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ASSESSMENT OF STINK BUG FEEDING DAMAGE IN LOUISIANA SOYBEAN: USE OF A NO-CHOICE FEEDING FIELD PROTOCOL

A Thesis

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Louisiana State University and
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by
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ABSTRACT

Numerous arthropods are pests of soybean in the subtropical environment of Louisiana. The most important pod-feeding guild includes a complex of stink bugs (Hemiptera: Pentatomidae). One of these species, redbanded stink bug (*Piezodorus guildinii* [Westwood]), has emerged as a significant pest in Louisiana soybean. Currently, limited information is available describing soybean plant injury from this pest in the United States or compares injury relative to native stink bugs. Therefore, a field protocol was modified to assess injury to soybean seeds. Studies conducted in Louisiana during 2009-2011 used procedures to isolate the effects of infestation duration (24-96 h after infestation [HAI]) on seed injury at selected pod stages (R5-R6.5). In addition to redbanded stink bug; seed injury from brown stink bug, *Euschistus servus* (Say); and *E. quadrator* (Rolston) also was evaluated. For the infestation duration experiments, all injury to immature seed was significant between treatments (non-infested and infested pods). Injury to immature seed in R5.5 stage pods peaked (P=0.0102) by 72 HAI for redbanded stink bug. Injury to immature seed in R5 stage soybean pods peaked (P=0.0084) by 96 HAI for brown stink bug. For mature seed injury and weights significant differences (P<0.0001) between non-infested and infested pods were detected for all species. A second experiment evaluated seed injury among pod stages (R5-R6.5). Immature and mature seed injury was not significantly different (P>0.05) among pod stages for any species. Weights of mature seed were significantly lowest for redbanded stink bug (P=0.0177) and *E. quadrator* (P=0.0261) infestations during the R5 and R5.5 stages. Based upon mature seed weights similar levels of injury were produced by native infestations and caged stink bugs. Non-injured seed did not compensate for injured seed within the same pod. These results add to our understanding of stink bug-induced seed injury during selected pod stages and will allow further improvement to Louisiana soybean IPM recommendations.

INTRODUCTION

Soybean, *Glycine max* (L.) Merrill, is the primary oilseed crop consumed in the world (Wilcox 2004). The United States is the global leader in soybean production with 31.3 million hectares, producing 90.6 million metric tons during 2010 (USDA-NASS 2011). Louisiana ranked 17th in total production among 32 soybean producing states with a total grain yield of 1.1 million metric tons (USDA-NASS 2011).

There are several soybean arthropod pests capable of influencing Southern United States soybean production. These include the velvetbean caterpillar, *Anticarsia gemmatalis* Hübner; soybean looper, *Chrysodeix includens* (Walker); corn earworm, *Helicoverpa zea* (Boddie); bean leaf beetle, *Cerotoma trifurcata* (Förster); lesser cornstalk borer, *Elasmopalpus lignosellus* (Zeller); threecornered alfalfa hopper, *Spissistilus festinus* (Say); southern green stink bug, *Nezara viridula (L.)*; green stink bug, *Acrosternum hilare* (Say); and brown stink bug, *Euschistus servus* (Say) (Funderburk et al. 1999). In addition, the redbanded stink bug, *Piezodorus guildinii* (Westwood), has recently become a significant pest in Louisiana and surrounding states (Musser et al. 2010, Temple et al. 2011).

Among these pests, the phytophagous stink bug complex is one of the most detrimental to soybean in the Southern United States (Funderburk et al. 1999). The southern green stink bug, green stink bug, and brown stink bug are annual pests. Economic losses due to a specific stink bug species are difficult to estimate. Currently, all stink bug species are combined into a single category, or are included with other phytophagous heteropterans (McPherson and McPherson 2000a). The annual impact of stink bugs on soybean yield and quality across the southern region consists of decreased profitability from direct yield losses, reduced seed quality, and increased management inputs (Funderburk et al. 1999). The redbanded stink bug has become the dominate stink bug species in Louisiana soybean. Though still present, native stink bug populations have not been as consistent as that observed in previous years (Temple et al. 2011). Correa-Ferreira and Azevado (2002) have shown that redbanded stink bug can decrease soybean seed quality, with no loss in yield. Current action thresholds in Louisiana do not consider this fact.

Soybean is a primary host for stink bugs, and is used as a food source for summer reproduction. Multiple life stages (eggs, nymphs, and adults) of stink bugs are common in many soybean fields throughout the growing season. Adults and/or fifth instars are the most damaging development stages (McPherson and McPherson 2000a). Stink bugs prefer to feed on pod and seed structures, making soybean plants most susceptible to injury during reproductive stages, particularly during seed development (McPherson and McPherson 2000b). Stink bug feeding on soybean can cause extensive seed injury (McPherson and McPherson 2000b). Injury is caused by the insertion of the stylets (piercing-sucking mouthparts) through the pod wall into the seed, and the extraction of plant fluids. On immature seed, discolored necrotic areas may surround the puncture (McPherson and McPherson 2000a). Mature seed injury is characterized by discoloration, puncture marks, and internal irregular white spots which may have a chalky appearance (Miner 1961). Furthermore, intense feeding on mature seed may produce small, irregular seed, which are wrinkled where the stlyet insertion occurred and seed contents were removed (Miner 1961, Miner and Dumas 1980). Stink bug-injured soybean has been associated with a decrease in pod number, fewer seed per pod, lower seed weight, decrease in oilseed content, increase in protein content, and lower soybean quality (Jensen and Newsom 1972, Todd and Turnipseed 1974, Thomas et al. 1974). In addition, percent germination of stink bug-injured seed is lower than non-injured seed (Daugherty et al. 1964, Jensen and Newsome 1972, Todd 1981).

Soybean production in Louisiana has changed over the past decade due to a shift to an early production system (Heatherly 1999, Baur et al. 2000). Currently, a large portion of the state is planted to earlier maturing varieties (MG III and IV), some of which express an indeterminate growth trait (R. Levy personal communication). Many of the action thresholds being used are based on an entirely different system. Changes in pest composition and production practices suggest that action thresholds should be revised.

REVIEW OF LITERATURE

General Stink Bug Biology

Stink bugs are “true bugs” in the class Insecta, order Hemiptera and suborder Heteroptera (Drake 1920). The latter is characterized by two key features, (1) a segmented beak arising from the front of the head; and (2) forewings that are leathery basally and membranous distally, along with fully developed membranous hindwings, all which are folded flat over the abdomen (McPherson and McPherson 2000a). Further classification shows that stink bugs are in the family Pentatomidae. This family is known as stink bugs due to the characteristic defense mechanism in which they emit a malodorous and ill-tasting substance (Drake 1920). Members of this family can also be identified by five segmented antennae and a well-defined scutellum (McPherson and McPherson 2000a).

All members of the order Hemiptera, including stink bugs, have modified piercing and sucking mouthparts (Todd 1981, McPherson and McPherson 2000a). The rostrum or “beak” is a four-jointed tube-like structure that contains four lance-like setae (Drake 1920). These “setae”, or stylets, include mandibles, which are comprised of the thicker and heavier pair, and maxillae, which are the thinner pair (Drake 1920). Stink bug feeding occurs by puncturing the plant’s reproductive structures with their sharp pointed mandibular and maxillary stylets. During this process, enzymes are injected to further break down the plant tissue, enabling the stink bug to ingest plant juices (Drake 1920, McPherson and McPherson 2000a). Although stink bugs are most often associated with feeding on the fruit of plants, they also are capable of feeding on stems and leaves (McPherson and McPherson 2000a).

Stink bug species complete development through five instars (Todd 1981, 1989; Drake 1920; McPherson 1982; McPherson and McPherson 2000a). First instars congregate on or near the egg mass following eclosion (Todd 1981, McPherson and McPherson 2000a). They do not feed on plants (Bowling 1980; Todd 1981, 1989; McPherson and McPherson 2000a), but are thought to acquire symbionts by ingesting secretions from the chorion (McPherson and McPherson 2000a). Adults and nymphs are usually found near the site on the plant where they prefer to feed. For stink bugs, these sites include tender growing shoots or fruit/seed structures (Todd 1981). Stink bugs usually overwinter as adults in reproductive diapause beneath leaf litter, emerging from a variety of hosts including grasses, herbaceous vegetation, shrubs and trees as temperatures warm up in the spring (McPherson and McPherson 2000a). The number of generations in North America varies from one (north) and five (extreme south) and is strongly influenced by environmental factors (McPherson and McPherson 2000a).

Soybean Stink Bug Complex Composition for the Southern United States

Each United States soybean producing region may be infested by one or more species of phytophagous stink bugs. These insects are usually among the most serious pests associated with this crop (Todd 1981, Kogan and Turnipseed 1987). Many of these species are not unique to soybean, but exist across the farmscape feeding on numerous crops and native plants.

A complex of stink bugs has historically been a key soybean pest complex throughout the Mid-South and Southeastern United States (Funderburk et al. 1999). McPherson et al. (1979) listed the southern green stink bug, *Nezara viridula* (L.); green stink bug, *Acrosternum hilare* (Say); brown stink bug, *Euschistus servus* (Say); and dusky stink bug, *Euschistus tristigmus* (Say) as being commonly collected species in Louisiana soybean. In another Louisiana soybean study, the principle species found were southern green stink bug, green stink bug, and brown stink bug (Boyd et al. 1997). Until recently, the southern green stink bug has been the most abundant species. However, during the last decade a new species, the redbanded stink bug, *Piezodorus guildinii* (Westwood), was detected in all Louisiana soybean producing regions, and was the primary species during 2006-2010 (Temple et al. 2011).

