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Re-evaluation of Late Season Action Thresholds and the Effect of Seed Treatments on Early Season Colonization of Threecornered Alfalfa Hopper, *Spissistilus festinus* (Say), in Louisiana Soybean, *Glycine max* (L) Merr

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RE-EVALUATION OF LATE SEASON ACTION THRESHOLDS AND THE
EFFECT OF SEED TREATMENTS ON EARLY SEASON COLONIZATION
OF THREECORNERED ALFALFA HOPPER, *SPISSISTILUS FESTINUS* (SAY),
IN LOUISIANA SOYBEAN, *GLYCINE MAX* (L) MERR

A Thesis

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ABSTRACT

Soybean, *Glycine max* (L.) Merr, is considered the most economically important oil and feed crop in the US and is grown on nearly 35,000,000 ha on an annual basis. Factors that limit yield include weed pressure, disease infection, and of particular interest to entomologists, insect herbivory. Soybean is targeted by a range of above and below ground pests which are primarily controlled by insecticides. Neonicotinoids are a recently popularized insecticide class that when applied as a seed treatment, can provide protection from early season pests. The most economically significant early season pest is threecornered alfalfa hopper, *Spissistilus festinus* (Say), whose feeding habit causes the formation of necrotic tissue known as a girdle. To understand the effect of neonicotinoid seed treatments (NSTs) on *S. festinus*, one greenhouse and two field studies were conducted from 2020–2021. An additional study was done to re-evaluate the Louisiana state recommended late-season action threshold (1 adult/sweep) over the same period. Hand-treated neonicotinoid seed treatments were found to significantly reduce survivorship of *S. festinus* infested on soybean under greenhouse conditions. However, when the same seed treatments were taken to the field level, NSTs did not significantly impact *S. festinus* cumulative insect days (CID). In additional field trials using Maturity Group (MG) IV and V soybean varieties commercially treated with different insecticide + fungicide seed treatments, only MGIV varieties significantly impacted *S. festinus* CID. Threshold re-evaluation field trials found no relationship between *S. festinus* CID and yield parameters (total seed and hundred seed weight (g), per five plants). Results of greenhouse and field trials suggest NSTs do not provide consistent control of *S. festinus*. Additionally, no evidence was found to change the Louisiana state recommended action threshold. It was concluded that rather than rely on NSTs, *S. festinus* is best managed according to traditional integrated pest management practices.

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CHAPTER 1. INTRODUCTION AND LITERATURE REVIEW

1.1. SOYBEAN

Soybean, *Glycine max* (L.) Merr, is one of the most important oil and feed crops grown in the US with 87,555,000 acres planted annually accounting for 90% of the US oilseed production (USDA-ERS). In Louisiana, soybean occupies the most acreage of any field crop with 1,100,000 acres planted in 2021 and remains one of the most economically important row crops grown (USDA/NASS 2021). Soybean alone contributed \$556,818,000 to the Louisiana agricultural economy in 2020, however, growers lose approximately \$56,000,000 - \$60,000,000 each year to insect pests (Musser et al. 2016, Musser et al. 2017, Musser et al. 2018, Musser et al. 2019, Musser et al. 2020).

To optimize yield potential, careful attention is placed on controlling weed, disease, and insect pests (Boerma and Specht 2004, Funderburk et al. 1999). Soybean fields are frequently scouted to confirm the presence of pests and determine economically tolerable population densities or damage levels (Stern 1973). Failure to accurately assess pest populations and take appropriate action could lead to significant economic losses characterized by yield loss and reductions in yield quality. Extensive research has been done to quantify the relationship between pest pressure and economic loss that can be practically applied at the field level. Efforts by Stern (1973) and Pedigo et al. (1986) led to the development of economic and action thresholds specifically for insect pests that allow growers to appropriately time corrective control tactics. Additionally, growers may implement precursory measures to inhibit pest population growth from reaching economically significant levels. In a classic example, Newsom and Herzog (1977) demonstrated in their “trap crop” concept that alternative hosts or earlier maturing soybean varieties planted along the field edge can draw insect pests away from the main crop,

thereby reducing the potential of observing economically significant pest populations in the main crop. Specifically, for the Midsouth, soybean production is characterized by raised or flat beds, wide (74 – 94 cm) or narrow (< 50 cm) row spacing, and the selection of specific cultivars best suited for the region (generally Maturity Group IV and V) (Heatherly 1998, Salmeron et al. 2014). Soybean is often incorporated into rotational and double cropping systems which additionally offer disease and weed management benefits by interrupting the seasonal cycle of a host-specific disease or suppressing weed pressure (Garbelini et al. 2020). However, such cropping practices can provide extended or overwintering habitat for insects and could lead to increased arthropod abundance and diversity (McPherson et al. 1982, Troxclair and Boethel 1984).

A wide variety of insects target soybean at every growth stage making soybean insect pest management complex and multifaceted (Boethel 2004). The sub-tropical climate and mild winters experienced in Louisiana offers a hospitable environment for insect pests which include armyworm, *Spodoptera* spp.; velvetbean caterpillar, *Anticarsia gemmatilis* (Hübner); green cloverworm, *Hypena scabra* (Fabricius); soybean looper, *Chrysodeixis includens* (Walker); corn earworm, *Helicoverpa zea* (Boddie); banded cucumber beetle, *Diabrotica balteata* (LeConte); and a host of stink bug species (Family: Pentatomidae) (Newsom 1978).

These pests are generally divided into pod feeders and defoliators with fewer pests targeting stems and roots. However, economic importance varies between pests when considering insect phenology, feeding habit, and specific plant parts targeted. Soybean tolerance to defoliation can vary depending on soybean growth habit and leaf morphology. Haile et al. (1998) reported that defoliation by *H. scabra* improved canopy light interception in wide-leaf isolines compared to narrow-leaf isolines of the same variety and showed no yield differences.

Wier and Boethel (1996) reported 74% and 94% defoliation by *C. includens* caused yield reductions of 800 kg/ha and 1,359 kg/ha, respectively, when defoliation occurred between full bloom and early seed development. This supports further reporting that yield loss from defoliation tends to be growth stage dependent as reduction in photosynthate production is most impactful during pod development (R3 – R5) (Boethel 2004, Fehr et al. 1971).

Pod feeding is considered more economically important as it causes direct damage to seed, impacting seed weight, quality, and overall yield (Daugherty et al. 1964, McPherson et al. 1979, Tood and Turnipseed 1974, Temple et al. 2011). Important pests in this category include the brown stink bug, *Euschistus servus* (Say); southern green stink bug, *Nezara viridula* (L.); green stink bug, *Acrosternum hilare* (Say); and redbanded stink bug *Piezodorus guildinii* (Westwood) (Temple et al. 2013). Daugherty et al. (1964) reported that feeding by *A. hilare* and *N. viridula* significantly impacted oil content and germination rate, as well as delayed maturity in field cage studies. Using an arbitrary damage index, McPherson et al. (1979) infested field-caged soybean with 0.5, 1, and 2 stink bugs/0.3 meters of row and determined that feeding during R5 reproductive stage resulted in the highest damage index, with 2 stink bugs/0.3 meters of row causing the most damage. This study suggested that stink bug management at R5 is most critical for yield potential. Tood and Turnipseed (1974) reported yield losses as high as 36 bu/acre when infesting 3 adult *N. viridula* per row/ft under field cages. Temple et al. (2011) reported significant yield reductions of 9.1% and 13% when infesting one and two adult *P. guildinii* per 0.6 row m, respectively. In terms of stem feeding, very few insects exist in this category compared to other modes of injury.

Larvae of the Dectes stem borer, *Dectes texanus* (LeConte), will tunnel into the pith of stems and petioles causing termination of the whole petiole as well as lodging (Hatchet et al.

1975). The kudzu bug is a relatively new pest of soybean in the US, being first reported in Georgia in 2009 (Suiter et al. 2010). Kudzu bug adults and larvae will feed on plant sap in the main stem and petioles which could lead up to 60% yield loss if left untreated (Seiter et al. 2013). However, the most economically significant stem feeder of soybean is the threecornered alfalfa hopper, *Spissistilus festinus* (Say), which constitutes anywhere from one to eight million dollars in economic loss plus cost of control annually (Beyer et al. 2017, Musser et al. 2016, 2017, 2018, 2019, 2020).

1.2. DISTRIBUTION AND BIOLOGY OF *S. FESTINUS*

The distribution of *S. festinus* ranges from the upper the Midwest to the southeast US with some estimates including parts of Canada and Mexico (Wildermuth 1915, Caldwell 1949). Adults are light green, 6 – 7 mm long, and possess a widened triangular pronotum which is where the name “threecornered” is derived (Beyer et al. 2017) (Image 1.1.). Adult and nymph *S. festinus* are typically found on stems and leaves of host plants (Beyer et al. 2017). Females use their ovipositors to create slits in the epidermis of plant tissue to oviposit (Beyer et al. 2017, Meisch and Randolph 1965, Wildermuth 1915). Meisch and Randolph (1965) reported that these slits can greatly reduce plant vitality during high infestations. Although this study did not describe the mechanisms of injury that lead to this phenomenon, exposing dermal tissue by damaging the epidermis can leave plants vulnerable to UV radiation, pathogens, water loss, and other environmental stressors (Lichtenthaler 1995, Javelle et al. 2010, Shepherd and Griffiths 2006). Females oviposit egg clusters into the base of the main stem as well as cotyledonary, unifoliate, trifoliate, and apical nodes of soybean (Daigle et al. 1988, Mitchell and Newsom 1984, Rice and Drees 1985). Eggs are white in color, cylindrically shaped, and approximately 0.9–1.3 mm long (Beyer et al. 2017).



Image 1.1. Adult *S. festinus*. Photo credit: T. Musgrove 2020.

Nymphs typically emerge 6 to 27 days after oviposition and it takes 32 days, on average, for a 1st instar to become an adult with five instars in total (Meisch and Randolph 1965, Wildermuth 1915). The 1st through 3rd instar nymphs are initially light brown and change to light green in the 4th and 5th instars (Beyer et al. 2017). *Spissistilus festinus* nymphs do not have a developed triangular pronotum like adults but instead have rows of pronounced setae that begin at the anterior end of the thoracic tergum and continue to the apex of the abdomen (Beyer et al. 2017) (Image 1.2.). Nymphs begin feeding at the 1st instar, but this feeding is often random and sparse (Moore and Mueller 1976). Johnson and Mueller (1989) reported that *S. festinus* will travel only 33 cm above the soil surface when in localized flight with males being more active

than females.



Image 1.2. Fifth instar *S. festinus*. Photo credit: T. Musgrove 2020.

However, this discrepancy could be due to increased male to female seasonal sex ratios during times of increased flight activity throughout the year. In soybean, males typically have higher numbers during the growing season peaking in June and July (Mitchell and Newsom 1984).

1.3. HOST PLANTS OF *S. FESTINUS*

Although *S. festinus* has more than twenty different possible host plants (Table 1.1.) (Mueller and Dumas 1987), its preferred hosts are of the family Fabaceae. The three most economically important field crops in the US that *S. festinus* will target as hosts include soybean, peanut, and alfalfa (Wildermuth 1915, Beyer et al. 2017, Mitchell and Newsom 1984). In all three species, *S. festinus* can feed and reproduce, resulting in multiple overlapping generations (Mitchell and Newsom 1984, Beyer et al. 2017). In Louisiana, *S. festinus* overwinters in pine,

Pinus taeda L., as a maintenance host and infests *T. repens* and *V. villosa* prior to early season soybean planting dates (Newsom et al. 1983). Although there are typically two generations during a growing season, host prevalence may allow three or four (Mitchell and Newsome 1984).

The complete host range of *S. festinus* is not limited to legumes but extends into other families which include several weeds species found in soybean production systems (Beyer et al. 2017, Moore and Mueller 1976, Mueller and Dumas 1987). Some alternative hosts include cocklebur (*Xanthium strumarium* L.), prickly sida (*Sida spinosa* L.), dock (*Rumex* spp. L.), lespedeza (*Lespedeza* spp. (Michx)) vetch; and several species of clover (*Trifolium* spp. L.) (Moore and Mueller 1976, Mueller and Dumas 1987). Prickly sida and cocklebur are typically found in soybean as weed pests; however, *S. festinus* is capable of reproducing on cocklebur, whereas prickly sida is considered a feeding host rather than a reproductive host (Moore and

Table 1.1. Selected hosts of *S. festinus*.

Common Name	Scientific Name	Source
Alfalfa	<i>Medicago sativa</i> L.	Cockerell 1899
Mesquite	<i>Prosopis</i> spp. L.	Wildermuth 1915
Red clover	<i>Trifolium pratense</i> L.	Wildermuth 1915
Cottonwood	<i>Populus</i> spp. L.	Wildermuth 1915
Cowpea	<i>Vigna unguiculata</i> (L.) Walp	Wildermuth 1915
Bermudagrass	<i>Cydonon dactylon</i> (L.) Pers.	Wildermuth 1915
Johnsongrass	<i>Sorghum halepense</i> (L.) Pers.	Wildermuth 1915
Wheat	<i>Triticum aestivum</i> L.	Wildermuth 1915
Barley	<i>Hordeum vulgare</i> L.	Wildermuth 1915
Oats	<i>Avena sativa</i> L.	Wildermuth 1915
Bur clover	<i>Medicago denticulata</i> L.	Wildermuth 1915
Sweet yellow clover	<i>Melilotus officinalis</i> (L.) Pall.	Wildermuth 1915
Soybean	<i>Glycine max</i> (L.) Merr.	Wildermuth 1915
Cocklebur	<i>Xanthium strumarium</i> L.	Wildermuth 1915
Marestail	<i>Erigeron canadensis</i> L.	Wildermuth 1915
Peanut	<i>Arachis hypogaea</i> L.	King et al. 1961
Prickly side	<i>Sida spinosa</i> L.	Moore and Mueller 1976
Lespedeza	<i>Lespedeza</i> spp. (Michx)	Moore and Mueller 1976

(table cont'd)

Common Name	Scientific Name	Source
Dock	<i>Rumex</i> spp. L.	Mueller 1980
Vetch	<i>Vicia sativa</i> L.	Newsom et al. 1983
Clover	<i>Trifolium</i> spp. L.	Newsom et al. 1983
Pine	<i>Pinus</i> spp. L.	Newsom et al. 1983
Yellow jessamine	<i>Gelsemium sempervirens</i> L.	Newsom et al. 1983
Honeysuckle	<i>Lonicera japonica</i> L.	Newsom et al. 1983
Common grapevine	<i>Vitis vinifera</i> L.	Smith 2013

(table cont'd)

Mueller 1976). Of the hosts previously listed, *S. festinus* has been shown to reproduce on dock, lespedeza, vetch, and clover, but each vary in their status as feeding hosts (Mueller and Dumas 1987). Furthermore, *S. festinus* longevity is reduced and oviposition rates are lower in comparison to primary hosts (Moore and Mueller 1976, Mueller and Dumas 1987). Generally, leguminous hosts are superior feeding and reproductive hosts compared to non-leguminous hosts with soybean and peanut having the potential to facilitate the highest levels of fecundity and herbivory (Mueller and Dumas 1987).

