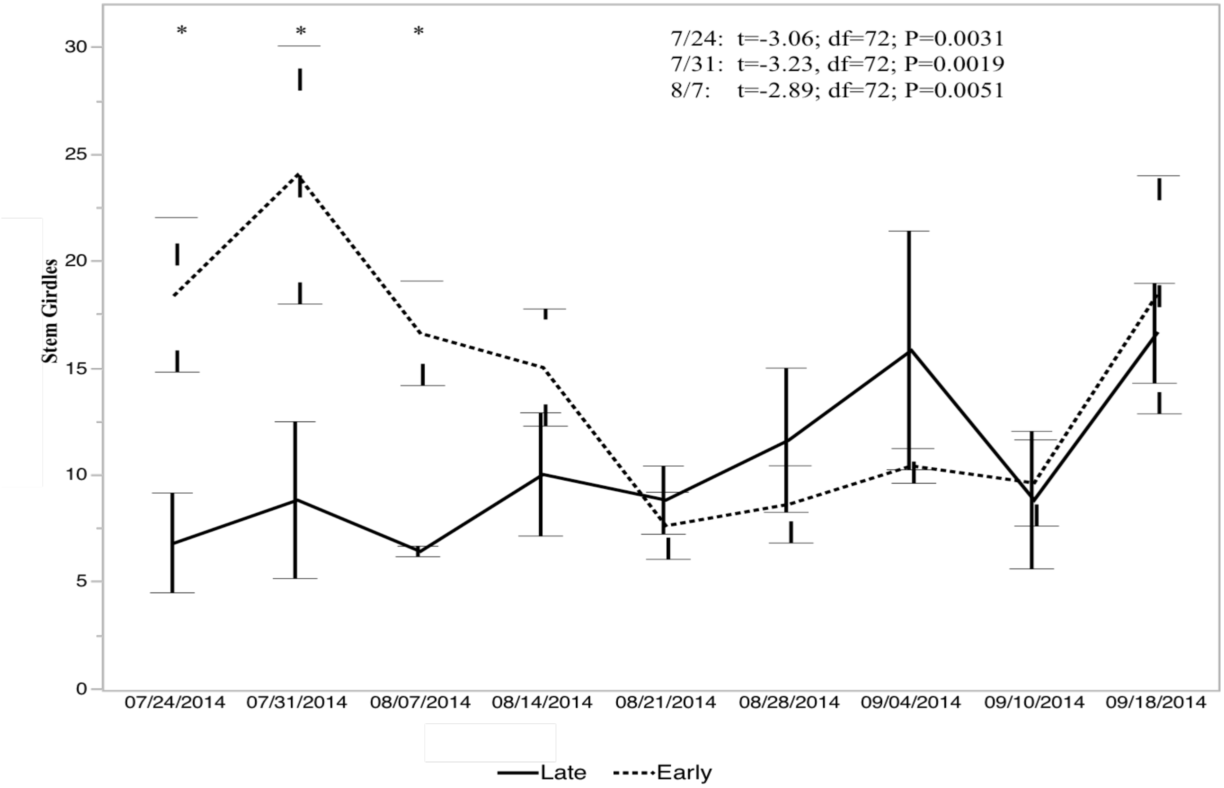

**Figure 3.8:** Means ± SEM of stem girdles between Late and Early fields in 2014. Dates with an * above indicate a significant different between the two field ages.