In a Georgia survey over four years, the southern green stink bug, green stink bug, and brown stink bug comprised 98% of stink bugs in soybean (McPherson et al. 1993). Gore et al. (2006) reported the soybean stink bug complex in Mississippi primarily to be composed of southern green stink bug, green stink bug, and brown stink bug, while *Thyanta* spp. were found in low numbers. During a 2003-2007 survey, the most abundant species in Arkansas soybean were southern green stink bug and brown stink bug (Smith et al. 2009). However, during 2006 and 2007 the redbanded stink bug was collected in Southern Arkansas soybean (Smith et al. 2009). A survey (three years) in southeast Texas established southern green stink bug to be the predominant species present (Drees and Rice 1990). Other stink bugs recorded in less abundance included green stink bug; brown stink bug; *Edessa bifida* (Say); *Euschistus crassus* (Dallas); *Euschistus ictericus* (L.); *Euschistus quadrator* (Rolston); *Oebalus pugnax* (F.); *Proxys punctulatus* (Palisot de Beauvois); and the redshouldered stink bug, *Thyanta accerra* (McAtee) (Drees and Rice 1990). The redbanded stink bug also has been found in damaging numbers in Southeast Texas soybean (Unpublished, M. Way, Texas AgriLife Research, Beaumont, TX).

Louisiana Soybean Stink Bugs

The southern green stink bug is the most broadly adapted stink bug in North America (McPherson and McPherson 2000c). This species is highly polyphagous, feeding on over 30 families of dicotyledonous plants, several monocots, and exhibits a strong preference for legumes (Todd 1981, Panizzi 2000, Panizzi et al. 2000, Bundy and McPherson 2000a, McPherson and McPherson 2000c). This species is one of the most important stink bug pests in North America due to its highly adaptable nature (McPherson and McPherson 2000a).

The southern green stink bug feeds on several major cultivated crops, including cowpea, *Vigna unguiculata* (L.) Walpers; lima bean, *Phaseolus lunatus* L.; pecan, *Carya illinoensis* (Wangenheim) K. Koch; macadamia, *Macadamia integrifolia* Maiden and Betche; rice, *Oryza sativa* L.; wheat, *Triticum* spp.; sorghum, *Sorghum bicolor* (L.) Moench; corn, *Zea mays*; tomato, *Lycopersicon esculentum* Miller; tobacco, *Nicotiana tabacum* L.; and cotton, *Gossypium hirsutum* L.; but it is most recognized as a soybean pest (McPherson and McPherson 2000c). The southern green stink bug completes three or four generations per year in Louisiana (Todd 1989, McPherson and McPherson 2000c). The third generation begins to migrate into soybean fields by late July, where the fourth generation will be produced (McPherson and McPherson 2000c). The southern green stink bug was considered to be the most dominant stink bug affecting soybean across the majority of Southern soybean regions (Kogan and Turnipseed 1987).

The adult southern green stink bug is a large shield-shaped bug that is light green in color (Drake 1920). The southern green stink bug is about 1.27 cm long and 8.5 cm wide, though size is slightly variable and females are usually larger than males (Drake 1920). Distinguishing characteristics include a rounded abdominal spine and brownish-red rings on the antennae (Kamminga et al. 2009). Early instars of the southern green stink bug are dark brown in color (Kamminga et al. 2009). Late instars are green in color with white spots towards the middle of the abdomen and pink to red markings along the outer edge of the abdomen (Kamminga et al. 2009). Drake (1920) describes in further detail both the light and dark color forms of southern green immature nymphs. Adults lay yellowish-white to cream colored eggs in compact hexagonal clusters that are firmly glued together (Drake 1920, Bundy and McPherson 2000b). As the egg incubates, it turns pinkish with a red crescent on the operculum. These colors become more intense until hatch occurs (Drake 1920). Eggs are about 1.02-1.26 mm long and

0.73-0.83 mm wide, with 28-40 delicate chorionic processes around the operculum (Drake 1920, Bundy and McPherson 2000b).

The green stink bug, *Acrosternum hilare* (Say) (syn. *Chinavia hilaris*), is closely related to the southern green stink bug, but is more commonly found in the northern states of the U. S. (Drake 1920). Although both species resemble each other in form, color and size; they can readily be distinguished from each other by the shape of their osteolar canal (Drake 1920). Other distinguishing factors include the presence of an abdominal spine and black rings on the antennae (Kamminga et al. 2009). Green stink bug nymphs are mostly black with orange markings in early development and become greener throughout with dark markings on the middle of the abdomen (Kamminga et al. 2009). Eggs of the green stink bug are lemon yellow, which become rose-colored as they incubate; or yellow-green, which turn ash grey as they incubate (Esselbaugh 1946). Green stink bug eggs have about 45-65 chorionic processes around the operculum (Drake 1920, Bundy and McPherson 2000b). In a soybean field study, southern green stink bug and green stink bug were found to produce identical injury (Todd 1981). Injury to soybean produced by the green stink bug and southern green stink bug are both qualitatively and quantitatively comparable (Yeargan 1977).

Multiple brown-colored stink bug species in the genus *Euschistus* inhabit soybean fields. The brown stink bug, *E. servus* (Say); dusky stink bug, *E. tristigmus* (Say); and the onespotted stink bug, *E. variolarius* (Palisot de Beauvois) are capable of producing injury that could be of economic importance in soybean (McPherson and McPherson 2000d).

The brown stink bug occurs throughout North America and is highly polyphagous, feeding on grasses, shrubs, and trees (McPherson 1982, McPherson and McPherson 2000d). In addition to soybean, other cultivated crops of economic importance include: alfalfa, *Medicago* *sativa* L.; peach, *Prunus persica* (L.) Batsch; pear, *Pyrus communis* L.; apple, *Malus domestica* Borkhauser; cotton; pecan; sorghum; and corn (McPherson and McPherson 2000d). This species may be predaceous in some situations. Records of predation include the mountain ash sawfly, *Pristiphora geniculata* (Hartig); and the cabbageworm, *Pieris rapae* (L.) (McPherson and McPherson 2000d).

The brown stink bug is considered to be the most economically important species in the *Euschistus* genus across North America (Panizzi et al. 2000). The brown stink bug is bivoltine and overwinters as an adult (McPherson and McPherson 2000d). Crop residues, leaves, grass bunches, piles of wood, bark, and other similar objects in open fields are preferred overwintering habitat (Jones and Sullivan 1981, McPherson and McPherson 2000d). Brown stink bug adults are mottled brown, have rounded shoulders, and lack an abdominal spine (Kamminga et al. 2009). Early instars are dark brown on the head and pronotum (Kamminga et al. 2009). Late instars are green-brown in color and have light brown spots down the middle of their abdomen (Kamminga et al. 2009). Eggs of the brown stink bug are white to cream colored and are 0.98-1.18 mm in length and 0.86-1.10 mm in width (Bundy and McPherson 2000b). These eggs have 26-29 porous micropylar processes that are weakly clavate and 0.05 mm in length. Egg masses usually consist of 25-55 eggs per cluster (Esselbaugh 1946, Bundy and McPherson 2000b).

Other minor brown-colored stink bug species are considered of economic importance in soybean. The physical characteristics of the remaining *Euschistus* species are very similar to brown stink bug in the egg and nymphal stages (McPherson and McPherson 2000d, Kamminga et al. 2009), but there are key physical traits among adults that make them readily distinguishable. The dusky stink bug has pointed shoulders and a light colored abdomen with one or more dark spots on the ventral surface of the abdomen (Kamminga et al. 2009). *E.*

quadrator (Rolston) adults are similar to dusky stink bug adults, except have a convex pronotum and lack abdominal spots (Kamminga et al. 2009). Adults of the onespotted stink bug superficially resemble the spined soldier bug, *Podisus maculiventris* (Say) (McPherson and McPherson 2000d).

A field study conducted by Miner in 1966 found brown stink bug caused less injury to soybean than southern green and green stink bugs (Todd 1981). In a study by McPherson et al. (1979) late instars and adults of brown stink bug could cause as much or more injury to soybean when compared to fourth instars of southern green stink bug. In this same study, injury produced by all stages of *E. tristigmus* was comparable to that produced by southern green stink bug third instars (McPherson et al. 1979).

The redbanded stink bug, *Piezodorus guildinii* (Westwood), is a neotropical species which completes five generations per year in South American soybean. Its geographical distribution ranges from Argentina, where it is a serious pest of soybean, north into the Southern United States (McPherson and McPherson 2000e). This insect has been in the United States for many years, but initially was not considered an economic pest (McPherson et al. 1993). Not much is known about its biology in the Mid-Southern United States.

This insect can cause severe injury in soybean, alfalfa and other legumes (Panizzi and Slansky 1985). It has occasionally been reported on sunflower, *Helianthus annuus* L.; cotton; and guava, *Psidium guajava* L.; but is not believed to be a serious pest on these crops (Panizzi and Slansky 1985). Non-crop hosts of this species include indigo, *Indigofera spp*. in the Southern United States, Columbia, and Brazil. It also feeds on wild native legumes in the genera *Sesbania* and *Crotalaria* (Panizzi et al. 2000).

The redbanded stink bug was first reported in South Louisiana during 2000, and has since become widely distributed throughout the state. Initially, it was misidentified as the redshouldered stink bug, *Thyanta accerra* (McAtee). Redbanded stink bug feeds on soybean pods similar to other pentatomids, causing injury symptoms comparable to the southern green stink bug (Panizzi et al. 2000). In 2002, the redbanded stink bug was classified as a primary economic soybean pest in Louisiana and Texas (Temple et al. 2011). The redbanded stink bug appears to be better adapted to feeding on flowering plants compared to other pentatomids (Panizzi et al. 2000). This species does not require soybean with reproductive structures in order to survive and reproduce, unlike the southern green stink bug that exhibits poor survivorship on vegetative and early reproductive stage plants (Panizzi et al. 2000, McPherson and McPherson 2000c). Costa and Link (1982) found that redbanded stink bug adults are more mobile than southern green stink bug adults.