1.4. FEEDING INJURY OF *S. FESTINUS*

Spissistilus festinus feeds on soybean by using its piercing-sucking mouth parts to extract sap from phloem tissue (Beyer et al. 2017, Andersen et al. 2002, Mitchell and Newsom 1984). This feeding habit is characterized by a ring of necrotic tissue around the circumference of a stem or petiole known as a girdle (Image 1.3.) (Mitchell and Newsom 1984). Girdling tends to disorganize internal vascular bundles resulting in blockage of nutrient flow and photosynthate translocation (Spurgeon and Mueller 1991, Hicks et al. 1984). This eventually causes nutrient assimilates to collect above the girdle creating a nutrient sink (Mitchell and Newsom 1984). Nymphs prefer to feed at the nutrient sink due to a higher concentration of phloem assimilates

which can persist at these sites for up to four days after girdling (Andersen et al. 2002, Mitchell and Newsom 1984).



Image 1.2. Girdle by *S. festinus* on soybean.
Photo credit: T. Musgrove 2020.

Effects of girdling can include reduced plant vigor, stem breakage, node termination, disease vectoring, and reduced root nodulation and dry weight (Wilson and Quisenberry 1987, Beyer et al. 2017, Mitchell and Newsom 1984, Mueller and Jones 1983, Padgett 1992, Mueller and Dumas 1975, Sparks and Boethel 1987). In small plot field trails, Sparks and Newsom (1984) reported yield reductions of 396.8 – 1,189.5 kg/ha due to late season damage when adult populations ranged from 48 – 476.5 insects per 100 sweeps. In attempting to formulate a damage threshold, Muller and Jones (1983) did not observe significant yield reductions until >65% of plants (V3 – V6) (Fehr et al. 1971) were girdled. Both studies used ‘Bragg’ MGVII soybean

variety. Cook et al. (2015) simulated stand loss by removing individual plants at R1 – R2 (Fehr et al. 1971) (R1 = one bloom at any node, R2 = full bloom) and found yield was significantly reduced for full season indeterminate (when stand loss was >30%) and full season determinate soybeans (when stand loss was >10%).

Girdling injury can also cause soybean to become lodged and entangled with adjacent plants due to either stem breakage or stem weakening (Tugwell et al. 1972, Mitchell and Newsom 1984, Mueller and Jones 1983). Lodging is a major concern regarding mechanical harvest potential and is characterized by prostrate, spindly plants. Lodged soybean is often more difficult to harvest because combine headers are only designed to harvest upright plants (Weber and Fehr 1966). Using MGVI ‘Lee 68’ soybean, Bailey (1975) observed that lodging in the late season correlated with girdling damage before the plant reached 25.4 cm in height. If girdling occurred after this height, it had little contribution to lodging.

Both adults and 3rd to 5th instar nymphs can girdle soybean (Mitchell and Newsom 1984). Jordan (1952) stated that nymphs are responsible for most girdling occurrences due to their lack of mobility, thus facilitating an increased likelihood of forming girdles. Sparks (1986) further supported this claim by demonstrating that nymphs caused 45 – 60% of yield loss in field studies. Mueller and Jones (1983) observed that the greater plant growth of non-girdled V3-V6 (Fehr et al. 1971) stage soybean can compensate for stand loss due to girdling if less than 65% of plants were girdled. *Spissistilus festinus* does not feed on pods but has been documented girdling pod stems; however, it is not their preferred feeding site (Mitchell and Newsom 1984). Both 3rd and 5th stage instar nymphs as well as adults tend to feed on upper petioles, but Moore and Mueller (1976) reported 4th stage instars tend to feed on the base of the main stem (Moore and Mueller 1976). This led them to conclude that the 4th instar can be the most damaging and

principal contributor to main stem breakage. Nymphal feeding does not become damaging until the 3rd instar. Furthermore, in ranking developmental stages from most damaging to least based on greenhouse observation, Moore and Mueller (1976) reported that the order should be 4th, 3rd, 5th, and then adult. Adding to this, Sparks (1984) stated that only 3rd to 5th instars should be considered in a management recommendation.

Girdling can cause slight, although significant, increased severity of diseases such as stem canker (*Diaporthe phaseolorum* var. *caulivora*) and stem blight (*Phomopsis sojae* Leh.) in soybean (Russin et al. 1987). In field trials, Padgett (1992) reported a significant increase in *D. phaseolorum* var. *caulivora* measured in surface area between gridled and non-girdled soybean (9.4 vs 6.2 cm²) as well as significantly reduced yield (5.6 – 9.0 g of seed/plant). Although the mechanism that links girdling to disease severity was never determined, Russin et al. (1987) theorized the effect was likely physiological since plants were artificially inoculated with *D. phaseolorum* var. *caulivora* and not vectored by *S. festinus*. Lastly, *S. festinus* was recently reported to be a vector of *Grapevine red blotch-associated virus* (GRBaV) in grapevine; however, *S. festinus* is not known to vector any diseases specific to soybean (Bahder et al. 2016). Regarding alternative crops, girdling has reduced or differing effects on yield. Peanut is the most resilient host to *S. festinus* feeding as there is no known correlation to injury and yield loss even though peanut is a feeding and reproductive host (Beyer et al. 2017). In alfalfa, Wilson and Quisenberry (1987) observed a reduction in root dry weight due to *S. festinus* feeding. Since alfalfa is harvested multiple times a year as a forage crop, reduction in root carbohydrate storage can significantly impact regrowth throughout the growing season, reducing dry matter yield (Wilson and Quisenberry 1987, Beyer et al. 2017).

1.5. CONTROL AND MANAGEMENT OF *S. FESTINUS*

1.5.1. Thresholds

Spissistilus festinus was first described as a pest of soybean by Wildermuth in 1915; however, a threshold was not established until 1984 by Sparks and Newsom. In conventional plantings of later maturing determinate soybean ('Bragg' MGVII), Sparks and Newsom (1984) reported late season *S. festinus* populations caused a yield reduction of 1514.6 kg/ha as mean adult populations approached 162 insects per 100 sweeps. However, yield loss did not correlate with early season populations. It was theorized that *S. festinus* damage during vegetative growth stages had little impact on yield due to compensatory physiological response to stand loss by soybean. This agrees with previous research by Caviness and Miner (1961) in which they reported that 45% stand reductions two weeks before bloom did not result in statistically significant yield loss. Furthermore, Muller and Jones (1983) and Tugwell and Miner (1967) also made similar conclusions with respect to soybean stand loss compensation. Efforts by Sparks and Newsom (1984) led to the current accepted action threshold of one adult per sweep after pod set. This threshold assumes nymphs already contribute to feeding damage; however, they are not specifically included since they are more difficult to accurately sample (Sparks and Newsom 1984). Moreover, the authors did not incorporate economic analysis of treatment costs or monetary impact of yield loss. Therefore, this cannot be considered an economic threshold.

1.5.2. Sampling Methods

A critical component of soybean insect management is to accurately estimate pest populations as a pretext to informed decision making. However, insect sampling tends to be inundated with error and bias depending on sampling methodology and natural variation within the sampling environment such as growth habit of plants and environmental conditions (Fowler and Witter 1982). Previous research has been done to evaluate the accuracy of different sampling

methods. Shepard et al. (1974) compared sweep-net, vacuum-net, and ground cloth methods for soybean insects and found significantly higher sample means with the ground cloth method. Turnipseed (1974) reported similar findings regarding ground cloth method. In addition, the authors stated that the vacuum-net method was best for sampling minuscule, saltatorial hemipterans. However, sampling population densities may not be as effective as sampling for damage. For example, Mueller (1980) proposed that given the difficulty of sampling for *S. festinus* nymphs, girdling damage estimates may be more reliable in determining *S. festinus* population pressure.

Despite previous research focusing on alternative methods, a 38 cm diameter sweep net is the standard method used to sample *S. festinus* in soybean (Sparks and Newsom 1984, Sparks 1986). While nymphs are more difficult to accurately sample with a sweep net, it remains the accepted standard simply due to practicality and ease of use compared to other techniques (Spurgeon and Mueller 1991). Spurgeon and Mueller (1991) reported that sampling for nymphs tends to be heavily influenced by age-bias as smaller instars are less likely to release from stems and petioles when disturbed. Additionally, alternative ways were tested to sample nymphs and concluded that, of the methods tested, whole plant bagging was best able to reflect the absolute number of nymphs in the field. Similarly, Sparks (1986) compared the ground cloth, vertical beat sheet, and beat net techniques for nymph sampling and determined the beat net was the most efficient in estimating nymph populations given its low coefficient of variance between samples and high number of insects per sample. Thus, the beat net technique was concluded to be the most accurate method to sample *S. festinus* nymphs. However, in terms of practicality, the sweep net remains the standard sampling method.

1.5.3. Chemical Control

Chemical control is the primary way to control *S. festinus* in soybean. Previous studies have shown that *S. festinus* is highly susceptible to multiple classes of insecticides. Pyrethroid and organophosphate broadcast spray applications offer effective control within 10 DAT (days after treatment); however, *S. festinus* can rebuild populations quickly, and multiple applications are needed throughout the growing season (Jackson 2000, Fitzpatrick et al. 2001, Price et al. 2008, Fontenot et al. 2009). Jordan (1952) first reported that applications of toxaphene, DDT (dichloro-diphenyl-trichloroethane), toxaphene-DDT (2-1) mixture, and parathion (5-6 gallons per acre total volume for each treatment) failed to significantly reduce adult populations under field conditions but were all highly effective in controlling nymphs. Rohlf et al. (1983) tested acephate (0.66 lbs a.i./a), permethrin (0.04 – 0.1 lbs a.i./a), and carbaryl (1.0 lbs a.i./a) and reported all treatments significantly reduced adult *S. festinus* populations 7 days after treatment (DAT). Wilson et al. (1985) reported acephate (0.75 lbs a.i./a), β -cyfluthrin (0.025-0.0125 lbs a.i./a), carbofuran (0.5 lbs a.i./a), and methyl parathion (0.5 lbs a.i./a) significantly reduced adult *S. festinus* populations below 50% of pre-spray levels 14 DAT. Fitzpatrick et al. (2000) reported foliar applications of thiamethoxam (0.0625 lbs a.i./a) significantly reduced adult populations by 78% compared to the control 10 DAT. Howard and Akin (2011) tested high and low dose applications of several pyrethroids and one organophosphate and reported that λ -cyhalothrin, β -cyfluthrin, esfenvalerate, z-cypermethrin, bifenthrin, and acephate significantly reduced *S. festinus* populations below threshold levels compared to the untreated check 7 DAT with exception of low dose lambda-cyhalothrin (0.0156 lbs a.i./a). Currently, there is no known pesticide resistance reported among labeled insecticides against *S. festinus*.

1.5.4. Host Plant Resistance

Host plant resistance (HPR) refers to the ability of plants to reduce susceptibility to pests through tolerance, antixenosis (reducing host desirability), or antibiosis (causing direct harm or reducing pest reproductive capacity) (Painter 1951). However, little is known of how effective host plant resistance can be in controlling *S. festinus* in soybean. Herbivory induced host-plant responses in soybean are characterized by increases in oxidative enzymes (Felton et al. 1994). These enzymes are thought to interfere with digestion in the insect gut, thereby reducing the nutritive quality of plant tissue (Felton et al. 1989). Felton et al. (1994) further discussed that competing pests may be mediated in the presence of increased oxidative responses due to *S. festinus* feeding but was never tested to verify. Additionally, this study examined herbivory of the Family Noctuidae but did not confirm whether this mechanism of host-plant resistance in soybean is a viable control method for *S. festinus*. Alternatively, Moellenbeck and Quisenberry (1993) reported that alfalfa has shown potential for host-plant resistance in 5 of 17 cultivars tested for stand resilience against *S. festinus* girdling, but the mechanisms of resistance were never defined.

1.5.5. Cultural Control

Spissistilus festinus is not controlled effectively by manipulating cultivation practices such as tilling and row spacing (Troxclair and Boethel 1984). Tilled systems can ensure that insect pests have no opportunity to overwinter in stubble from the previous season; however, when comparing *S. festinus* populations in till versus no-till systems, Troxclair and Boethel (1984) showed that altering tillage practices do not have a significant impact. They reported that although early season populations tend to be higher in no-till versus tilled plots, no significance is found when accounting for populations over an entire season. Likewise, row spacing (18 cm, 51 cm, and 76 cm) had no effect on population distribution (Troxclair and Boethel 1984).

1.5.6. Biological Control

Predatory hemipterans as well as certain species of Strepsiptera have been known to prey upon and parasitize *S. festinus* (Jordan 1953, Medal et al. 1997, Newsom et al. 1983). Medal et al. (1995) reported that nymphs are most susceptible to predation by beneficial hemipterans due to lack of mobility and rigid exoskeleton compared to adults. However, of the three hemipteran species used in this study, *Nabis roseipennis* (Reuter), *Geocoris punctipes* (Say), and *Orius insidiosus* (Say), *N. roseipennis* was the only natural enemy that preyed upon adults. In addition to predation, parasitism by family specific Strepsiptera was reported by Jordan (1952, 1953) and Pierce (1952). *Membracixenos jordani* (Jordan) is the only known Strepsipteran to parasitize *S. festinus* in North America (Pierce 1952). As many as six Strepsipteran larvae can be found within one adult; however, *M. jordani* has not been shown to cause mortality but rather inhibit the ability of females to produce eggs (Jordan 1952). As a result, it was theorized that *M. jordani* may act as a limiting factor in *S. festinus* population growth (Jordan 1953).