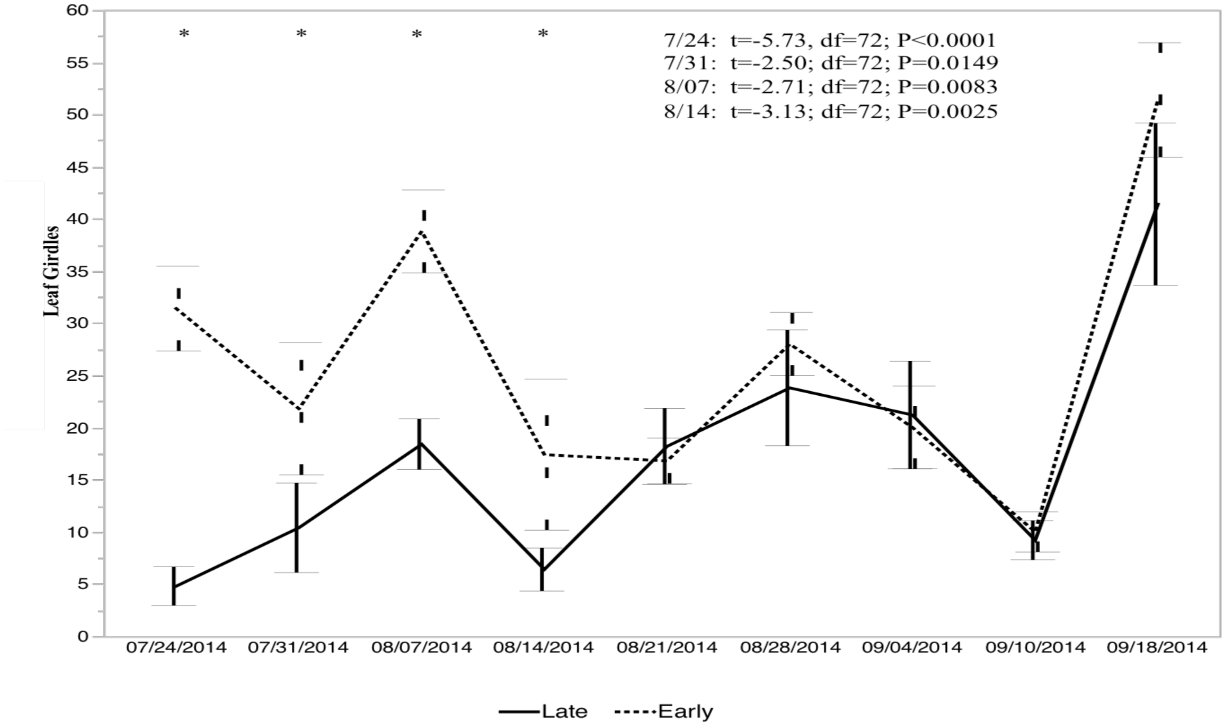

**Figure 3.9:** Means ± SEM of leaf girdles between Late and Early fields in 2014. Dates with an * above indicate a significant different between the two field ages.

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# CHAPTER 4

# CONCLUSIONS

*Spissistilus festinus* is currently treated as a pest of peanut in the southeastern United States, though there are no established Economic Thresholds and its pest status has not been quantified. *Spissitilus festinus* has historically been considered an economic pest of soybean and alfalfa. Its pest status in soybean is attributed to yield loss resulting not from direct inhibition of phloem flow, but lodging or premature leaf drop. Its pest status in alfalfa results from reduced forage quality of the hay, lowering the value of the product. Neither of these issues are concerns in peanut. Peanut is not susceptible to lodging due to its relatively prostrate growth habit and presence of many lateral stems. Likewise, the registration restrictions associated with many of the pest management chemicals used in peanut renders most peanut hay unsalable for forage.

The data presented here regarding the ability of *S. festinus* to affect peanut yield suggests that the insect is not or is only rarely an economically important pest of peanut. A significant negative correlation between damage and yield loss was observed, but only a small portion of the variation in yield was explained by the model. Our data suggest that damage occurring in the later part of the season, during pod set and maturation, had more impact on yield and dry weight vegetative biomass than damage that occurred earlier in the season.

The seasonal abundance of *S. festinus* in peanut depends on the availability of the crop. The majority of girdle damage occurred in late August for peanut seeded in late May. Monitoring adult numbers to predict girdle damage involves a very small period where treatment would be effective to prevent damage. Monitoring nymphs in peanut requires multiple beat sheet samples to accurately predict nymphal populations. Despite this, monitoring nymphs with the beat sheet sampling method provides the best predictor of future injury.

In conclusion, this research shows that *Spissitilus festinus* has the potential to reduce yield in peanut. The studies performed were limited to one cultivar; *S. festinus* may have more or less impact in other peanut cultivars. However, the preliminary EIL calculated from these data estimates that the damage levels seen commonly in peanut fields are not negatively impacting peanut. Nevertheless, targeting the first generation of nymphs as monitored by beat sheet sampling would provide the best control.