Southern green stink bug was the dominant stink bug pest across Argentina and Southern Brazil during the expansion of the soybean crop until the late-1970's when redbanded stink bug began to displace the southern green stink bug in Brazil (Kogan and Turnipseed 1987). This trend of redbanded stink bug displacing southern green stink bug as the major species also may be evolving in Louisiana (Temple et al. 2011). Several biological and ecological factors may be responsible for providing redbanded stink bug an advantage over other stink bugs in soybean. Smaller size, higher mobility, adaptation to warm climates, lower parasitization rates, and reduced susceptibility to commonly used insecticides likely contribute to this change in species composition (Kogan and Turnipseed 1987).

Redbanded stink bug adults are described as shiny yellow-green to light green in color (Kamminga et al. 2009). They are characterized by two transverse stripes across the base of the scutellum; the first a yellowish color and the second a dark red color (Grazia et al. 1980, Kamminga et al. 2009). Another unique characteristic that aids in distinguishing this species from *Thyanta* spp., or other similar stink bugs, is the presence of a long ventral abdominal spine that extends into a thoracic groove between the metathoracic and mesothoracic coxae (Greene et al. 2006, Kamminga et al. 2009). Eggs produced by this species are initially white but progressively become black throughout the incubation period. Eggs are laid in two parallel rows on leaves, stems, and pods (Panizzi et al. 2000).

Summary of Mid-South Stink Bug-Induced Yield Losses and Control Costs

The stink bug complex is the number one pest impacting soybean production in the Mid-South, accounting for the loss of 4.22 million bushels of soybean in Mississippi, Tennessee, and Arkansas during 2009(Refer to Table 1.1). A total of 2.37 million acres of soybean, nearly half of the total soybean acreage planted in these three states, required an insecticide application to control the stink bug complex. Total loses (yield and chemical control) amounted to a cost of $9.48 an acre.

Stink Bug Seasonal Occurrence

During a seasonal survey of Louisiana soybean, McPherson et al. (1979) found that southern green stink bug populations reached peak levels in early November, while brown stink bug and green stink bug populations reached peak levels in late September. Seasonal data collected in Louisiana during 2009 showed redbanded adults in maturity group (MG) IV soybean peaked in July, while in MG V soybean a later peak during September was seen (Temple et al. 2011). Schumann and Todd (1982) found soybean growth stage to be a predominant factor affecting southern green stink bug populations in soybean. Southern green stink bug oviposition was initiated during the late vegetative stage. Third generation southern green stink bugs

Table 1. Stink bug-induced yield losses and cost of chemical control for Mid-South states (Data adapted from Musser et al. 2010, 2011)

	Stink bug-induced soybean losses in the Mid-South									
	Mississippi			**Tennessee**			**Arkansas**		**Overall**	
	2008	**2009**	**2010**	**2008**	**2009**	**2010**	**2009**	**2010**	**2009**	**2010**
Bushel loss	1,441,625	1,085,905	75,106	1,117,878	890,695	1,760,480	2,224,468	4,522,171	4,214,668	6,859,323
Yield (bu/ac^2)	40	38	39	34	45	32	38	35	39	35.55
Acres treated	1,250,000	950,000	550,000	800,000	320,000	775,000	1,100,000	1,100,000	2,370,000	2,425,000
No. appl./total acre	0.933	0.924	0.385	0.64	0.204	0.641	0.322	0.379	0.478	0.438
Cost/acre	$8.40	$9.24	$3.85	$5.44	$1.94	$4.97	$2.33	$2.84	$4.13	$3.57
Loss + cost/acre	$14.85	$13.76	$6.32	$13.64	$7.04	$17.11	$8.51	$17.02	$9.58	$13.90

developed on early maturing cultivars, while fourth and fifth generations could be found in later-maturing cultivars (Schumann and Todd 1982). These populations increased during the R3 to R5 growth stages with peak numbers occurring at R6 (Schumann and Todd 1982). Populations collected during late vegetative stages or early reproductive stages were comprised of a higher frequency of females than males (Schumann and Todd 1982).

A three year study in a Georgia cotton-soybean ecosystem evaluated the effects of stink bugs on MG V and MG VII soybean (Bundy and McPherson 2000a). The most abundant stink bug species included southern green stink bug, green stink bug, and brown stink bug. Stink bug infestations were most common during the period of pod formation (R3) to full seed development (R6). Peak populations occurred during the R6 to beginning R7 stages. Stink bug that infested early MG V soybean did not migrate into the later MG VII variety until plants had developed to the R6 growth stage. In Louisiana, crops are planted sequentially beginning with winter wheat, then corn, followed by early soybean and cotton, and finally late-planted soybean and late-planted cotton. Stink bugs prefer soybean over cotton for feeding and reproduction (Bundy and McPherson 2000a).

Stink Bug-Induced Injury to Soybean

Adults and most nymphal stages of stink bug feed on soybean. The most common injury is produced by a puncture of the pod/seed structure with modified piercing-sucking mouthparts, followed by the injection of digestive enzymes for tissue breakdown, and finally the extraction of plant liquids (Todd 1981). Stink bug feeding can result in an inferior grade of seed, or if injury is severe and seed are deemed worthless, they may be discarded for no value (Todd 1981). Adults and fifth instars are capable of causing significantly more injury than earlier stages (McPherson et al. 1979). Simmons and Yeargan (1988) evaluated the feeding duration and frequency for life stages of green stink bug. Fifth instars had the highest daily frequency (50%) of feeding, which was similar to that for adults (Simmons and Yeargan 1988).

The wound caused by insertion of the mouthparts on immature seed is characterized by minute, brownish-black spots (Todd 1981). These wounds further develop into black or dark brown areas around the puncture site as the seed matures, most likely due to seed decomposition caused by plant pathogens (Miner 1961, McPherson and McPherson 2000a). The seed coat may become uneven, sunken, and wrinkled above the feeding site; due to the displacement of the plant material that has been removed (Miner 1961, Miner and Dumas 1980). The type and severity of injury is highly dependent on the stage of the pod in which the stink bug feeds (Todd 1981). Feeding at early seed development can result in aborted pods; or shriveled, deformed, and undersized seeds (Todd 1981).

Depieri and Panizzi (2011) confirmed a relationship between stink bug feeding time and the injury produced to soybean. Feeding durations for the redbanded stink bug and the southern green stink bug were similar. For the redbanded and southern green stink bugs, a positive correlation was found between feeding time and degree of injury. The redbanded stink bug produced the more severe injury when compared to the three other species. The redbanded sink bug was capable of creating more seed tissue injury than the other species. This increased injury may be due to a combination of physical injury to the seed and increased chemical dissolution from salivary enzymes.

Injury on mature seeds can easily be observed by dissecting a seed. Irregular white splotches with a chalky texture indicate the site of injury. According to Todd (1981) the white chalky areas on the flesh of injured cotyledons is a result of the removal of cell contents during stink bug feeding. Miner (1961) describes this chalky appearance as being a result of air bubble production during the stink bug feeding process. Todd (1981) described four categories of stink bug damage: (1) Light damage - seed with puncture marks, but no deformity of the seed coat or endosperm; (2) Medium damage - seed with puncture marks and mild deformity but no reduction in size; (3) Heavy damage - seed with puncture marks, gross deformity, and some reduction in size and weight; and (4) Severe damage - seed with puncture marks, gross deformity, and drastically reduced size and weight. The last two categories are sometimes combined, and seed in these categories are of no value for oil, meal, or planting (Todd 1981).

Stink Bug Effects on Soybean Seed Composition, Quality and Yield

The primary effects of stink bug injury to soybean are a direct loss of crop yield and reduction in seed quality. Stink bug seed feeding results in poor germination and low seedling survivorship (Daugherty et al. 1964, Jensen and Newsom 1972, Todd 1981). Stink bug-injured seed is associated with a decrease in oil content, but an increase in protein levels (Jensen and Newsom 1972, Thomas et al. 1974, Todd and Turnipseed 1974). Plant maturity also may be delayed in those instances of significant seed injury (Leonard et al. 2011).

Feeding injury produced by southern green stink bug, green stink bug, brown stink bug, and dusky brown stink bug caused significant yield losses in Louisiana soybean (McPherson et al. 1979). Significant differences in seedling emergence on injured seed can be directly correlated with the intensity of the injury produced by a stink bug (Jensen and Newsom 1972).

During a six-week period of soybean pod seed development, a significant yield reduction resulted from an infestation of 1.8 stink bugs/row-m (McPherson et al. 1979). The presence of southern green stink bug injury at the beginning of the seed fill stages significantly decreased soybean seed yield and quality. However, soybean plants that reached full maturity (R8) were not significantly impacted by these insects (Thomas et al. 1974). Todd and Turnipseed (1974) reported significant reductions in grain yield and significant increases in seed injury from southern green stink bug population densities of 3.3, 9.8, and 16.4/row-m (Todd 1981). Soybean seeds harvested from cages that contained 40 infested pods with a single bug (fourth instar through adult) resulted in 63.9 to 78.5 percent injured pods (Todd and Turnipseed 1974, Todd 1981). Yeargan (1977) reported similar observations when caging two or four green stink bug adults/0.3 row-m on soybean produced in Kentucky. Yield losses were the result of a combination of reduced seed size and reduced seed produced by the soybean plants (Yeargan 1977).

Heavy stink bug infestations during seed maturation can reduce seed germination, as well as, seedling emergence and vigor (Todd 1981). Daugherty et al. (1964) found that heavy brown stink bug infestations resulted in reduced germination which directly correlated to the number of feeding punctures per seed (Todd 1981). Germination is affected more by the location of punctures than the quantity of punctures (Jensen and Newsom 1972). Punctures to the radicle-hypocotyl axis can prevent germination. Punctures to the cotyledons may affect vigor, but will not necessarily prevent the seed from germinating (Jensen and Newsom 1972, Todd 1981). A quick and easy test used for identifying non-living and living areas of a seed is the tetrazolium test, which is used for assessing soybean seed germination (Todd 1981).

Daugherty et al. (1964) demonstrated that two to four pairs of brown stink bugs on plants increased seed injury in the middle third of the plant when compared to the non-infested. Injured seed were significantly smaller compared to non-injured seed (Daugherty et al. 1964). Seed germination decreased as the stink bug infestations increased. Plants produced about the same number of seeds, but plants with higher infestations matured later as a result of plant compensation for seed injury (Daugherty et al. 1964).