1.6. JUSTIFICATION

Current management practices and the effect on the establishment, colonization, and late season disruption of *S. festinus* have not yet been fully elucidated. Manipulation of planting dates, application of insecticide seed treatments, and knowledge of nymphal feeding have not been evaluated collectively when researching *S. festinus* management. Sparks and Newsom (1984) observed that late season *S. festinus* damage had a significant effect on reducing final yield but found no correlation between yield and early season feeding. It was concluded that soybeans may compensate for early season stand loss due to *S. festinus* injury if damage occurred in the early vegetative stages. However, two more recent studies, Pulakkatu-thodi (2010) and Ramsey (2015), observed no significant impact on yield, even at infestation levels two to three

times the current threshold of one adult per sweep. However, the difference in research methods across the three studies may highlight gaps in the current knowledge of *S. festinus* life history and management.

Firstly, studies by Sparks and Newsom (1984) accounted for infestation time over an entire season. Experiments in these studies allowed native populations to colonize from overwintering sites and progress through their generational development in the field. However, the two studies by Pulakkatu-thodi (2010) and Ramsey (2015) artificially infested test plots with adults only for two weeks at most. This may be the reason why the findings of each study were significantly different. In addition to infestation time, none of the previous studies made any effort to investigate the effect of neonicotinoid seed treatments on *S. festinus*. These systemic pesticides are applied to the surface of seed prior to planting and are absorbed by the plant after germination (Jeschke et al. 2010). Neonicotinoid concentrations within plants generally persist for approximately thirty days after planting (DAP); therefore, the range of insects controlled is limited to the early season (Magalhaes et al. 2009, Whalen et al. 2021). However, little is known of seed treatment efficacy in controlling early season *S. festinus*. Davis et al. (2009, 2010) reported no yield differences between non-treated seed but did not determine whether NSTs impacted girdling activity. Even still, it is reasonable to assume that NSTs have some effect particularly during early season colonization and oviposition.

Another concept to consider is the effect of planting dates on *S. festinus* early season colonization. Soybean production systems have adopted improved cultural strategies to increase yield. One of the most significant changes is the Early Season Production System (ESPS) (Heatherly and Spurlock 1999). Before the widespread adoption of ESPS, conventional soybean plantings for Maturity Group IV, V, and VI occurred mid to late May; this was to avoid any early

season cold snaps or freezes (Heatherly and Spurlock 1999). However, it became clear that late season drought had a significant negative impact on yields (Heatherly and Spurlock 1999). To address this issue, growers began planting as early as possible so that growth stages critical to reproductive development receive adequate rainfall during the season. Studies have shown that although the likelihood of encountering early season cold damage is increased, the return on final yield is significant and often attractive enough to incur this risk (Heatherly and Spurlock 1999). Bowers (1995) demonstrated that ESPS can aid in achieving acceptable yields while avoiding late season drought damage. However, early planting risks exposing vegetative stage soybean to early season pest pressure. Certain hemipteran pests tend to establish high populations in the presence of early planted soybean. Baur et al. (2000) observed significantly higher populations as well as frequency of threshold populations for *Nezara viridula* (L.) and *S. festinus* in early plantings when compared to conventional plantings. Therefore, it is important to understand early season planting and colonization interactions of soybean pests when re-evaluating pest threshold.

The following research objectives were proposed:

Objective 1: Evaluate neonicotinoid seed treatment efficacy against *S. festinus*.

Objective 2: Determine effect of neonicotinoid seed treatments on *S. festinus* field colonization.

Objective 3: Re-evaluate literature-stated action threshold in soybean.

CHAPTER 2. EFFECTS OF NEONICOTINOID SEED TREATMENTS ON THREECORNERED ALFALFA HOPPER, *SPISSISTILUS FESTINUS* (SAY), IN LOUISIANA SOYBEAN, *GLYCINE MAX* (L.) MERR

2.1. INTRODUCTION

Soybean [*Glycine max* (L.) Merr.] is the most important oilseed crop grown in the United States. With 36,000,000 ha planted annually, this accounts for 90% of US oilseed production (USDA-ERS). In Louisiana, soybean is one of the most economically important row crops grown, with 450,000 ha planted in 2021 (USDA/NASS 2021). Soybean alone contributed \$556,818,000 to the Louisiana agricultural economy in 2020, however, growers lose approximately \$56,000,000 - \$60,000,000 each year to insect pests (Musser et al. 2016, 2017, 2018, 2019, 2020).

One of these early season pests, the threecornered alfalfa hopper, *Spissistilus festinus* (Say), is a small, saltatorial hemipteran (Family: Membracidae). Adults are light green, 6–7 mm long, and possess a widened triangular pronotum from which the name “threecornered” is derived (Beyer et al. 2017). *Spissistilus festinus* feeds on soybean by using its piercing-sucking mouth parts to extract sap from primary and secondary phloem tissue (Mitchell and Newsom 1984, Andersen et al. 2002, Beyer et al. 2017). This feeding habit is characterized by a ring of necrotic tissue around the circumference of a stem or petiole known as a girdle (Mitchell and Newsom 1984). Girdling disorganizes internal vascular bundles resulting in blockage of nutrient flow and photosynthate translocation (Hicks et al. 1984, Spurgeon and Mueller 1991).

Effects of girdling include reduced plant vigor, stem breakage, node termination, disease vectoring, and reduced root nodulation and dry weight (Mueller and Dumas 1975, Mueller and Jones 1983, Mitchell and Newsom 1984, Wilson and Quisenberry 1987, Padgett 1992, Sparks and Boethel 1987, Beyer et al. 2017). In small plot field trials, Sparks and Newsom (1984)

reported yield reductions of 396.8–1,189.5 kg/ha due to late season damage when adult populations ranged from 48–476.5 insects per 100 sweeps with a 38 cm diameter sweep net. Attempting to formulate a damage threshold, Muller and Jones (1983) did not observe significant yield reductions until >65% of plants (V3–V6) (Fehr et al. 1971) were girdled early season. Thus, the current action threshold for *S. festinus* in Louisiana is 1 adult per sweep or 100 adults per 100 sweeps (Towles et al. 2022).

Neonicotinoid seed treatments (NSTs) are generally thought to be effective against early season sap-sucking insect pests (Magalhaes et al. 2009, Reisig et al. 2012, North et al. 2016). Bayer Crop Science released the first commercially available neonicotinoid, imidacloprid, for agricultural use in the early 1990s (Jeschke et al. 2010). Currently, neonicotinoids include clothianidin, acetamiprid, dinotefuran, nitenpyram, imidacloprid, thiacloprid, and thiamethoxam (Nauen et al. 2003, Tomizawa and Casida 2005). When applied as a seed treatment, neonicotinoids provide systemic protection from early season pests, which may also reduce non-target effects (Nauen et al. 2003). However, these insecticides have come under scrutiny for potential pollution of waterways as well as impacts to native pollinators (Decourtye and Devillers 2010, Goulson 2013, Hladik et al. 2014, Stewart et al. 2014). Although NSTs are labeled for controlling *S. festinus* in soybean, little is known on how these products impact mortality and girdling. Therefore, the usefulness of seed treatments in context with early season control for *S. festinus* must be confirmed.

For early season control of *S. festinus*, current management practices advise using foliar sprays or NSTs. Neonicotinoid concentrations within plants generally persist for approximately thirty days after planting (DAP); therefore, the range of insects controlled is limited to the early season (Magalhaes et al. 2009, Krupke and Alford 2017, Whalen et al. 2021). However, little

literature is available regarding seed treatment effect on *S. festinus* colonization, establishment, or efficacy duration against this pest. Nunez et al. (2003) evaluated Cruiser 5FS (a.i. thiamethoxam) and Gaucho 600 (a.i. imidacloprid) NST impacts on *S. festinus* girdling, insect numbers, stand count, nodes per plant, yield, and seed quality in plants sampled at R1. The authors reported no significant differences in any of the parameters sampled. However, they reported low overall insect numbers throughout the season, especially for *S. festinus*. Davis et al. (2009) and Davis et al. (2010) observed no significant reduction in treated soybean yield but did not determine whether NSTs impacted girdling activity. If NSTs alter feeding or oviposition behavior, it may cause the disruption of early season populations attempting to colonize soybean. This could, in turn, have a temporal effect on later season populations. Thus, the objective of these experiments was to evaluate the influence of soybean NSTs on *S. festinus* mortality, girdling, and population growth.

2.2. MATERIALS AND METHODS

2.2.1. Seed treatment and test plant preparation for greenhouse assays

Soybean variety UA5715GT (University of Arkansas, Foundation Seed Program, Fayetteville, AR) was treated in the laboratory with Cruiser 5FS (thiamethoxam, Syngenta, Greensboro, NC) at 1.137 mL per 1.36 kg of seed or Admire Pro (imidacloprid, Bayer Crop Science, St. Louis, MO) in the greenhouse at 0.055 mL per 500 mL of water (concentration based on soil-application field rate) as per the labeled rates. Cruiser 5FS was prepared as a slurry, mixed with 1.36 kg of seed in a plastic bag, and shaken for 30 seconds. After seeds were thoroughly coated, they were placed under a fumigation hood to dry for 24 hr prior to planting. Admire Pro was prepared as a solution and applied directly to seed in-furrow using a hand-held spray bottle during the planting process. After which, seed was covered with soil.

2.2.2. *S. festinus* no-choice on-plant greenhouse assays

Greenhouse trials were conducted at the LSU Plant Materials Center in Baton Rouge, LA. Treatments consisted of Cruiser 5FS, Admire Pro, or a non-treated check all evaluated at V1, V3, and V5 growth stages. Soybean was planted under greenhouse conditions [28°C, 75% RH, and 14:10 (L:D)] into 90 individual 1.89 L plastic nursery pots in Miracle-Gro Potting Mix (Scotts Miracle-Gro, Maryville, OH) at 4 seeds per pot with ten pots per treatment per growth stage. Each experiment was replicated twice. Once soybean produced its first, third, or fifth fully opened trifoliate, pots were thinned to one plant per pot and placed under screen mesh cages (rearing and observation cage, Bioquip, catalog # 1466BV). Adult *S. festinus* were field-collected with a 38 cm diameter sweep net from R2–R6 soybean at Doyle Chambers Central Research Station, Baton Rouge, LA. To reduce stress induced mortality, insects were placed into a screen mesh cage with a diet of green beans and distilled water and held for 24 hr in a walk-in rearing room [26°C, 50% RH, and 14:10 (L:D)]. After 24 hr, insects were removed and placed in test cages, with three randomly selected adults per cage. Number of alive and dead *S. festinus* was monitored for seven days, and number of girdles was taken on the final day. The trial was considered finished if all insects in both treatments were deceased. Insects were considered deceased if no coordinated locomotion was re-established after being disturbed with a fine-tipped paintbrush. Insect data was calculated as percent survival and subjected to a Two-Way analysis of variance (ANOVA) using PROC MIXED (SAS 2021) with NST, growth stage, and their interaction being fixed effects. Girdling data was subjected to a Two-Way ANOVA with NST and growth stage being fixed effects. Normality testing was done using Shapiro-Wilks test for normality in PROC UNIVARIATE (SAS 2021). Means were separated using Tukey's HSD ($\alpha \leq 0.05$) when significance was found.

2.2.3. *S. festinus* field assay

Field trials were planted at the Doyle Chambers Research Station in Baton Rouge, LA on June 9th in 2021. Using a RCBD, soybean variety UA5715GT (University of Arkansas, Foundation Seed Program, Fayetteville, AR) treated with Cruiser 5FS, Admire Pro, or untreated was planted into 9.14 m² plots containing four 12.19 m rows on 0.91 m centers at a seeding rate of 8 seed/0.30 m of row. For Admire Pro treatments, non-treated seed was planted by hand and treatment was applied in furrow using a CO₂ backpack sprayer calibrated to deliver 140.2L/hectare at 241.3 kPa using four T-jet 80015VS flat-fan nozzles before seed was covered. Beginning 2 weeks after planting and every week thereafter, *S. festinus* was collected from soybean plots using a D-Vac (John W. Hock Company, model #1612) (Dietrick 1961). Samples were collected from three randomly selected one-meter sections of row per plot, stored in plastic bags, and taken back to the lab for evaluation. Sampling concluded at harvest maturity (R8) (Fehr et al. 1971). Girdling counts were taken from five randomly selected plants per plot at R6. Once soybean reached harvest maturity, five randomly selected plants per plot were destructively sampled and placed in a walk-in propane seed dryer at the Doyle Chambers Research Station Seed Warehouse set to 43° C for 5–7 days to lower seed moisture to 13%. Harvest parameters evaluated were total number of seed per five plants and one-hundred seed weight in grams. Insect numbers were converted to cumulative insect days (CID) using the formula:

$$\Sigma\{(X_{i+1} - X_i)[(Y_i + Y_{i+1}) \div 2]\}$$

where X_i and X_{i+1} are adjacent points in time and Y_i and Y_{i+1} are corresponding number of insects sampled at each point (Ruppel 1983). Cumulative insect days and yield data were subjected to a One-Way ANOVA using PROC MIXED (SAS 2021) with NST being a fixed effect. Girdling data was subjected to a One-Way ANOVA with NST being the fixed effect.

Normality testing was done using Shapiro-Wilks test for normality in PROC UNIVARIATE (SAS 2021). In all analysis, means were separated using Tukey's HSD ($P \leq 0.05$) when significance was found.

2.3. RESULTS

2.3.1. *S. festinus* greenhouse assay

Analysis revealed NST and growth stage main effects as well as their interaction significantly impacted *S. festinus* percent survival on soybean grown in the greenhouse (Table 2.1.).