Soybean seed injury from the redbanded stink bug, southern green stink bug, and *E. heros* (F.) were compared in a Brazilian study (Correa-Ferreira and Azevado 2002). Field infestations were conducted for 15 days at levels of four stink bugs/row-m. Regardless of species, there was no difference in yield between the infested and non-infested treatments. Stink bug feeding by all species in this study resulted in inferior seed quality when compared to the control (Correa-Ferreira and Azevedo 2002). The redbanded stink bug injury resulted in significantly lower seed quality compared to the other two species.

Stink bug feeding on soybean results in only a small reduction in oil seed content, and a slight increase in protein content compared to non-injured soybean (Miner 1961, Todd 1981). Southern green stink bug feeding on soybean decreased oil content but increased protein content as the degree of injury increased (Todd and Turnipseed 1974). In Arkansas, injury produced by stink bugs on soybean did not result in appreciable yield losses, but did reduce seed quality (Miner 1961, Miner and Dumas 1980). No significant relationship was found between oil content or protein content and the degree of stink bug injury (Miner 1961). A study in Missouri correlated associated brown stink bug injury with lower oil content and higher protein content (Daugherty et al. 1964). Fatty acid composition directly correlated with the intensity of green stink bug feeding (Daugherty et al. 1964). Proportions of palmatic, stearitc, and oleic acid oil increased, while linoleic and linolenic acids decreased, as severity of stink bug injury increased (Todd et al. 1973, Todd 1981). In another study, infestations of 16 or 32 southern green stink bugs/4.5 row-m at the beginning of seed fill (R6) decreased oil content and increased protein content (Thomas et al. 1974). A significant increase in protein content was recorded for the highest infestation level of stink bug during the pod fill stage (Thomas et al. 1974).

Delayed plant maturity in soybean is defined as whole-fields or portions of fields where plants retain leaves, green stems, and/or green pods long after normal plant maturity should have occurred (Boethel et al. 2000). This can delay the time that crop harvest occurs, decrease the quality of seed that is harvested, result in seed with high moisture content that can lead to mill rejection, or entirely prevent harvest (Leonard et al. 2011). A study in Louisiana evaluated the potential causes of "green plant malady" including plant disease, environmental factors, fungicide use, and stink bug injury. It was noted that variety varied in predisposition to green stem, which unlike pod and leaf retention can be a normal varietal effect. Results showed that there was no correlation between presence of virus and delayed maturity. An inconsistent relationship between drought stress and delayed maturity was detected. Fungicide use increased occurrence of delayed maturity. This was expected because of healthier plants associated with fungicide applications. Stink bug injury was associated with green plant malady when populations were persistent at moderate levels near threshold and with the occurrence of population outbreaks reaching 2-3 times the action threshold (Leonard et al. 2011).

Daugherty et al. (1964) demonstrated that two to four pairs of brown stink bugs on plants increased seed injury in the middle third of the plant when compared to the non-infested. Injured seed were significantly smaller compared to non-injured seed (Daugherty et al. 1964). Germination of seed decreased as the stink bug infestations increased. Plants produced about the same number of seed, but plants with higher infestations matured later as a result of plant compensation for seed injury (Daugherty et al. 1964).

Delayed maturity of soybean as a response to stink bug infestations has been reported for southern green stink bug and brown stink bug in Louisiana, Georgia, and Arkansas (Daugherty et al. 1964, Duncan and Walker 1968, Todd and Turnipseed 1974, Boethel et al. 2000). In a caging study with southern green stink bug infestations above the economic threshold, delayed leaf senescence was consistent throughout the R3-R5 stages, though the effect at R5 was most pronounced (Boethel et al. 2000). Delayed maturity was also seen at the R5.5 stage, but was not detected at R2 and R6. The greatest impact on seed yield and quality occurred during R3-R5.5 stages (Boethel et al. 2000). Bailey (2007) demonstrated that green stink bug adults and nymphs that fed during the late flowering and pod fill reproductive stages delayed leaf senescence on infested plants. Soybean with a longer period from flowering, to maturity was associated with green stink bug injury (Daugherty et al. 1964). The most crucial time period to protect soybean and reduce the probability of delayed plant maturity from southern green stink bug injury seems to be the pod elongation (R3) through late pod filling stages (R6) (Boethel et al. 2000).

Stink bug feeding can indirectly affect plant health by initiating a point of entry for soybean pathogens. Daugherty et al. (1964) showed that six species of pentatomids were capable of vectoring the leaf spot disease pathogen, *Nematospora coryli* Peglion, to immature soybean seed (Miner and Dumas 1980, Todd 1981). In addition, several species of bacteria have been isolated from organs of the southern green stink bug (Ragsdale et al. 1979). In subsequent research, this species was able to transmit five pathogenic bacteria to soybean (Ragsdale et al. 1979, Todd 1981). Research in Puerto Rico showed southern green stink bug may be involved in transmission of the bacterial blight pathogen, *Xanothonomas* spp.; in common beans, *Phaseolus vulgaris* L. (Todd 1981). Southern green stink bug was often collected from bacterial blight-infected common beans. Infection of soybean plants resulted from artificial infestation of southern green stinkbug with bacterial isolates following stink bug feeding on the leaves and pods (Todd 1981).A similar insect/pathogen relationship was found for redbanded stink bug in soybean (Panizzi et al. 1979). Over 30% of stink bug-injured seed were infected with several pathogens including those in the genera *Fusarium*, *Phomopsis*, *Diaporthe*, *Colletotrichum*, *Cercospora*, *Rhizoctonia*, and *Macrophomina*.

Numerous pathogens that infect soybean plants do not require stink bug injury for infection. Several fungal pathogens have been detected on plants in the absence of stinkbugs (Todd 1981). The presence of a stink bug wound is sufficient to increase the likely-hood of plant pathogen entry (Musser et al., MSU Department of Entomology and Plant Pathology, unpublished data).

No stink bug infestations in stored soybean seed has been reported (Todd and Womack 1973). However, cigarette beetles, *Lasioderma serricorne* (F.), do infest stored seed of many cereals, small grains, vegetables, including processed products, such as soybean meal (Todd and Womack 1973). Laboratory observations suggested cigarette beetles exhibited a strong preference for stink bug-injured seed in storage (Todd and Womack 1973, Todd 1981). Non-stink bug-injured soybean were completely free of cigarette beetle injury. The frequency of cigarette beetle-injured seed directly correlated with higher southern green stink bug injured seed (Todd and Womack 1973).

Miner and Dumas (1980) evaluated mature soybean seed prior to and after 6-10 months in storage. The seed had multiple levels of stink bug injury and were at multiple moisture concentrations. There was no significant relationship between seed oil level, protein content, and storage (Miner and Dumas 1980)

Stink Bug IPM

The quality of soybean as a host for pests are affected by multiple management techniques, such as switching from multiple to single cropping, row spacing, tillage, planting date, crop rotation, and pesticides use patterns (Kogan and Turnipseed 1987). These production practices can affect plant susceptibility to stink bugs, as well as, influence the overall integrated pest management (IPM) strategy for soybean.

In most southern field crops, insecticides are important tools in a successful IPM system. However, there are several alternative strategies that can reduce the reliance on insecticides and augment the treatments that are applied. Some of these strategies include an early season production system, trap cropping, host plant resistance, and biological control.

Soybean production in Louisiana has changed over the past decade. Historically, Louisiana planted later maturing varieties (MG's V, VI and VII), but has since transitioned to an early soybean production system during the late 1990's and early 2000s (Heatherly 1999, Baur et al. 2000). For the growing season of 2010, a significant portion of Louisiana soybean acreage was dedicated to early maturing varieties (64% MGs III and IV), while later maturing varieties accounted for the later portion (35% MG V and 1% MG VI) (R. Levy personal communication). Adoption of the early soybean production system occurred for many reasons including a reduction of late season drought stress, insect pest problems, and inclement weather that is often associated with early August and mid-September (Heatherly 1999).

Producers can manipulate a series of agronomic practices to avoid significant problems with stink bugs. The goal of these strategies is to manage crop maturity in such a way that plants are in stages of development that are less attractive or susceptible when peak populations occur. Stink bug populations are highest at the end of the season. Earlier in the season the amount of acreage attractive to stink bugs results in more widely distributed populations (Paxton et al. 2007). However, during the latter part of the production season, late-maturing soybean will concentrate higher stink bug populations compared to numbers on a larger acreage of early-planted soybean. All soybean are potential targets for attack by stink bugs, but fields that reach maturity late in the growing season, when stink bugs are at high levels, are especially vulnerable. The late-maturing fields have plants in early seed development stages, which are highly attractive to these pests. The surrounding crops in the area should be more mature and in less attractive stages of development (Paxton et al. 2007). The use of planting date and MG III and MG IV soybean can be used to manipulate crop maturity to escape these late-season concentrated populations.

The winter wheat and soybean double cropping system serves as an example of the difficulty in controlling late-season populations of stink bugs. Soybean planted following winter wheat (harvested around late May/early June) is generally planted well-beyond the optimum planting window for the early season soybean production system. Delayed planting usually results in lower yields, but it also increases the potential for stink bug injury, and increased inputs for chemical control strategies (Paxton et al. 2007).

The use of a crop to attract and concentrate the initial populations migrating into an area has been useful managing stink bugs. This strategy will be successful if it provides a place that will be both more attractive and require only a small area to prevent or reduce pest movement into adjacent soybean fields. The coordination of early and late maturing soybean cultivars by varying MG and planting date has been integrated into a management program for trap cropping and reduced the area needed for insecticide sprays (Bundy and McPherson 2000a).