Table 2.1. Two-Way analysis of variance table for percent survival of *S. festinus* on soybean grown in greenhouse.

Effect	<i>df</i>	<i>F</i>	<i>P</i>
Treatment	2, 89	21.52	<0.0001
Growth stage	2, 89	39.75	<0.0001
Treatment x Growth stage	4, 89	3.10	0.0194

The main effect of treatment resulted in significantly lower percent survival in Admire Pro ($9.4 \pm 2.6\%$) and Cruiser 5FS ($19.4 \pm 4.1\%$) treated soybean compared to the control ($40.0 \pm 4.7\%$).

When analyzed by growth stage main effect, percent survival averaged $2.8 \pm 1.4\%$, $21.1 \pm 4.0\%$, and $45.0 \pm 4.6\%$ for V1, V3, and V5 growth stages, respectively. These means were all significantly different from each other. Percent survival means resulting from the interaction of treatment and growth stage are presented in Fig. 2.1. Significance was not found between treatments at V1. At V3, percent survival means in non-treated soybean ($40.0 \pm 6.7\%$) were significantly higher than soybean treated with Admire Pro ($5.0 \pm 3.6\%$) but not from Cruiser 5FS treated soybean ($18.3 \pm 7.5\%$). At V5, the effect of treatment resulted in greater percent survival means in non-treated soybean ($71.7 \pm 6.5\%$) compared to soybean treated with Admire Pro ($23.3 \pm 6.0\%$) and Cruiser 5FS ($40.0 \pm 7.5\%$). However, neither NST was different from each other.

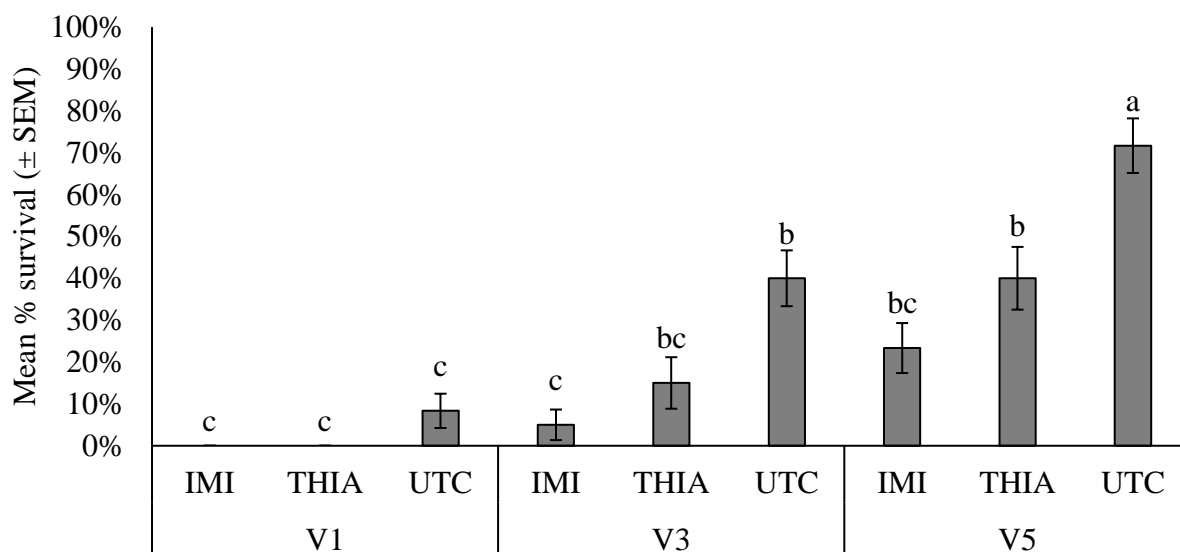


Figure 2.1. The interaction effect of treatment and growth stage on *S. festinus* percent survival on soybean grown in greenhouse. Bars followed by the same letter are not significantly different. ($P \leq 0.05$, Tukey's HSD)

Analysis of girdling revealed the main effects of treatment and growth stage as well as their interaction significantly impacted the number of girdles found per plant (Table 2.2.).

Table 2.2. Two-Way analysis of variance table for number of girdles per plant on soybean grown in greenhouse.

Effect	df	F	P
Treatment	2, 89	6.99	0.0015
Growth stage	2, 89	17.99	<0.0001
Treatment x Growth stage	4, 89	2.84	0.0288

The main effect of treatment resulted in significantly higher mean girdles per plant in non-treated soybean (0.5 ± 0.1) compared to both Admire Pro (0.1 ± 0.1) and Cruiser 5FS treated soybean (0.2 ± 0.1), although neither insecticide treatment was different from each other. Similarly, the effect of growth stage resulted in greater mean girdles per plant at the V5 growth stage (0.6 ± 0.2) compared to both V1 and V3 (0.0 ± 0.0 and 0.2 ± 0.1 , respectively). Both V1 and V3 growth stages were not different. The interaction of treatment and growth stage are presented in Fig. 2.2.

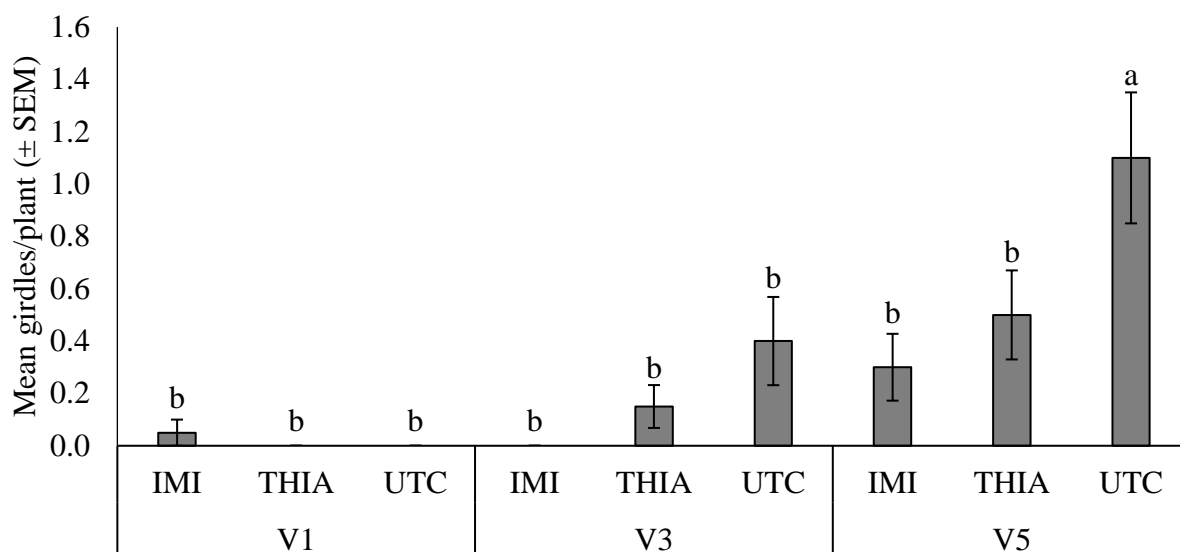


Figure 2.2. The interaction effect of treatment and growth stage on mean *S. festinus* girdles per plant on soybean grown in greenhouse. Bars followed by the same letter within columns are not significantly different. ($P \leq 0.05$, Tukey's HSD)

Analysis revealed treatments were not different from the control in V1 or V3 growth stages. At V5, non-treated soybean averaged greater mean girdles per plant (1.1 ± 0.3) compared to soybean treated with Admire Pro (0.3 ± 0.1) and Cruiser 5FS (0.5 ± 0.2), with both NSTs not being different from each other.

2.3.2 *S. festinus* field assay

Analysis revealed treatments had no significant effect on adult CID means ($F = 0.77$; $df = 2, 9$; $P = 0.4919$). Cumulative insect days per treatment averaged 52.9 ± 17.6 , 48.8 ± 11.0 , and 68.8 ± 2.0 for Admire Pro, Cruiser 5FS and non-treated soybean, respectively. In addition, nymph CID was also unaffected by treatment ($F = 0.01$; $df = 2, 9$; $P = 0.9928$). For nymphs, CID per treatment averaged 40.4 ± 12.2 , 39.8 ± 16.1 , and 38.3 ± 8.3 for Admire Pro, Cruiser 5FS and non-treated soybean, respectively. Gridling was also unaffected by treatment ($F = 1.19$; $df = 2, 44$; $P = 0.3147$). Number of girdles per plant averaged 5.5 ± 0.7 , 6.8 ± 1.3 , and 5.5 ± 0.8 for Admire Pro, Cruiser 5FS, and non-treated soybean, respectively. The effect of treatment on yield

parameters per five plants is presented in Table 2.3. Neonicotinoid seed treatments did not impact either yield parameter evaluated (total seeds, $F = 0.14$; $df = 2, 9$; $P = 0.8704$; hundred seed weight, $F = 0.06$; $df = 2, 9$; $P = 0.9456$).

Table 2.3. The effect of treatment on yield parameters of soybean at the field level. Means in this table are not significantly different. ($P \leq 0.05$, Tukey's HSD)

Treatment	Total Seed	100 seed wt (g)
IMI	665.3 \pm 137.1	12.5 \pm 0.5
THIA	622.0 \pm 7.0	12.4 \pm 0.5
UTC	598.7 \pm 71.7	12.6 \pm 0.3

2.4. DISCUSSION

This study evaluated neonicotinoid seed treatments as a potential contributor to *S. festinus* management in soybean. Neonicotinoid seed treatments have been reported to be effective against sap feeding soybean pests, like *S. festinus*, but none have confirmed efficacy through observations on treated soybean plants in a controlled setting. Previous studies determined NST efficacy indirectly by observing changes in *S. festinus* field population means (Nunez et al. 2003, Davis et al. 2009, Davis et al. 2010). Experiments in this study conducted in the greenhouse demonstrated NSTs cause mortality and alter girdling behavior upon direct exposure to systemic concentrations. These results are the first reported greenhouse observation of *S. festinus* being susceptible to NSTs because of direct exposure through herbivory. In addition to NSTs, the growth stages tested were initially included because V1 to V5 spans approximately 30 days. Previous research has identified this period to be the optimal window of efficacy for NSTs before systemic concentrations are metabolized and eliminated by the plant (Magalhaes et al. 2009, Seagraves and Lundgre 2011). Incidentally, the effect of growth stage was found to be just as deleterious to *S. festinus* survival as NSTs. The difference in survival between growth stages is made more intriguing given the time between each growth stage being

no more than a week. This would imply physiological changes between V1, V3, and V5 are significant enough to impact *S. festinus* survival. However, upon further consideration, a more likely explanation would be stress induced by the greenhouse environment is responsible for reduced survivorship. Given the highly active/mobile nature of *S. festinus* adults, being caged in a greenhouse without optimal sheltering and feeding options may induce undue stress on the insect. In addition, the lack of girdling in V1 and V3 soybean may also indicate that *S. festinus* were too stressed to feed. Therefore, the effect of growth stage on *S. festinus* survival and girdling behavior may be more due to experimental methodology rather than growth stage itself. However, further research would be needed to determine the effect of growth stage in the absence of NSTs.

Given results in the greenhouse study, field trials were expected to follow similar trends. Since previous literature has correlated yield damage to both population density and feeding injury (Mueller and Jones 1983, Sparks and Newsom 1984), each was included as a response variable in this study. With the understanding that NSTs lose efficacy ~30 days after planting (Magalhaes et al. 2009), CID was used to reflect the overall population over time. Cumulative insect day was originally developed to reflect population density and time within a single value (Ruppel 1983). This can be especially useful in studies that follow a pest population for an extended period of time such as an entire field season. Although, CID has not been used in previous literature to estimate *S. festinus* populations, it has been used frequently with aphid pests, specifically soybean aphid *Aphis glycines* (Matsumura) (Hanafi et al. 1989, McCornack and Ragsdale 2006, McCornack and Ragsdale 2007).

Neonicotinoid seed treatments failing to impact *S. festinus* CID and girdling frequency at the field level may be due to a variety of factors. Field populations enjoy more uninhibited

movement throughout the environment compared to caged insects (Sparks and Boethel 1987, Jonson and Mueller 1989, Trichilo et al. 1993). If *S. festinus* is negatively affected by environmental stressors like crowding, this can be overcome by movement within the field. Such behavior is not possible in greenhouse cages. Additionally, the small plot design used in the field study may reduce the likelihood of detecting differences in NSTs since *S. festinus* is known to reinfest soybean rather quickly (Sparks and Boethel 1987). Furthermore, reinfestation may be facilitated to a greater degree in seed treated soybean since the residual activity of foliar applied insecticide is not present. Therefore, if NSTs did have an effect, it may not have been detected given the study design. Alternatively, if the magnitude of NSTs' impact on *S. festinus* was not strong enough to be identified with the methods in this study, it may provide evidence that NSTs as a control tactic are not a reliable strategy. This conclusion is highlighted by the fact that girdling at R6 (considered late-season) was not impacted by NSTs since the product was no longer present in the plant.

Significant research has been done to determine the overall value neonicotinoid seed treatments provide to growers. In a large, multi-year study that included 170 trials between 2005–2014, North et al. (2016) reported NSTs (plus a base fungicide within each trial) were associated with positive overall economic returns in 67% of trials with an average yield increase of 132 kg/ha per trial. However, there is still discrepancy between studies when taking specific pests into account. McCormack and Ragsdale (2006) reported that thiamethoxam seed treatments reduced season-long soybean aphid (*Aphis glycines* Matsumura) populations by 40–66% but did not report significant yield increases. Reisig et al. (2012) also reported seed treatments reduced thrips populations in the early season but was not associated with significant yield increases. Our field study revealed similar findings regarding yield loss prevention associated with NSTs.

Experiments in this study utilized single cropping where no crop was previously present in the same season. However, in mid-late June or later double-crop plantings, NSTs may have an immediate effect on resident *S. festinus* populations. Insects would already be prevalent in the environment at that point in the season rather than in the beginning stages of colonization. However, in single cropped soybean, traditional integrated pest management strategies should be considered for control of *S. festinus* rather than relying on preventative measures like NSTs.