Bundy and McPherson (2000a) showed that stink bugs migrating into early maturity soybean did not migrate into an adjacent late-maturing variety until plants developed full pods (R6 stage). Therefore, using an early maturing soybean variety as a trap crop could be used to concentrate populations away from the later maturing crop. This practice could reduce the quantity of insecticide necessary to provide sufficient control of the pest (Bundy and McPherson

2000a). Trap crop areas consisting of earlier maturing soybean comprising about 5% of the total acreage have been shown to be highly effective as a trap crop for stink bugs (Newsom and Herzog 1977). This technique is most feasible as a component of area-wide pest management, which would require cooperation from all growers. Benefits of this technique include minimum adverse effects to natural biological control agents, minimal environmental pollution, delay of the build-up of resistant pest populations, and favorable economics (Newsome and Herzog 1977).

Host plant resistance (HPR), is an important IPM tool that uses genetic resistance or yield tolerances to reduce the economic impact of pests. In the presence of target pest populations, crops expressing HPR traits usually have higher yields or higher quality seed compared to susceptible cultivars. Plant resistance in soybean is defined relative to that of insect-susceptible plants (McPherson et al. 2007).

The focus for HPR in soybean is to target foliage feeding pests and reduce plant defoliation (McPherson et al. 2007). Soybean resistance to defoliators has steadily improved, but adoption of these HPR expressing lines has been somewhat limited due to the lower yields and later maturity related to these traits (McPherson et al. 2007). Any defoliation in soybean can be caused by a number of different factors including hail, insect injury, and disease. Defoliation reduces photosynthetic supply, which in turn decreases the size and number of the seed produced (Board et al. 2010). Reductions in seed quantity and size could have negative effects when combined with direct injury caused by stink bugs and other soybean insect pests.

Soybean lines transformed to express *Bacillus thuringiensis* (Bt) are currently being studied for control of lepidopteran pests. MacRae et al. (2005) conducted research in this area in the United States using soybean expressing a Bt *Cry1A* protein (*tic107*) in both greenhouse and field trials. Both studies evaluated the efficacy of the Bt soybean for controlling velvetbean caterpillar, *Anticarsia gemmatalis* Hübner; and soybean looper, *Chrysodeix includens* (Walker). Complete control of both pests was achieved with the Bt soybean, while the negative controls experienced significant injury (MacRae et al. 2005). The efficacy of the same Bt soybean was evaluated against native populations of lepidopteran pests in field trials (McPherson and MacRae 2009). Populations of velvetbean caterpillar, soybean looper, and green cloverworm, *Hypena scabra* (F.) were essentially absent in Bt soybean throughout the growing season, while infestations in the negative control were moderately high (5-10 larvae/row-m) to very high (20-30 larvae/row-m) (McPherson and MacRae 2009). The lepidopteran pests in the control plots were managed with two or three foliar insecticide treatments and preformed just as well compared to the Bt soybean plots (McPherson and MacRae 2009). Bt soybean provided season-long control of lepidopteran pests while producing yields equal to that of standard non-Bt cultivars suggesting there is great potential for this transgenic technology to be incorporated into sustainable and profitable soybean IPM programs.

Considerable soybean line selection and conventional (non-transgenic) plant breeding work also has been done for HPR to stink bugs. Some soybean varieties less susceptible to stink bug injury include IAC-100 (released from the Instituto Agronomica de Campinas at San Paulo, Brazil) and ‘Hutcheson’ (McPherson et al. 2007). The IAC-100 strain has demonstrated resistance to redbanded stink bug, southern green stink bug, and *Euschistus spp*. (McPherson et al. 2007). Further development will hopefully lead to economical, higher yielding, stink bug-resistant soybean and should be accepted by producers.

Stink bugs are attacked by numerous invertebrates. Parasites have been the most successful and include several hymenoptera that attack eggs (Girault 1907, McPherson and

McPherson 2000a) and dipterans (Tachinidae) that attack adults and nymphs (McPherson and McPherson 2000a). An example of an important tachinid is *Trichopoda pennipes* (F.), because its primary host is the southern green stink bug (McPherson et al. 1982). Stink bug egg parasitism rates in Southern Louisiana were evaluated for egg parasitic wasps with all species occurring in the family Scelionidae (Orr et al. 1986). *Telenomus podisi* Ashmead was the most prevalent which coincided with its primary host *Euschistus* spp. being the most heavily parasitized. *Trissolcus basalis* (Wollaston) populations were highest in years where its primary host the southern green stink bug was most prevalent. Both species were noted for host switching when all host numbers increased. Usually cases of double parasitism involved *T. podisi* and *T. basalis* (Orr et al. 1986). One of the most effective predators is a member of the order hymenoptera and family Formicidae, the red imported fire ant, *Solenopsis invicta* Buren. This species preys heavily on stink bug eggs, nymphs and adults (McPherson and McPherson 2000a). In addition, several species of birds have been reported to be predators of stink bugs (Knowlton 1944, McPherson and McPherson 2000a).

Effects of field applications of insecticides intended to control pest insects are important when considering biological control options. In a Louisiana soybean field study, effects of methyl parathion and permethrin on the green stink bug egg parasitoid *T. baslis* were evaluated (Orr et al. 1989). This parasitoid was not affected by permethrin, but highly affected by methyl parathion for a short period of time (within 6h of application). Neither insecticide reduced adult eclosion from the host egg, though some mortality occurred after the adult wasp chewed its way out in the methyl parathion treatment (Orr et al. 1989). Natural biological control is limited in environments where beneficial insects are unable to suppress pest populations below their respective economic threshold, which then requires an insecticide application (Boyd and Boethel

1998). In a study evaluating Hemipteran predator susceptibility to insecticides, newer compounds (emamectin benzoate, imidacloprid, spinosad) were generally found to be more selective compared to standard chemistries (methyl parathion, permethrin, thiodcarb). More selective compounds enable producers to conserve beneficial insect populations, which can in turn help pest resurgence and secondary pest outbreaks (Boyd and Boethel 1998).

Chemical control is the primary means of managing stink bugs in soybean. Brown stink bugs are known to typically be harder to control compared to the southern green stink bug and green stink bug (Willrich et al. 2003). However, redbanded stink bug have been found to be more difficult to control than other stink bugs in Louisiana soybean, especially in late-planted fields (Temple et al. 2011). In Louisiana insecticide efficacy trials, southern green stink bug were more susceptible to both pyrethroids and organophosphates compared to the redbanded stink bug (Temple et al. 2009). Only a limited number of insecticides have proven to be effective for control of this insect pest (Paxton et al. 2007). Selected insecticide use strategies can be effective against redbanded stink bug, but re-infestations are frequent, severe, and require more frequent insecticide applications compared to that of other species (Paxton et al. 2007). Monitoring stink bug infestations in fields is accomplished using sweep nets and shake sheets. Action thresholds are the foundations of IPM programs and serve to justify the use of chemical control strategies only when needed.

Action thresholds for the common stink bug complex, which includes green stink bug, southern green stink bug, and brown stink bug, currently is 36 insects/100 sweep samples or one insect/0.3 row-m in Louisiana, Texas, Tennessee, and Arkansas (Gouge et al. 1999, Lorenz et al. 2006, Baldwin et al. 2009, Stewart et al. 2008). For these same species, action thresholds in Mississippi are 12 insects/100 sweep samples for R1-R5.5 stage soybean, and 36 insects/100 sweep samples for the remainder of the season (Catchot 2007). In Louisiana soybean, action thresholds (24 insects/100 sweep samples) for redbanded stink bug are lower compared to other species (Baldwin et al. 2009). This change evolved as the redbanded stink bug was found to be more difficult to control with insecticides than other stink bugs (Paxton et al. 2007).

Action thresholds do not always take seed quality into consideration, only seed yield. Yeargan (1977) found no significant differences in yield between treatments while conducting an artificial infestation study, although there were significant differences in injury produced between treatments. Correa-Ferreira and Azevado (2002) found no significant differences in yield among species evaluated, though there were significant differences in quality that resulted from the feeding of the different species. A stink bug-caging study demonstrated non-significant overall yield responses, but stink bug feeding decreased in soybean seed quality (Musser et al., MSU Department of Entomology and Plant Pathology, unpublished data). As stink bug infestation levels increased seed weight decreased, while heat injury and total injury increased (Musser et al., MSU Department of Entomology and Plant Pathology, unpublished data). Although yield may be consistently affected by significant seed injury, quality is factored into the soybean seed grade, and in turn, influences seed value (Guinn 2002). A loss in grade quality can result in a substantial loss of profit. Therefore, an improved action threshold calculation should factor in seed quality, in addition to crop yield.

HYPOTHESES AND OBJECTIVES

The following hypotheses were developed for this work.

> H_0: No differences in feeding injury will be detected among species, pod stages, or post-treatment cage durations.
>
> H_A: Significant differences in feeding injury will be detected among species, pod stages, or post-treatment cage durations.

The objectives for this project are as follows:

1. To modify a field protocol for the evaluation of stink bug injury on soybean pods/seed.
    - 1.1. Determine the number of stink bugs needed for infestations to produced consistent injury.
2. Establish differences among infestation feeding durations (24h, 48h, 72h, and 96h).
    - 2.1. Evaluate injury immediately following caging period on immature seed.
    - 2.2. Evaluate injury at harvest on mature seed.
3. Establish feeding intensity differences among R5-R6.5 stage pods.
    - 3.1. Evaluate injury immediately following caging period on immature seed.
    - 3.2. Evaluate injury at harvest on mature seed.

This study was done to develop a protocol to isolate the effects of species specific stink bug feeding on soybean yield and seed quality. This protocol enabled the comparison of selected species of stink bug during discrete reproductive growth stages of soybean in a no-choice feeding study. It also allowed for a determination of injury intensity at multiple infestation intervals. This study will document near immediate injury on immature seed and effects on mature seed at the time of harvest. Results will provide a more complete understanding of stink bug feeding on soybean and can serve as reference information to improve soybean integrated pest management (IPM) recommendations.

MATERIALS AND METHODS

Trial Locations and Plot Management

Experiments were conducted at the Louisiana State University Agricultural Center's Macon Ridge Research Station near Winnsboro, LA (Franklin Parish) during the summers of 2009 and 2010 and at the Ben Hur Research Station near Baton Rouge, LA (East Baton Rouge Parish) during the summer of 2011 (Figure 1). Maturity Group (MG) 4 and MG 5 soybean were planted every two weeks to ensure the availability of plants with soybean pods at multiple development stages. Varieties included Asgrow (AG4404) and (AG5506) in 2009, Asgrow (AG4404) and (AG5506) in 2010, and Asgrow (AG4303) and (AG5503) in 2011. This approach also provided multiple sites to ensure plot availability between scheduled insecticide applications for control of pests other than stink bugs.

Soybean plots were maintained under LSU Ag Center recommended management practices. Once a test plot was selected, alternating rows were mowed to facilitate easy access to plants that were to be caged. Routine insecticide treatments were applied to maintain native stink bug numbers at low levels and reduce seed injury in control pods. Application of short residual insecticides allowed for quick re-entry to utilize more pods in the infestation area. Multiple products were rotated as a good practice for sustainable agriculture (Orthene at 0.75 lb AI/acre, acephate, Makhteshim Agan of North America, Inc., 4515 Falls of Neuse Road Suite 300, Raleigh, NC 27609; Baythroid at 0.022 lb AI/acre, beta-Cyfluthrin, Bayer CropScience, 2T.W. Alexander Drive, Research Triangle PK, NC 27709). LSU AgCenter recommended integrated pest management (IPM) practices were followed before and after pod infestations were done.

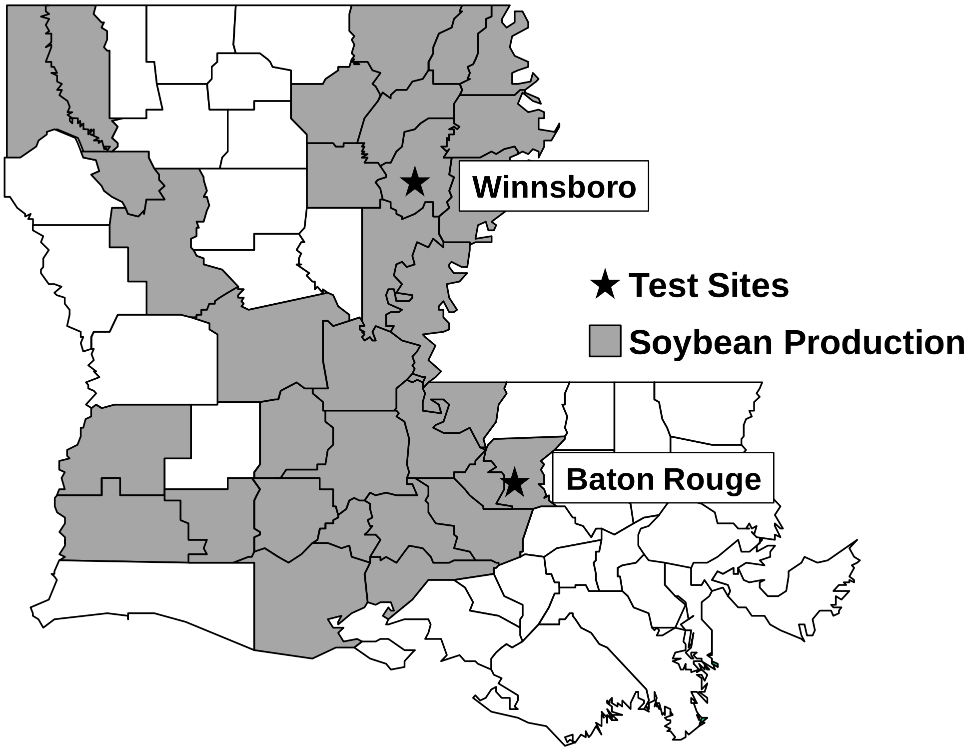

Figure 1. Louisiana soybean producing parishes and test sites.

Stink Bug Collection and Colony Maintenance

Stink bugs were collected from soybean fields on the Macon Ridge Research Station and the Ben Hur Research Station throughout the soybean production season. Collections were made one day prior to infestation using a standard 38.1cm sweep net. All insects were held in polypropylene cages (30.0 x 30.0 x 30.0cm, BugDorm, Megaview Science Education Services co. ltd., Taichung, Taiwan) for ~24h to ensure healthy insects. Stink bugs were provided a sugar:water solution with soybean pods or green beans in the laboratory. All common stink bug species occurring in significant numbers were used in these studies. This complex included the redbanded stink bug, *Piezodorus guildinii* (Westwood); southern green stink bug *Nezara viridula* (L.); brown stink bug, *Euschistus servus* (Say), and *Euschistus quadrator* (Rolston). Redbanded, brown, and *E. quadrator* adults were the primary stages used in this test, sexes were not separated.

Soybean Pod Development Characterization

A soybean plant's reproductive, or "R stages", are described as: (R1) beginning bloom, (R2) full bloom, (R3) beginning pod on the four uppermost nodes, (R4) full pod with no seed on the four uppermost nodes, (R5) beginning seed on the four uppermost nodes, (R6) full seed on the four uppermost nodes, (R7) beginning maturity with one or more pods reaching maturity, and (R8) full maturity when 95% of pods have reached maturity (Fehr et al. 1971, McPherson and McPherson 2000b).

The guide for describing reproductive soybean plant stages does not account for the variability in multiple pod stages present on a plant. Soybean reproductive stages occur from emergence of the first flower until the end of plant maturity. This period is divided into the following four general phases: 1) Flowering (R1 and R2), 2) Pod development (R3 and R4), 3) Seed growth (R5 and R6), and 4) Seed/plant maturation (R7 and R8) (Fehr et al. 1971). Stink bugs prefer to feed on soybean pods containing seed. The primary pod stages that were caged with stink bugs ranged from R5 to R6 (Figure 2). The R5 stage, with initial seed development, has a seed that is 3 mm long in a pod at one of the four most uppermost nodes on the main stem with a fully developed leaf as described by Fehr et al. (1971). The R6 plant stage (full pod) is described as a pod containing immature seed that fill the pod cavity at one of the four uppermost nodes on the main stem with a fully developed leaf (Fehr et al. 1971). To examine the effects of stink bug feeding injury on soybean seed within different levels of development, infestations were quantified at different pod stages, rather than plant stages. Four different pod stages were evaluated: R5, R5.5, R6, and R6.5. Pod stages were characterized as follows: R5 pod with beginning seed, R5.5 pod with spaces present in between larger seed, R6 pod with seed that encompass the entire space, R6.5 pod with swollen seed that are beginning to separate, R7 pod with yellow seed beginning to shrink, and R8 pod with fully mature dry brown seed.

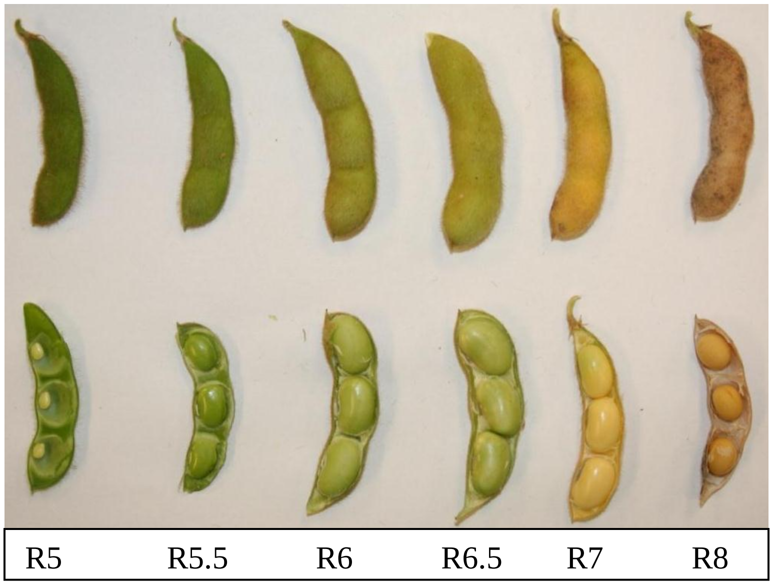

Figure 2. Soybean pod development characterization.

Infestation Procedures

Infestation procedures were modeled after the no-choice feeding assay described by Russell et al. (1999) for caging tarnished plant bugs, *Lygus lineolaris* (Palisot de Beauvois), on cotton fruiting forms. Stink bugs were individually placed in 20 ml scintillation vials, and held in a chilled cooler to prevent mortality from heat exhaustion during transportation to the field site. In the field plots, a single adult or nymph was placed in 15 cm x 11.5 cm nylon No. 280 mesh bag (Figure 3) and then caged on a single soybean raceme with two pods that were in the same pod development stage (R5, R5.5, R6, R6.5, or R7). If excess pods occurred on the raceme they were manually removed at the peduncle. For each stink bug-infested cage, there was a non-infested control cage on the nearest same age-cohort of pods. The bag was secured tightly around the peduncles of the two pods with a drawstring. At the time of caging a yellow "snapon-tag" (A. M. Leonard, Inc. Piqua, OH) was placed on the stem above the infested or non-infested cages. Tags were marked with infestation number, infestation date, stink bug species/stage and treatment (infested vs. non-infested). One group of infested and non-infested pods were removed after 7d, and one companion group remained until harvest. At the end of the caging period (7d), the cohort of immature pods was removed and transported to the laboratory for evaluation. To distinguish which pods were to be removed, the tag also was assigned with an A or B. Cages classified with A's had the green pods removed seven days after infestation (DAI), whereas those labeled with B's were mature pods removed at harvest.

Figure 3. Stink bug infestation procedures.