2.5. SUMMARY

Most commercially available soybean seed has been previously treated with a combination of fungicide and insecticide seed treatments, thereby raising the question as to the effect magnitude on early season pests (Gaspar et al. 2014). In this study, *S. festinus* was exposed to imidacloprid and thiamethoxam seed treatments to evaluate survivorship and girdling effects in the greenhouse. Since results showed that NSTs influenced survivorship in the greenhouse, an additional field study was done to evaluate potential temporal effects to later season populations. However, no differences were detected in population density, girdling, or yield. Given results of the field trial, it is recommended that traditional integrated management tactics would be more effective to control *S. festinus* in soybean.

CHAPTER 3. INFLUENCE OF SOYBEAN VARIETIES AND SEED TREATMENTS ON INFESTATIONS OF THREECORNERED ALFALFA HOPPER, *SPISSISTILUS FESTINUS* (SAY), IN LOUISIANA SOYBEAN, *GLYCINE MAX* (L.) MERR

3.1. INTRODUCTION

Soybean growers are presented with a range of options when it comes to combatting yield limiting disease and insect pests. Additionally, seed companies have developed various technologies that allow growers to maximize yields. Among these technologies are fungicide and insecticide seed treatments. Both can have a significant effect on yield, and careful consideration must be taken to ensure the appropriate technology is chosen according to the grower's specific needs (Boyd et al. 1997). The practice of applying systemic pesticides to the surface of seed is a common component of soybean yield protection throughout the midsouth (North et al. 2016, Musser et al. 2020). By incorporating fungicides and insecticides into a single seed treatment product, seedling soybean can be simultaneously protected from soil-borne pathogens and insect pests. Recent estimates report 59% of all soybean acreage planted in the midsouth have seed treatments applied to them, 90% in Louisiana (Musser et al. 2020). Since these pesticides tend to be highly water soluble, they are readily absorbed and translocated within the plant (Elbert et al. 2008, Krupke and Alford 2017). This allows active ingredients to be delivered directly to target pests without relying on broadcast spray applications. The most commonly used seed treatments in the US are thiamethoxam (insecticide) and mefenoxam (fungicide), although many more exist (Gaspar et al. 2015, Camargo et al. 2019). In addition, insecticide seed treatments are heavily dominated by neonicotinoids, which is a relatively new insecticide class that acts on acetylcholine receptors in the insect nervous system (Maienfisch et al. 2001, Elbert et al. 2008, Douglas and Tooker 2015). Neonicotinoids have gained wide acceptance as seed treatments given the low mammalian toxicity and efficacy on sap sucking insects (Nauen et al. 2003, Jeschke et al. 2011). However, they have come under scrutiny over suspected negative impacts

to pollinators, soil microbial communities, and waterways (Doublet et al. 2015, Pisa et al. 2015, Tosi et al. 2017, Coulon et al. 2019, Yu et al. 2020).

The widespread adoption of fungicide and neonicotinoid soybean seed treatments in the US has been driven by several factors. Soybean production has shifted to early planting which means seed are planted into colder, wetter soils, increasing the likelihood of infection by seed-borne pathogens (Dorrance et al. 2009). This shift was due to previous research that indicated soybean yield benefits from early plantings and late season drought stresses can be avoided (Bowers 1995, Heatherly 1999, Robinson et al. 2009). Additionally, early planting exposes seedling soybean to early season pests which include wireworms, *Limonius spp.*, *Melanotus spp.*, and *Agiotes mancus* (Say); banded cucumber beetle, *Diabrotica balteata* LeConte; and the threecornered alfalfa hopper, *Spissistilus festinus* (Say) (Baur et al. 2000, Davis et al. 2009, Davis et al. 2010). The combination of disease and arthropod pest challenges that arise in the early season have likely led to seed companies expanding their product lines to include seed protectant products (Esker and Conley 2012, Gaspar et al. 2015). Field crops including grain sorghum, *Sorghum bicolor* (L.); corn, *Zea maize* (L.); cotton, *Gossypium hirsutum* (L.); rice, *Oryza sativa* (L.), and soybean have a range of insecticide and fungicide seed treatments labeled for each crop (US-EPA 2022). In addition to control of insects and disease, previous research has indicated certain neonicotinoid seed treatments can improve crop performance (increased germination rates, improved seedling vigor) amid environmental stressors such as excessive heat and drought conditions (TeKronney et al. 1974, Stamm et al. 2014, Campobenedetto et al. 2020).

Currently, there is mixed consensus on the potential value seed treatments provide to growers. In a 2013 grower survey, yield gains were reported between \$22–48 ha when using seed treatments (Hurley and Mitchell 2016). North et al. (2016) reported yield gains in 67% of

trials surveyed ranging from 70–203 kg/ha across Arkansas, Louisiana, Mississippi, and Tennessee with neonicotinoid treated soybean compared to soybean treated with fungicide only. Research by Mourtzinis et al. (2019) suggested benefits from fungicide + insecticide seed treatments were marginal across fourteen states with yield benefits from 10–220 kg/ha. The study also reported break-even costs were too inconsistent and postulated that observed yield benefits were likely more effected by variations in farm-specific management practices such as planting date and seeding rate. Aside from yield benefit, there is also inconsistent reporting on NST efficacy in controlling sap sucking insects. Reisig et al. (2012) reported thiamethoxam and imidacloprid seed treatments significantly reduced thrips abundance five weeks after planting, but the reduction in thrips populations provided no yield benefit. In a study evaluating NST impacts to the soybean aphid, *Aphis glycines* (Matsumura) and its natural enemies, none of the NSTs tested had a detrimental effect to *A. glycines* but significantly reduced natural enemy abundance in field plots, specifically *Chrysoperla* (Neuroptera: Chrysopidae) and *Nabis americanoferus* (Hemiptera: Nabidae) (Seagraves and Lundgren 2011). Two studies by McCornack and Ragsdale (2006) and Johnson et al. (2008) both reported NSTs did not provide consistent control of aphids nor effective yield protection.

In addition to control failures, NSTs have gained notoriety for potential impact to non-target native pollinators. Camargo et al. (2019) demonstrated that residues of thiamethoxam and mefenoxam (fungicide) accumulate in floral tissue sampled at early flowering (R1–R2) which native pollinators may encounter. Additionally, clothianidin was the primary metabolite detected, which previous research has shown to be more toxic to insects than thiamethoxam (Nauen et al. 2003). Doublet et al. (2015) discovered evidence of synergism between thiacloprid and two common pathogens of *Apis mellifera* (L.), causing increased viral loads in *A. mellifera* larvae.

Posi et al. (2017) reported chronic sublethal thiamethoxam concentrations significantly reduced *A. mellifera* flight duration and flight distance. However, it is difficult to make determinations of the true impact of NSTs on native pollinators at the field level since the window in which NSTs cause mortality is 30 days after planting (Magalhaes et al. 2009). Most soybean begins flowering ~60 and ~70 days after planting (MGIV and MGVI, respectively) which is longer than the time NSTs remain at effective levels (Purcell et al. 2014). Therefore, in theory, native pollinators should not encounter detrimental concentrations of NSTs by the time of field arrival.

In addition to seed treatments, soybean production in the US has shifted to earlier planting dates in concert with earlier maturing varieties (Heatherly 1999). This was done to avoid late season drought pressure, which research has shown to provide significant yield benefits (Heatherly et al. 1998). Maturity groups typically grown in the midsouth include MGIII, MGIV, and MGVI (Salmeron et al. 2014, 2015). Although previous research has identified differences in insect populations between traditional vs. early planting systems, little is known on the effect of maturity group on insects. Lee et al. (2021) reported higher hemipteran pest populations in MGVI compared to MGIII from 2012–2018 but not compared to MGIV. Alternatively, hemipteran pests tend to establish high populations in early planted soybean. Baur et al. (2000) observed significantly higher populations as well as increased frequency of threshold populations for southern green stink bug, *Nezara viridula* (L.), and threecornered alfalfa hopper, *Spissistilus festinus* (Say), in early plantings when compared to conventional plantings.

The efficacy of current management practices on the colonization and establishment of early season pests, such as *S. festinus*, have not yet been fully elucidated. The shift to earlier planting dates, introduction of neonicotinoid seed treatments (NSTs), and maturity group

selection have not been evaluated collectively when researching *S. festinus* management. Therefore, experiments were done using a range of soybean varieties complete with commercially treated combinations of fungicide and insecticide seed treatments to identify potential effects to *S. festinus* populations.

3.2. MATERIALS AND METHODS

All research was conducted at the Doyle Chambers Research Station in Baton Rouge, LA. Field trials spanned two years and incorporated ten soybean varieties per year, each treated with different fungicide/neonicotinoid STs (Tables 3.1.–3.3.). In the field, varieties were separated by MG and arranged in separate RCBDs with four blocks. Experiments were replicated twice per year. Soybean was planted in 27.87 m² plots containing four 9.1 m rows on 0.91 m centers at a seeding rate of 8 seed/0.30 m of row.

Table 3.1. Summary of planting dates.

Year	Planting date
2020	May 7 th
	May 22 nd
2021	April 29 th
	June 9 th

Beginning three weeks after planting and every week thereafter, weekly sweep net samples (25 sweeps) were taken from the first two rows per plot, alternating with the second two rows per plot the following week. This was done to ensure a representative sample would be taken since the frequency of sampling would likely disturb the *S. festinus* population. Insect numbers were converted to cumulative insect days (CID) using the formula:

$$\Sigma\{(X_{i+1} - X_i)[(Y_i + Y_{i+1}) \div 2]\}$$

where X_i and X_{i+1} are adjacent points in time and Y_i and Y_{i+1} are corresponding number of insects sampled at each point (Ruppel 1983). Mean plant height was taken from five randomly selected plants per plot at R3. The same sampling method was used at R6 when taking mean girdling counts.

Since varieties changed from 2020 to 2021, analysis was done per year. Cumulative insect days, plant height, and girdling data were each subjected to a One-Way analysis of variance (ANOVA) using PROC MIXED (SAS 2021) to test the effect of variety. Normality testing was done using Shapiro-Wilks in PROC UNIVARIATE (SAS 2021). Means were separated using Tukey's HSD ($P \leq 0.05$) when significance was found.

Table 3.2. Summary of 2020 soybean varieties and respective insecticide/fungicide seed treatments.

Brand	Variety	MG	Product name	Seed treatment package (insecticides listed first)
Armor	48-D25	MGIV	Warden CX	thiamethoxam, fludioxonil, mefenoxam, sedaxane
AsGrow	AG48X9		Acceleron Elite	imidacloprid, fluxapyroxad, metalaxyl, pyraclostrobin
BASF	CZ4570X		Poncho Votivo + Obvious Plus	clothianidin, <i>Bacillus firmus</i> I-1582, thiophanate-methyl, metalaxyl, fluxapyroxad, pyraclostrobin
Pioneer	P46A86X		Lumigen + Gaucho 600	imidacloprid, metalaxyl, penflufen, prothioconazole
Progeny	4821RX		Poncho Votivo + Obvious Plus	clothianidin, <i>Bacillus firmus</i> I-1582, thiophanate-methyl, metalaxyl, fluxapyroxad, pyraclostrobin
Armor	55-D57	MGV	Warden CX	thiamethoxam, fludioxonil, mefenoxam, sedaxane
BASF	CZ5299X		Poncho Votivo + Obvious Plus	clothianidin, <i>Bacillus firmus</i> I-1582, thiophanate-methyl, metalaxyl, fluxapyroxad, pyraclostrobin
AsGrow	AG53X0		Acceleron Elite	imidacloprid, fluxapyroxad, metalaxyl, pyraclostrobin
Pioneer	P54A54X		Lumigen + Gaucho 600	imidacloprid, metalaxyl, penflufen, prothioconazole
Progeny	5252RX		Poncho Votivo + Obvious Plus	clothianidin, <i>Bacillus firmus</i> I-1582, thiophanate-methyl, metalaxyl, fluxapyroxad, pyraclostrobin

Table 3.3. Summary of 2021 soybean varieties and respective insecticide/fungicide seed treatments.

Brand	Variety	MG	Product name	Seed treatment package (insecticides listed first)
Syngenta	S49-F5X	MGIV	CruiserMaxx	thiamethoxam, mefenoxam, fludioxonil
Pioneer	46X86X		Lumigen + Gaucho 600	imidacloprid, metalaxyl, penflufen, prothioconazole
Delta Grow	46X65RR2X		Magnum	thiamethoxam
Progeny	P4806XFS		Poncho Votivo + Obvious Plus	clothianidin, <i>Bacillus firmus</i> I-1582, thiophanate-methyl, metalaxyl, fluxapyroxad, pyraclostrobin
Bayer	AG48X9		Acceleron Elite	imidacloprid, fluxapyroxad, metalaxyl, pyraclostrobin
Pioneer	P54A54X	MGV	Lumigen + Gaucho 600	imidacloprid, metalaxyl, penflufen, prothioconazole
Delta Grow	52X05		Magnum	thiamethoxam
Dyna-Gro	S56XT99		CruiserMaxx	thiamethoxam, mefenoxam, fludioxonil
Progeny	P5554RX		Poncho Votivo + Obvious Plus	clothianidin, <i>Bacillus firmus</i> I-1582, thiophanate-methyl, metalaxyl, fluxapyroxad, pyraclostrobin
Bayer	AG53XF2		Acceleron Elite	imidacloprid, fluxapyroxad, metalaxyl, pyraclostrobin

3.3. RESULTS

Results of height analysis are presented in Fig. 3.1. In 2020, height was significantly influenced by variety ($F = 21.93$; $df = 9, 359$; $P < 0.0001$). Results of CID analysis by variety are presented in Fig. 2. In 2020, *S. festinus* populations were significantly impacted by variety ($F = 4.96$; $df = 4, 34$; $P = 0.0004$). Progeny 5252RX in MGIV averaged lower mean CID compared to Pioneer P54A54X and Armor 55-D57. However, Progeny 5252RX was not different from BASF CZ 5299X, Asgrow AG53X0, or any of the MGIV varieties. Overall mean *S. festinus* populations in 2021 experienced a three-fold decrease from 2020. No differences in variety were observed in 2021. For this reason, height data for 2021 varieties is not presented as it provides no inferential value. When pooling variety, the effect of year as well as the interaction of MG and year on overall *S. festinus* CID were significant (year, $F = 198.62$; $df = 1, 140$; $P < 0.0001$; MG x year, $F = 7.19$; $df = 1, 140$; $P = 0.0082$) (Table 3.4.).