Stink Bug Infestations

Stink bugs were caged on soybean pods for multiple time intervals (24h, 48h, 72h, or 96h). Insects were removed from infested cages after the assigned hours after infestation (HAI) had elapsed, at which point their condition (alive, dead, moribund, or missing) was recorded. Insects were considered dead if they could not right themselves up and showed no signs of movement. Insects were considered moribund if they could not right themselves up, but were able to move. Data was not included if there was no damage and the insect was dead, moribund, or missing. At the end of the prescribed infestation period, both infested and non-infested cages were maintained on pods until a total of 7 DAI had elapsed (time after stink bug removal: 7d, 6d, 5d, 4d, or 3d). Cages were replaced on pods to allow injury to become more apparent, and to prevent injury produced by native stink bug infestations before green pods were harvested. Green pods were hand harvested at this time if indicated by an "A" on the tags, all others with "B" tags remained until full seed maturity. Similar methods were used to study the effects of stink bugs on pods in selected growth stages (R5, R5.5, R6, and R6.5).

Soybean Seed Injury

Green pods with immature seed were harvested, transported to a laboratory, and stored in a refrigerator. Pods were examined for injury within 3d after removal from the field. Each pod was examined for external feeding symptoms and was then dissected at the suture to evaluate the interior pod wall and seed coat for evidence of feeding. Symptoms of stink bug feeding on green pods included discoloration and puncture marks. Further signs of feeding on the seed included shriveling and disfiguration. Data was recorded as percent injured green pods and immature soybean seed.

Mature pods with seeds were harvested after whole plants developed to the R8 stage. If necessary, seed was further dried to ensure <13% moisture for storage. Mature pods were dissected for examination of the interior pod wall for evidence of punctures and to expose seed. External seed injury was characterized by shriveling of the seed, indentions, discoloration, and punctures. The presence of internal injury to mature seed was examined by cross-sectioning each seed with a razor. The presence of a chalky puncture wound was characteristic of internal mature seed injury. Data was recorded as percent injured soybean pods and seeds. Mature seed weights were recorded for each of the following categories: 1) non-infested pods non-injured seed; 2) non-infested pods injured seed; 3) infested pods non-injured seed; and 4) infested pods injured seed.

Data Analyses

The experimental design for this study was completely randomized. Each infestation was considered a replication. The effects of infestation duration on seed injury and a component of grain quality (mature seed weights) were subjected to analysis of variance (ANOVA) using the GLIMMIX procedure (SAS Institute Inc. 2009). Normal distribution was assumed and date of infestation was considered as a factor influencing variability. Significant effects of treatments, infestation durations, and the treatment by infestation duration interactions were evaluated for a single pod stage.

The effects of pod stage on seed injury and grain quality (mature seed weights) for a single infestation duration also were subjected to an analysis of variance (ANOVA) using the GLIMMIX procedure (SAS Institute Inc. 2009). Normal distribution was assumed and date of infestation was considered as a factor influencing variability. Significant differences between treatments, pod stages, and treatment by pod stage interaction were evaluated for single infestation duration. Whenever appropriate, means were compared using Fisher's protected LSD test (P=0.05). In some instances, the data for each date and pod stage was corrected for any injury or associated effects in non-infested cages using Abbott's formula (Abbott 1925).

RESULTS

Infestation Duration

Stink bug injury to soybean was evaluated for infestation duration effects by examining immature and mature seed. For redbanded stink bug, *Piezodorus guildinii* (Westwood), caged on R5.5 stage pods, there was a significant difference (*P*<0.0001) detected between the stink bug-infested and non-infested treatments on immature seed injury at all intervals (Figure 4). Significant seed injury differences also were detected for infestation duration (*P*=0.0002) and for treatment by infestation duration effects (*P*=0.0102). Seed injury in the infested pods peaked 72 HAI and was not significantly different from seed injury at 96 HAI. Both values were significantly higher than seed injury produced at earlier infestation durations. Seed injury at 48 HAI was significantly higher than that produced 24 HAI. There were no significant differences in seed injury for non-infested pods across all time periods.

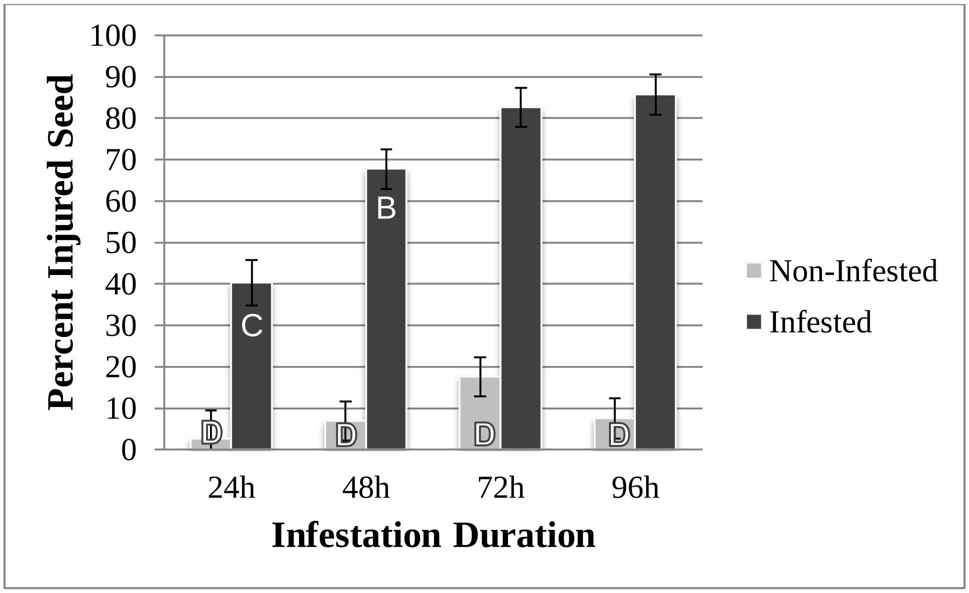

Figure 4. Immature seed injury (mean ± S.E.) from redbanded stink bug (*P. guildinii*) adults caged on R5.5 pods. Means with a common letter are not significantly different (LSD, *P*≤ 0.05).

Mature seed injury in stink bug-infested pods was significantly (*P*<0.0001) higher than seed injury in non-infested pods (Figure 5). Significant differences were detected for the effect of infestation duration (*P*=0.0351) but no significant differences were detected for the treatment by infestation duration interaction (*P*=0.9045). There were no significant differences in seed injury among infestation durations for non-infested pods. However for infested pods, mature seed injury 24 HAI was significantly lower than seed injury 72 and 96 HAI (Figure 5).

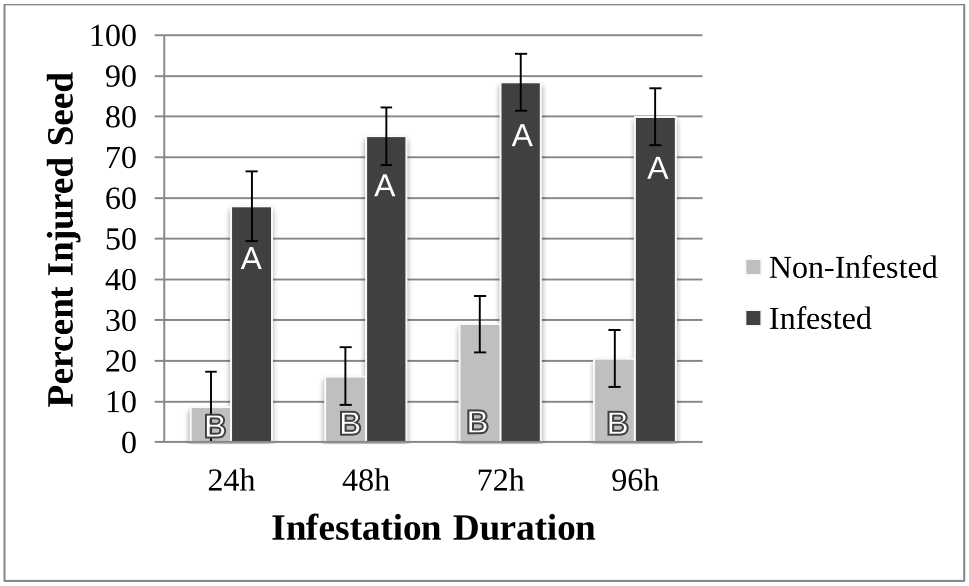

Figure 5. Mature seed injury (mean ± S.E.) from redbanded stink bug (*P. guildinii*) adults caged on R5.5 pods. Means with a common letter are not significantly different (LSD, *P*≤ 0.05) for treatment (non-infested vs. infested) effects.

Mature seed weights were categorized by treatment (infested vs. non-infested) and the presence of injured seed (Figure 6). Significant differences were detected between the mean weights of injured and non-injured seed (*P*<0.0001). However, no significant differences were detected among infestation durations for injured or non-injured seed (*P*=0.7674). No significant

(*P*=0.6437) interaction was detected between treatment and infestation duration. Weights for non-infested pods and non-injured seed ranged from 155-174mg/seed. Weights of injured seed in the non-infested pods ranged from 24 to 71mg/seed. Weights of non-injured seed from infested pods ranged from 169 to 198mg/seed. Weights of injured seed from infested pods ranged from 33 to 62mg/seed. There also were no significant differences between the weights of the non-injured seed (infested vs. non-infested) and no significant differences between the injured seed (infested vs. non-infested) weights.