Table 3.4. Effect of maturity group and year on *S. festinus* CID. Means followed by the same letter within columns are not significantly different ($P = 0.05$, Tukey's HSD).

Year	MG	<i>S. festinus</i> CID (\pm SEM)
2020	MGIV	458.9 \pm 17.7b
	MGV	554.5 \pm 27.7a
2021	MGIV	167.9 \pm 8.3c
	MGV	165.3 \pm 5.5c

Results of girdling analysis are presented in Fig. 3.3. Variety had a significant effect on mean number of girdles per plant at R6 in 2020 ($F = 7.48$; $df = 4, 34$; $P = 0.0002$). In MGIV varieties, BASF CZ5299X and Progeny 5252RX averaged significantly lower mean girdles per plant compared to Pioneer P54A54X but not to Armor 55-D57, Asgrow AG5X0, or any of the MGIV varieties. In 2021, no differences in girdling were observed between varieties.

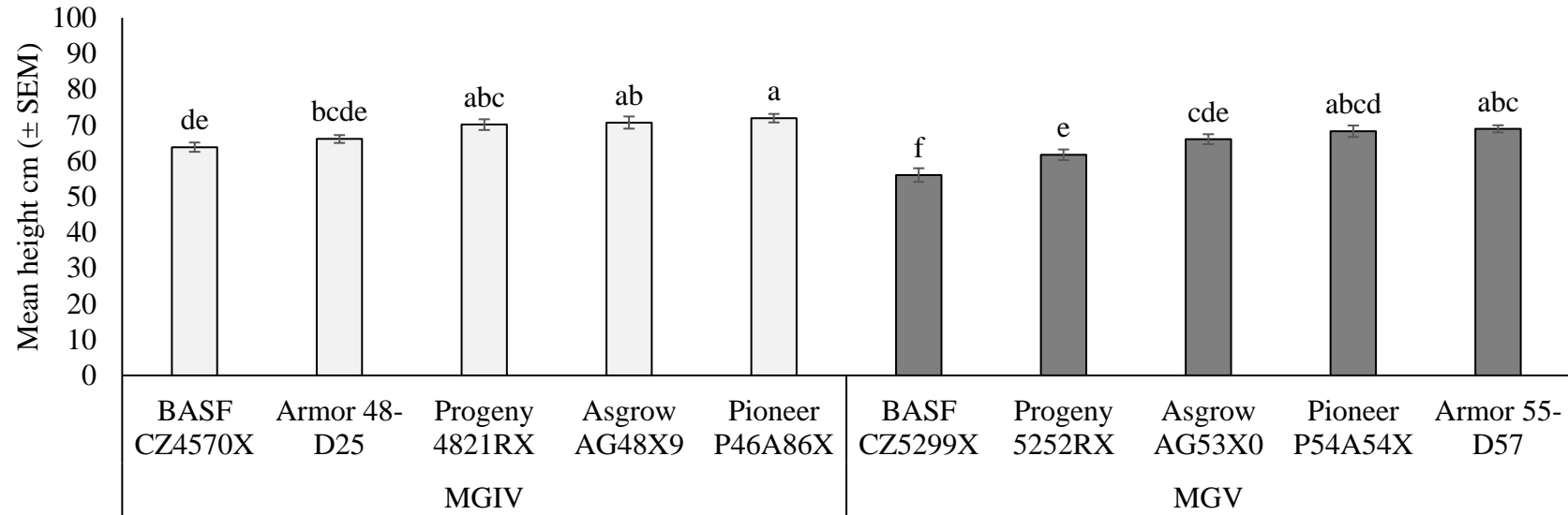
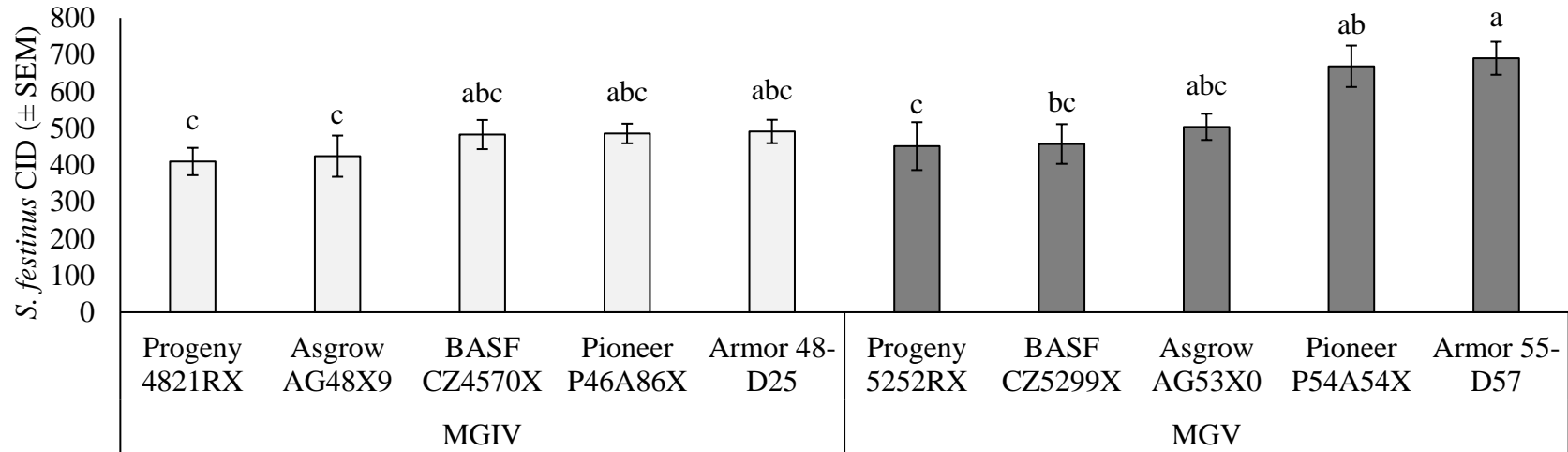


Figure 3.1. Comparison of height among 2020 soybean varieties sampled at R3. Bars with the same letter are not significantly different. (P = 0.05, Tukey's HSD)

a)



b)

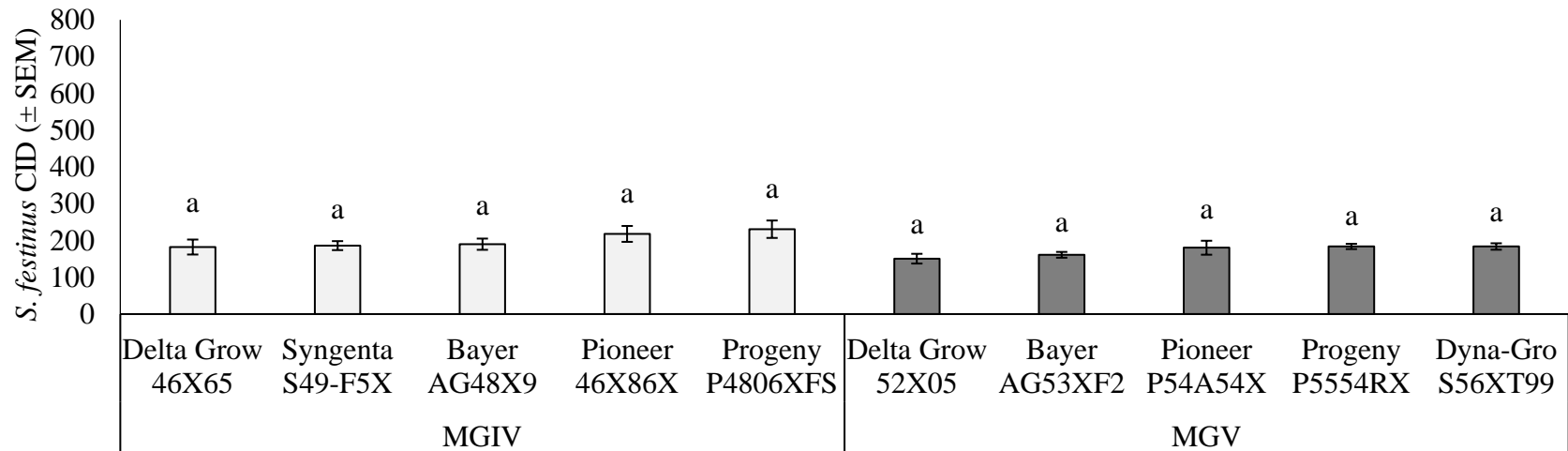
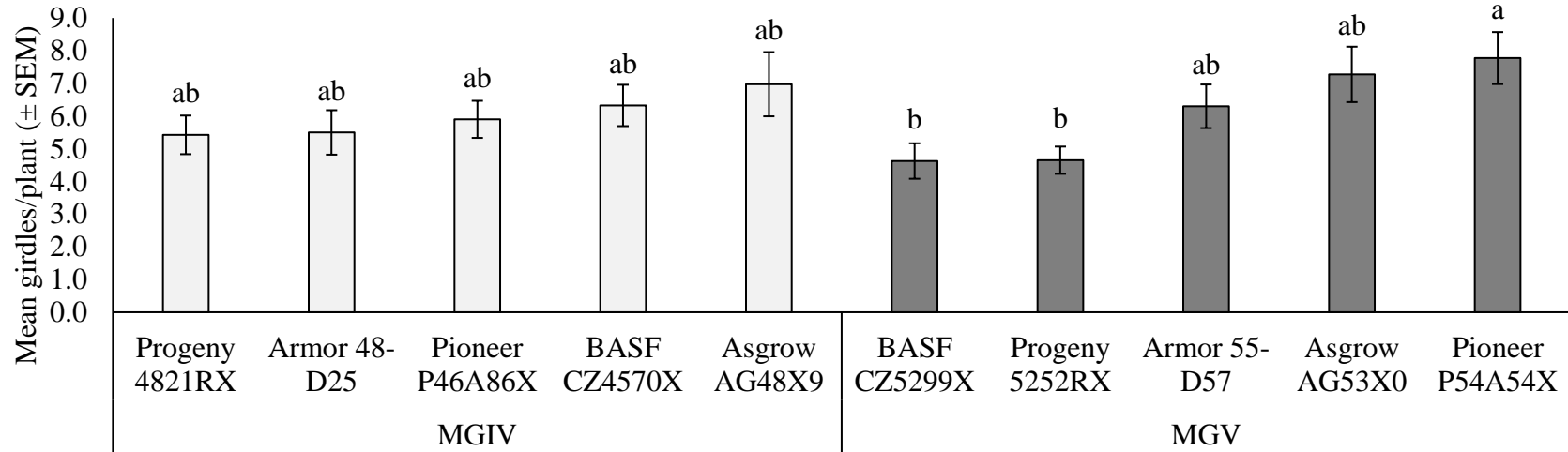


Figure 3.2. Effect of selected soybean varieties on *S. festinus* CID in 2020 (a) and 2021 (b). Soybean was sampled from V4 to R8. Bars followed by the same letter within maturity group are not significantly different ($P = 0.05$, Tukey's HSD).

a)



b)

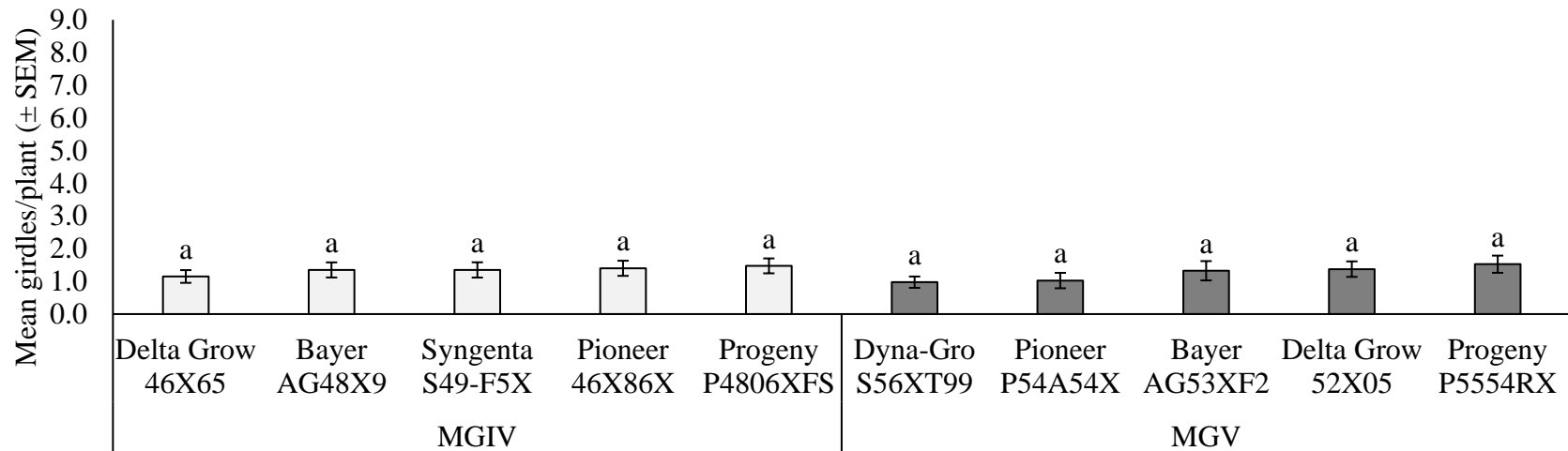


Figure 3.3. Effect of selected soybean varieties on number of girdles per five plants at R6 in 2020 (a) and 2021 (b). Bars followed by the same letter within maturity group are not significantly different ($P = 0.05$, Tukey's HSD).)

3.4. DISCUSSION

The soybean varieties selected in this study span a range of maturation rate, growth habit, herbicide traits, and seed treatment packages. Each variety was commercially treated with a combination of neonicotinoid insecticide and fungicide seed treatments. This provides a representative range of soybean technologies currently used in midsouth production that may have an impact on insect pest populations. Previously, no known mechanism of varietal resistance has been identified regarding *S. festinus* in soybean but has been investigated in alfalfa (Mollenbeck and Quisenberry 1992, Mollenbeck and Quisenberry 1993). Although our study did not investigate resistance in the traditional sense given the inclusion of seed treatments, the potential effect of variety must not be ignored since seed treatments do not provide season long protection against soybean pests (Magalhaes et al. 2009, Reisig et al. 2012). Additionally, our findings are the first reports of soybean variety in conjunction with commercial seed treatments having an effect on *S. festinus* populations. The only maturity groups missing from our study are MGIII and MGVI. However, these are used in reduced hectares in Louisiana compared to those used in our study (Temple et al. 2013).

This study reveals the status of selected varieties + seed treatments used in Louisiana against *S. festinus* seasonal populations. Analysis of plant height was done to provide insight into why population density and girdling were affected by variety. It was discovered that the varieties averaging the lowest CID means in 2020 were also among the shortest compared to other varieties and the reverse can be said of the tallest varieties. This raises the question as to the effect of plant height on *S. festinus* in-field movement when staggered plant heights are present. Johnson et al. (1997) reported that localized flight of *S. festinus* was primarily within the first 30 cm above the soybean canopy when using sticky trap cards as a sampling method. In a field with

staggered plant height, taller plants may act as a trap for *S. festinus* when migrating out of shorter soybean. This could result in greater populations being sampled in taller soybean which may also contribute to the clustered spatial distribution of *S. festinus* reported in previous research (Sparks and Boethel 1987, Spurgeon and Muller 1991, Trichilo et al. 1993, Preto et al. 2019). However, if plant height is the reason differences were observed, this effect may disappear in a field of uniform height. As a consequence, plant height may not affect *S. festinus*. Therefore, future research is needed to understand how plant height affects *S. festinus* movement in the field.

Other than overall MGIV soybean supporting greater populations than MGIV, results showed no clear trend in CID among individual varieties tested in 2020. In general, most varieties supported similar *S. festinus* populations. All MGIV varieties were not different from each other and only one MGIV variety ('Armor 55-D57') was different from two other MGIV varieties ('Progeny 525RX' and 'BASF CZ5299X'). Note that differences in population were only found in MGIV soybean in 2020, which observed greater overall pest pressure than 2021. However, comparison of varieties between years cannot be made since varieties + seed treatments changed. When using girdling as a proxy for *S. festinus* populations, 'BASF CZ5299X' as well as both 'Progeny' varieties supported reduced CID as well as girdles per plant in 2020. However, correlating population density to girdling was not a component of the study design, so inferences regarding potential correlation cannot be made. In addition, the intermittent economic significance of *S. festinus* across the midsouth may not provide the incentive to select resistance to this pest in soybean breeding programs. Therefore, the results of this study may only be useful in predicting which varieties may support higher *S. festinus* populations in a given season.

The impact of respective seed treatment packages is difficult to divorce from varietal effects and vice versa since no non-treated check was included in this study. Nonetheless, it is

worth noting that ‘BASF CZ5299X’ was found to average low CID and girdling. In addition to being a shorter variety, it was treated with clothianidin which is known to be significantly more toxic to insects compared to other NSTs (Nauen et al. 2003). However, one other MGIV variety in 2020 was also treated with clothianidin and *S. festinus* populations were not different from all other varieties tested.

Lower populations in 2021 may be attributed to an unprecedented freezing event in early spring of 2021 where daytime temperatures fell to 0 °C over a five-day period (NWS-NOAA 2022). This may have slowed *S. festinus* movement from overwintering sites into early season soybean, thereby delaying colonization until after insecticide seed treatments have lost efficacy. However, previous reports by Newsom et al. (1983) stated highly active adults were observed overwintering in piney underbrush (*Pinus* spp.) in subfreezing temperatures (°C). Nonetheless, near freezing climactic conditions may still have affected *S. festinus* movement in 2021.

3.5. SUMMARY

The objective of this study was to compare the effects of common soybean varieties treated with commercially available seed treatments on resident *S. festinus* populations. Results indicate that certain varieties combined with respective seed treatment may have an effect on CID and feeding behavior, particularly in MGIV soybean. However, this was only observed in 2020. The results of this study indicate that variety as well as commercially available seed treatment packages offer inconsistent control of *S. festinus* and, therefore, emphasis should be placed on pest monitoring, especially in MGIV soybean.

CHAPTER 4. LATE SEASON ACTION THRESHOLD RE-EVALUATION OF THREECORNERED ALFALFA HOPPER, *SPISSISTILUS FESTINUS* (SAY), IN LOUISIANA SOYBEAN, *GLYCINE MAX* (L.) MERR

4.1. INTRODUCTION

Approximately 90% of all plant oil in the US is produced from soybean, *Glycine max* (L.) Merr. (USDA-ERS). It is considered one of the most important oil and feed crops grown in the US with 35,000,00 ha planted annually (USDA-ERS). In Louisiana, soybean is the most planted field crop with 45,000 ha planted in 2021 and provides significant contributions to the Louisiana agricultural economy (\$556,818,000 in 2020) (USDA/NASS 2021). However, losses in yield due to insect pests and associated control costs range from \$56,000,000 - \$60,000,000 each year (Musser et al. 2016, Musser et al. 2017, Musser et al. 2018, Musser et al. 2019, Musser et al. 2020).

To optimize yield potential, careful attention is placed on controlling weed, disease, and insect pests (Boerma and Specht 2004, Funderburk et al. 1999). Soybean fields are frequently scouted to confirm the presence of pests, identify the major species, and determine population levels (Stern 1973). Failure to accurately assess pest populations and take appropriate action leads to significant losses. For many insects, research has been done to describe the relationship between pest pressure and economic loss. Efforts by Stern (1973) and Pedigo et al. (1986) led to the development of economic and action thresholds that allow growers to apply tactics prior to losses.

Spissistilus festinus was first described as a pest of soybean by Wildermuth in 1915, however, a threshold was not established until 1984 by Sparks and Newsom. In conventional plantings of later maturing determinate soybean ('Bragg' MGVII), Sparks and Newsom (1984) reported late season *S. festinus* populations caused a yield reduction of 1514.6 kg/ha as mean adult populations approached 162 insects per 100 sweeps with a standard 38 cm diameter sweep

net. However, yield loss did not correlate with early season populations. It was theorized that *S. festinus* damage suffered during vegetative growth stages had little impact on yield due to the compensatory physiological response to stand loss by soybean. This agrees with previous research by Caviness and Miner (1961) in which they reported that 45% stand reductions two weeks before bloom did not result in statistically significant yield loss. Furthermore, Muller and Jones (1983) and Tugwell and Miner (1967) also made similar conclusions with respect to soybean stand loss compensation. Efforts by Sparks and Newsom (1984) led to the current accepted action threshold of one adult per sweep after pod set (R3) (Fehr et al. 1971). This threshold assumes nymphs already contribute to feeding damage; however, they are not specifically included since they are more difficult to accurately sample (Sparks and Newsom 1984). There is no reported economic threshold for *S. festinus* in soybean.

Across the midsouth, there is no consensus on the pest status of *S. festinus* in soybean. The Arkansas Cooperative Extension Service recommends insecticide applications when >50% of plants are girdled and does not recommend further monitoring after plants have surpassed 25 cm in height (Studebaker et al. 2022). The Alabama, Georgia, and Mississippi State Cooperative Extension Services all recommend treatment at 2 adults/sweep (Ramsey 2015, Roberts et al. 2022). In North Carolina, *S. festinus* is rarely considered an economic pest and insecticide applications are only advised in seedling soybean (Reisig et al. 2022). The action threshold provided by the Tennessee Extension Service is when >50% of plants are infested with adults or nymphs only when soybean is less than 30 cm in height (Stewart and McClure 2020). The Virginia Cooperative Extension service recommends treating when >10% of seedling plants are infested (Cahoon et al. 2022). The action threshold provided by the Texas A&M Extension Service is when “main stem girdling threatens stand” (Vyavhare et al. 2022). The Clemson

Extension Service recommends treatments when adults are “more than several per sweep” (Greene 2022). As mentioned previously, Louisiana maintains the action threshold of 1 adult/sweep, which remains unchanged since it was developed in the mid-80s (Sparks and Newsom 1984).

Sparks and Newsom (1984) laid the framework for effective management of *S. festinus* in Louisiana. However, two more recent studies, Pulakkatu-thodi (2010) and Ramsey (2015), have reported late season thresholds two to three times the current recommendation of one adult per sweep have no relationship with yield loss. Ramsey (2015) even argues that *S. festinus* should no longer be considered an economically significant pest of soybean. However, the difference in research methods across the three studies highlight gaps in the current knowledge of *S. festinus* life history and management.

Firstly, studies by Sparks and Newsom (1984) accounted for infestation time over an entire season. Experiments in these studies allowed native populations to colonize from overwintering sites and progress through by generational development in the field. However, studies by Pulakkatu-thodi (2010) and Ramsey (2015) artificially infested test plots with adults only for, at most, two weeks. Research is needed that incorporates not only natural colonization by native populations, but also modern maturity groups and planting dates. Thus, to address these needs, experiments were conducted to re-evaluate current action thresholds using current maturity groups grown in Louisiana under natural infestations.

4.2. MATERIALS AND METHODS

Field trials were conducted from 2020 to 2021 at the Doyle Chambers Research Station (DC) in Baton Rouge, LA and the Dean Lee Research Station (DL) in Alexandria, LA. Summary of varieties and planting dates are presented in Table 4.1. Soybean was planted in a RCBD using

27.87 m² plots containing four 9.1 m rows on 0.91 m centers at a seeding rate of 8 seed/0.30 m of row.

Table 4.1. Summary of planting dates, locations, and varieties. UA varieties were purchased from University of Arkansas, Foundation Seed Program in Fayetteville, AR.

Year	Variety	Planting date	Location
2020	UA5414RR	May 7 th	Doyle Chambers Research Station
	UA5414RR	May 22 nd	Doyle Chambers Research Station
2021	UA5715GT	April 29 th	Doyle Chambers Research Station
	UA5715GT	June 9 th	Doyle Chambers Research Station
	CZ4689X	June 4 th	Dean Lee Research Station

Experiments at Doyle Chambers Research Station were replicated twice each year. Experiments at Dean Lee Research Station were not replicated and only took place in 2021. Beginning at R3 (beginning pod development) (Fehr et al. 1971), weekly sweep net samples (25 sweeps) were taken from the first two rows per plot, alternating with the second two rows per plot each consecutive week. This was done to ensure a representative sample would be taken each week since the frequency of sampling would likely disturb the *S. festinus* population. Sampling duration was either five or eight weeks depending on location. To achieve a range of population densities as well as control of stink bugs and lepidopteran pests, tank mixes of Bracket 97 (acephate, 2.34 g/27.87 m²) and Grizzly Too (λ -cyhalothrin, 0.326 mL/27.87 m²) were applied to test plots immediately after sampling using a CO₂ backpack sprayer calibrated to deliver 140.2L/hectare at 241.3 kPa using four T-jet 80015VS flat-fan nozzles. Applications were initiated once *S. festinus* populations reached a predetermined action threshold treatment as indicated below. Treatments consisted of the following: the current action threshold (1

adult/sweep), two sub-thresholds (0.25x, 0.5x), two supra-thresholds (2x, 3x), a zero threshold (0x), and control (no threshold). Once soybean reached harvest maturity, five randomly selected plants per plot were destructively sampled and placed in a walk-in propane seed dryer at the Doyle Chambers Research Station Seed Warehouse set to 43° C for 5–7 days to lower seed moisture to 13%. Harvest parameters evaluated were total number of seed per five plants and one-hundred seed weight in grams.

Insect numbers and yield data were subjected to a One-Way analysis of variance (ANOVA) using PROC MIXED (SAS 2021) with treatment being the lone fixed effect analyzed by week. Since varieties changed at Doyle Chambers between years, results are presented by year. Results from Dean Lee were also presented separately for the same reason. When multiple plantings per variety occurred within year, they were treated as replicates and combined for analysis. Since sampling was cut short at three weeks for the June 9th, 2021 planting, it was excluded from analysis because comparisons with equal spray intervals (weeks) could not be made within that year. Normality testing was done using the Shapiro-Wilks test in PROC UNIVARIATE (SAS 2021). Means were separated using Tukey's HSD ($\alpha \leq 0.05$) when significance was found.

4.3. RESULTS

Threshold frequencies are presented in Tables 4.2. and 4.3. In all locations and years, 0x thresholds received the total amount of weekly applications that could be made over the study period. However, the number of plots receiving insecticide applications per all other treatments varied across locations and years. The true action threshold (1x) was reached five times across two plantings in 2020 at Doyle Chambers. However, it was not reached in 2021 in the same location. The highest threshold reached at Dean Lee was 25% of the action threshold and was

observed only five times. Across all locations and years, no threshold treatment greater than 1x was observed.

Table 4.2. Number of insecticide applications and percent of plots sprayed per treatment at Doyle Chambers. Both plantings are included for 2020 (64 total possible applications per treatment over 8 wk). One planting is included for 2021 (32 total possible applications per treatment over 8 wk).

Year	Treatment	No. of apps per treatment	% Plots sprayed
2020	0x	64	100%
	.25x	44	69%
	.5x	22	34%
	1x	5	8%
	2x	0	0%
	3x	0	0%
	UTC	0	0%
2021	0x	32	100%
	.25x	8	25%
	.5x	1	3%
	1x	0	0%
	2x	0	0%
	3x	0	0%
	UTC	0	0%

Table 4.3. Number of insecticide applications and percent of plots sprayed per treatment at Dean Lee in 2021. One planting is included (20 total possible applications per treatment over 5 wk).

Treatment	No. of apps per treatment	% Plots sprayed
0x	20	100%
.25x	5	25%
.5x	0	0%
1x	0	0%
2x	0	0%
3x	0	0%
UTC	0	0%

Analysis of weekly insecticide sprays are presented in Tables 4.4–4.6 . In 2020, insecticide applications at Doyle Chambers only impacted *S. festinus* populations in four of eight

weeks and only two of eight weeks in 2021 (Tables 4.4. and 4.5.). Weeks in which differences were found in 2020 were two ($F = 4.01$; $df = 6, 48$; $P = 0.0025$), five ($F = 6.49$; $df = 6, 48$; $P < 0.0001$), seven ($F = 6.93$; $df = 6, 48$; $P < 0.0001$), and eight ($F = 3.79$; $df = 6, 48$; $P = 0.0036$). Weeks in which differences were found in 2021 were one ($F = 2.54$; $df = 6, 21$; $P < 0.0522$) and six ($F = 3.18$; $df = 6, 21$; $P < 0.0223$). Insecticide applications did not impact *S. festinus* populations at any point at Dean Lee (Table 4.6.).

Analysis of hundred seed and weight and total seed per five plants across all locations and years are presented in Tables 4.7. and 4.8. No yield benefit to hundred seed weight or total seed as a result of insecticide applications was observed in any location or year.

Table 4.4. Effect of insecticide sprays in 2020 applied at different threshold levels on number of *S. festinus* caught in 25 sweeps at Doyle Chambers (\pm SE). Means followed by the same letter within week are not significantly different ($P = 0.05$, Tukey's HSD).

Week	0x	.25x	.5x	1x	2x	3x	UTC
1	3.1 \pm 0.4a	4.4 \pm 0.9a	4.8 \pm 1.3a	6.4 \pm 1.6a	5.9 \pm 1.6a	7.6 \pm 2.3a	7.6 \pm 2.8a
2	6.3 \pm 1.2bc	5.9 \pm 1.3c	7.6 \pm 0.8abc	8.1 \pm 1.1abc	12.1 \pm 1.1ab	10.1 \pm 2.0abc	12.6 \pm 1.8a
3	11.3 \pm 1.8a	8.0 \pm 1.0a	8.6 \pm 1.7a	13.0 \pm 2.4a	15.4 \pm 2.8a	16.4 \pm 2.7a	13.6 \pm 2.7a
4	10.1 \pm 4.4a	13.1 \pm 3.7a	16.4 \pm 3.0a	19.0 \pm 4.0a	17.6 \pm 3.1a	18.8 \pm 3.3a	16.5 \pm 4.1a
5	5.3 \pm 1.3c	8.4 \pm 1.9bc	9.6 \pm 1.7abc	14.1 \pm 1.8ab	17.9 \pm 2.3a	16.8 \pm 2.1ab	16.8 \pm 2.6ab
6	9.6 \pm 2.8a	12.4 \pm 2.4a	12.8 \pm 2.3a	16.0 \pm 3.2a	15.9 \pm 2.1a	14.9 \pm 1.5a	15.4 \pm 1.6a
7	3.8 \pm 0.9c	6.5 \pm 1.3bc	4.6 \pm 1.1bc	11.3 \pm 2.0ab	10.8 \pm 1.7abc	16.4 \pm 2.6a	11.6 \pm 1.8ab
8	4.4 \pm 0.8b	14.3 \pm 3.1a	13.6 \pm 2.6a	13.4 \pm 1.4a	13.1 \pm 1.9ab	15.0 \pm 1.6a	15.4 \pm 2.4a

Table 4.5. Effect of insecticide sprays in 2021 applied at different threshold levels on *S. festinus* caught in 25 sweeps at Doyle Chambers (\pm SE). Means followed by the same letter within week are not significantly different ($P = 0.05$, Tukey's HSD)

Week	0x	.25x	.5x	1x	2x	3x	UTC
1	0.0 \pm 0.0b	1.3 \pm 0.5ab	1.3 \pm 0.5ab	0.8 \pm 0.8ab	0.5 \pm 0.3ab	1.5 \pm 0.6ab	2.5 \pm 0.5a
2	1.5 \pm 0.6a	1.0 \pm 0.7a	2.8 \pm 0.8a	2.8 \pm 1.8a	2.0 \pm 0.9a	1.8 \pm 0.9a	2.0 \pm 1.1a
3	2.0 \pm 0.7a	3.3 \pm 1.4a	3.0 \pm 0.9a	2.8 \pm 0.8a	3.8 \pm 0.8a	1.5 \pm 0.9a	3.0 \pm 1.1a
4	2.0 \pm 0.7a	1.3 \pm 0.8a	3.8 \pm 1.1a	1.0 \pm 0.4a	3.3 \pm 1.5a	3.0 \pm 1.7a	2.5 \pm 0.6a

(table cont'd)

Week	0x	.25x	.5x	1x	2x	3x	UTC
5	2.3 ± 0.8a	1.8 ± 0.9a	1.8 ± 1.1a	1.8 ± 1.0a	3.0 ± 1.8a	2.5 ± 0.6a	5.8 ± 1.9a
6	1.3 ± 0.8b	7.5 ± 0.9a	6.8 ± 0.9a	6.5 ± 1.9ab	6.5 ± 1.0ab	7.0 ± 1.4a	6.5 ± 1.2ab
7	4.3 ± 1.3a	5.0 ± 1.1a	8.0 ± 1.1a	7.5 ± 1.3a	8.3 ± 1.0a	7.0 ± 1.4a	5.0 ± 1.6a
8	5.3 ± 2.5a	2.3 ± 1.6a	8.3 ± 2.6a	9.8 ± 1.4a	9.8 ± 2.0a	7.3 ± 1.3a	7.5 ± 1.7a

(table cont'd)

Table 4.6. Effect of insecticide sprays in 2021 applied at different threshold levels on *S. festinus* caught in 25 sweeps at Dean Lee (± SE). Means followed by the same letter within week are not significantly different ($P = 0.05$, Tukey's HSD)

Week	0x	.25x	.5x	1x	2x	3x	UTC
1	2.0 ± 1.1a	2.0 ± 0.4	4.8 ± 1.4a	2.5 ± 0.3a	4.0 ± 1.3a	4.3 ± 0.8a	3.0 ± 0.6a
2	4.0 ± 1.4a	4.8 ± 1.2a	2.8 ± 0.9a	2.0 ± 0.9a	5.3 ± 1.8a	6.5 ± 0.6a	5.0 ± 1.7a
3	11.0 ± 0.9a	8.3 ± 1.7a	6.3 ± 0.9a	10.8 ± 2.7a	11.0 ± 2.7a	8.0 ± 2.1a	9.0 ± 1.2a
4	3.0 ± 1.2a	4.8 ± 0.5a	5.8 ± 1.9a	7.3 ± 1.9a	6.0 ± 0.9a	10.0 ± 2.2a	7.3 ± 0.5a
5	3.5 ± 0.6a	3.8 ± 0.9a	1.5 ± 0.3a	3.3 ± 1.3a	5.3 ± 1.8a	3.5 ± 1.3a	4.8 ± 1.9a

Table 4.7. Effect of threshold-based insecticide applications on mean # of seeds per five plants (\pm SE). Means followed by the same letter within rows are not significantly different ($P = 0.05$, Tukey's HSD).

Year	Location	Threshold (\pm SEM)						
		0x	0.25x	0.5x	1x	2x	3x	UTC
2020	Doyle Chambers Research Station	1094.6 \pm 123.2a	987.3 \pm 100.0a	1083.4 \pm 91.5a	940.1 \pm 109.4a	882.6 \pm 97.9a	1135.1 \pm 71.3a	1029.6 \pm 113.5a
2021	Doyle Chambers Research Station	1029.8 \pm 122.6a	869.1 \pm 91.5a	814.9 \pm 136.0a	876.6 \pm 89.1a	907.5 \pm 116.3a	835.6 \pm 90.8a	802.1 \pm 67.4a
2021	Dean Lee Research Station	639.5 \pm 39.9a	684.3 \pm 80.1a	625.0 \pm 68.3a	610.5 \pm 80.1a	537.8 \pm 28.2a	588.8 \pm 43.7a	566.3 \pm 34.5a

Table 4.8. Effect of threshold-based insecticide applications on hundred seed weight (g). Means followed by the same letter within rows are not significantly different ($P = 0.05$, Tukey's HSD).

Year	Location	Threshold (\pm SEM)						
		0x	0.25x	0.5x	1x	2x	3x	UTC
2020	Doyle Chambers Research Station	12.5 \pm 0.3a	12.9 \pm 0.3a	12.5 \pm 0.1a	12.7 \pm 0.2a	12.6 \pm 0.2a	12.5 \pm 0.6a	12.4 \pm 0.2a
2021	Doyle Chambers Research Station	12.7 \pm 0.1a	12.9 \pm 0.2a	12.5 \pm 0.2a	12.9 \pm 0.2a	12.1 \pm 0.2a	12.7 \pm 0.2a	12.8 \pm 0.7a
2021	Dean Lee Research Station	14.5 \pm 0.3a	13.0 \pm 2.0a	14.1 \pm 0.4a	14.0 \pm 0.3a	14.1 \pm 0.4a	14.3 \pm 0.5a	16.3 \pm 1.3a

4.4. DISCUSSION

In all locations and years, maintaining different population densities throughout the season proved difficult as *S. festinus* quickly immigrated back to recently sprayed plots. Even though the zero threshold plots received weekly insecticide applications, populations within those plots were not consistently different from other sub-threshold plots in all locations and years. Failure to maintain a near-zero *S. festinus* population in 0x plots illustrates that *S. festinus* is highly mobile and can reinfest treated plots rather quickly. Sparks and Boethel (1987) reported high levels of reinfestation after applying short-residual organophosphate insecticides. Insecticides used in our study incorporated an organophosphate (Bracket 97) plus a pyrethroid (Grizzly Too). Both are labelled for *S. festinus* in soybean, yet the combination of both chemistries failed to provide consistent control. However, previous research has reported foliar applied pyrethroid and organophosphates offer effective control of *S. festinus*. Rohlf et al. (1983) tested acephate (0.66 lbs a.i./a), permethrin (0.04 – 0.1 lbs a.i./a), and carbaryl (1.0 lbs a.i./a) and reported all treatments significantly reduced adult *S. festinus* populations 7 days after treatment (DAT). Wilson et al. (1985) reported acephate (0.75 lbs a.i./a), β -cyfluthrin (0.025-0.0125 lbs a.i./a), carbofuran (0.5 lbs a.i./a), and methyl parathion (0.5 lbs a.i./a) significantly reduced adult *S. festinus* populations below 50% of pre-spray levels 14 DAT. Fitzpatrick et al. (2000) reported foliar applications of thiamethoxam (0.0625 lbs a.i./a) significantly reduced adult populations by 78% compared to the control 10 DAT. Howard and Akin (2011) tested high and low dose applications of several pyrethroids and one organophosphate and reported that λ -cyhalothrin, β -cyfluthrin, esfenvalerate, z-cypermethrin, bifenthrin, and acephate significantly reduced *S. festinus* populations below threshold levels compared to the untreated check 7 DAT with exception of low dose lambda-cyhalothrin (0.0156 lbs a.i./a). Currently, there is no known

pesticide resistance reported among labeled insecticides against *S. festinus*. The two insecticides used in our study were both generic formulations which may explain why poor control was observed. Our findings plus previous research indicates insecticide efficacy among labelled organophosphate and pyrethroid insecticides can vary.

In addition, our results revealed no meaningful relationship between *S. festinus* infestation and yield loss. In addition to populations never reaching levels greater than 1 adult/sweep, inconsistent control of *S. festinus* at sub-threshold levels across locations and years make conclusions regarding yield loss difficult. Moreover, overall *S. festinus* populations were so low in 2021 compared to 2020, it all but guaranteed *S. festinus* would never reach levels necessary to evaluate potential yield losses. This would have been overcome by artificial infestation under field cages, but that would eliminate the objective of incorporating native populations. The aforementioned shortcomings highlight that although *S. festinus* was not shown to affect yield in this study, changing the current action threshold may not be justified since certain conditions of the study were not met (such as populations never exceeding 1 adult/sweep).

Most state extension agencies throughout the midsouth currently recommend control strategies for *S. festinus*, although they range in terms of thresholds used and timing of insecticide applications. Compared to other midsouth states, Louisiana seems to be the most impacted by this pest given our low action threshold and focus on late season populations. However, our results indicate that populations greater than 1 adult/sweep are needed to affect the yield parameters evaluated in this study. This does not mean, however, that the action threshold should be refined. Other insect pests including soybean looper, *Chrysodeixis includens* (Walker); velvetbean caterpillar, *Anticarsia gemmatilis* (Hübner); redbanded stink bug, *Piezodorus*

guildinii (Panizzi); and southern green stink bug, *Nezara viridula* (Say); were present throughout all experiments with some species periodically reaching respective economic thresholds (personal observation). These pests have been extensively studied, and either action or economic thresholds have been described for each pest. Therefore, it should not be assumed that *S. festinus* should lose its pest status since that would mean each pest previously mentioned should also presumably lose their pest status. Therefore, until future research using native populations identifies soybean yield responses at greater than or less than action threshold levels, there remains no need to adjust the action threshold for *S. festinus* in Louisiana.

4.5. SUMMARY

The purpose of this study was to re-evaluate the *S. festinus* action threshold of 1 adult/sweep in context with current production practices such as early planting and the use of earlier maturing varieties compared to those used in older literature. Previous studies have demonstrated that *S. festinus* can induce significant yield losses and should retain its pest status. However, recent studies have challenged the historical narrative surrounding *S. festinus*. Field trials in this study conducted in 2020 and 2021 in multiple locations did not provide evidence that the current action threshold should be adjusted. Insecticide applications triggered by different sub- and supra-threshold treatments did not result in having any impact to hundred seed weight or total number of seed per five plants. Additionally, it was found that the insecticides used did not provide effective residual control of *S. festinus* which may have contributed to findings regarding yield. However, it was not clear why given previous research confirming the efficacy of organophosphates and pyrethroids against *S. festinus* to a convincing degree. Nonetheless, our results provide no evidence that the current action threshold in Louisiana of 1 adult/sweep should be adjusted.

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- (USDA-NASS) U.S. Departments of Agriculture. 2020.**

Vita

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