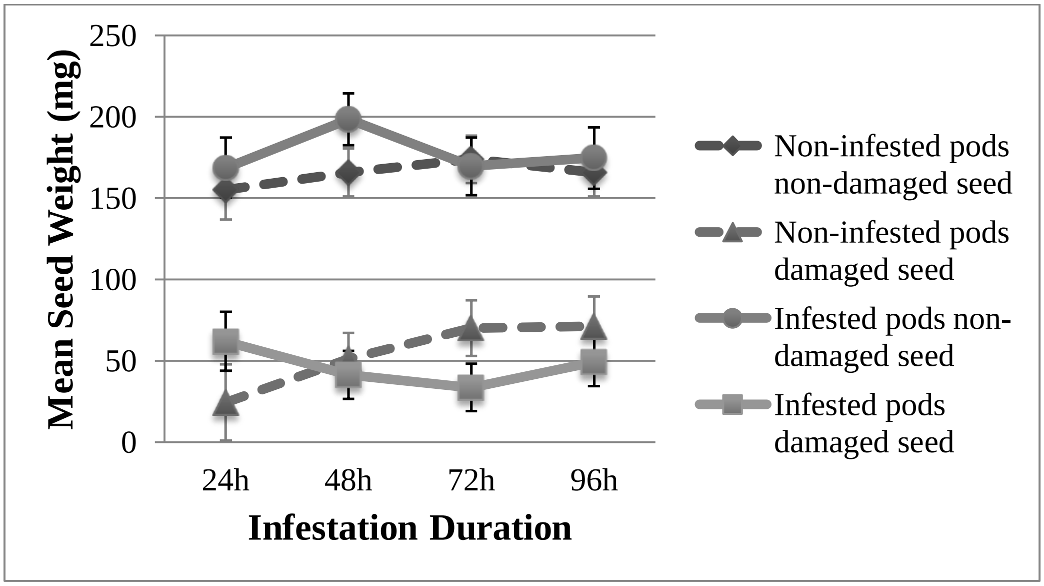

Figure 6. Mature seed weights (mean ± S.E.) from redbanded stink bug (*P. guildinii*) adults caged on R5.5 pods.

For brown stink bug, *Euschistus servus* (Say), caged on R5 pods, there was a significant difference in immature seed injury detected between the stink bug-infested and non-infested treatments (*P*<0.0001) at all intervals (Figure 7). Significant differences were detected among infestation durations (*P*=0.0170) and for the treatment by infestation duration interaction (*P*=0.0084). Seed injury in the infested pods peaked 96 HAI and was significantly higher than seed injury 72 HAI. At both periods, seed injury was significantly higher than seed injury produced at other infestation durations. No significant differences were detected in seed injury between 24 HAI and 48 HAI. There were no significant differences (*P*>0.05) in seed injury for non-infested pods across all time periods.

Mature seed injury from stink bug-infested pods was significantly higher than seed injury in the non-infested pods (*P*<0.0001) at maturity (Figure 8). No significant differences were detected for infestation duration (*P*=0.1200) and no significant differences were detected for the treatment (non-infested vs. infested) by infestation duration interaction (*P*=0.6228). There were no significant differences (*P*>0.05) in seed injury among infestation durations for non-infested pods or infested pods.

Mature seed weights were categorized by treatment (infested vs. non-infested) and the presence of injured seed for brown stink bug (Figure 9). Significant differences (*P*<0.0001) were detected between weights of injured and non-injured seed at all time intervals. No significant difference was detected for infestation duration (*P*=0.1389) or the treatment by infestation duration interaction (*P*=0.7692). Weights for non-injured seed from non-infested pods ranged from 141 to 202mg/seed. Weights for non-infested pods and injured seed ranged from 37 to 80mg/seed. Weights of non-injured seed from infested pods ranged from 142 to 203mg/seed. Weights from infested pods of injured seed ranged from 53 to 76mg/seed. Regardless of treatment (infested vs. non-infested), there also were no significant differences in the weights of the non-injured seed or injured seed.

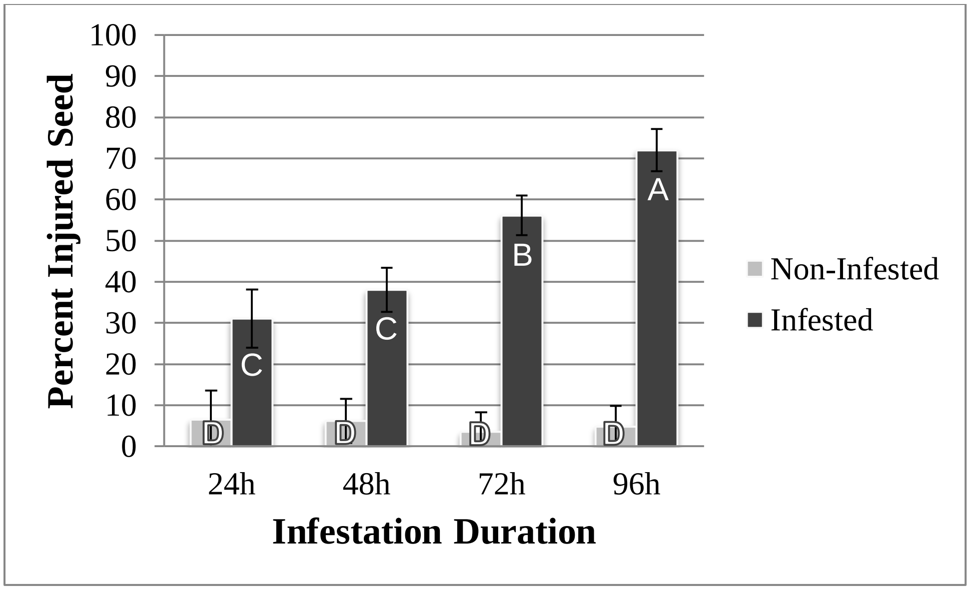

Figure 7. Immature seed injury (mean + S.E.) from Brown stink bug (*E. servus*) adults caged on R5 pods. Means with a common letter are not significantly different (LSD, *P*≤ 0.05).

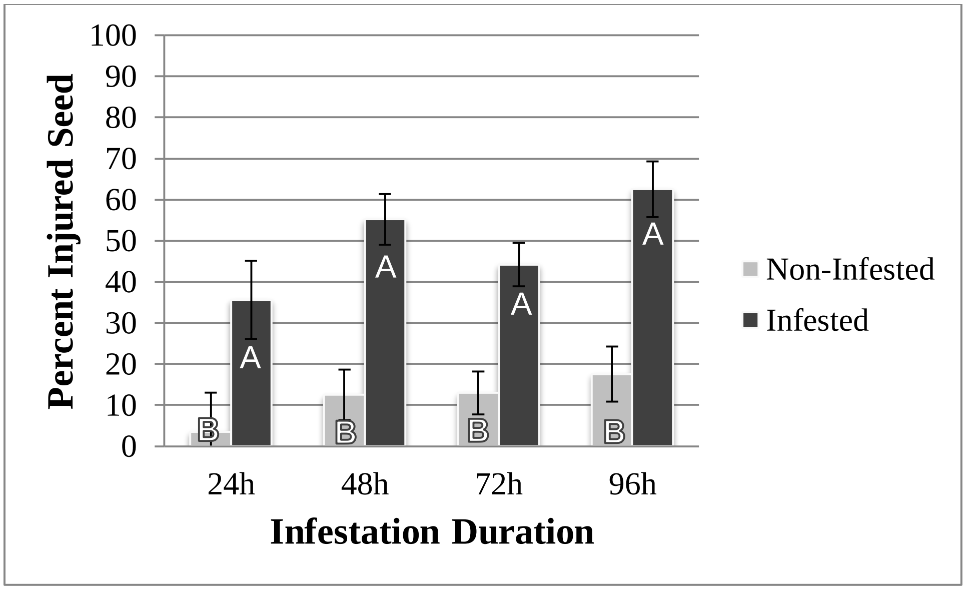

Figure 8. Mature seed injury (mean + S.E.) from brown stink bug (*E. servus*) adults caged on R5 pods. Means with a common letter are not significantly different (LSD, *P*≤ 0.05) for treatment (non-infested vs. infested) effects.

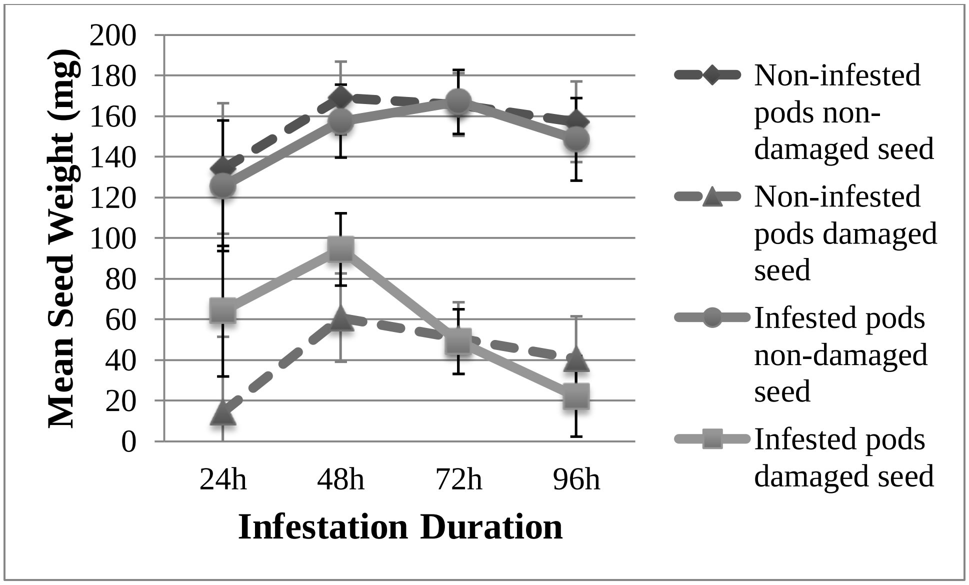

Figure 9. Mature seed weights (mean ± S.E.) from brown stink bug (*E. servus*) adults caged on R5 pods.

For brown stink bug caged on R5.5 pods there was a significant (*P*<0.0001) difference detected between the infested and non-infested treatments for immature seed injury at all intervals (Figure 10). No significant infestation duration effect (*P*=0.1422) or treatment by infestation duration interaction (*P*=0.1240) was detected. There were no significant differences (*P*>0.05) in seed injury for non-infested pods or infested pods across all time periods.

Mature seed injury from stink bug-infested pods was significantly higher (*P*<0.0001) than seed injury in the non-infested pods (Figure 11). No significant difference was detected for infestation duration (*P*=0.3432) or the treatment by infestation duration interaction (*P*=0.6730). There were no significant differences in mature seed injury among infestation durations for non-infested and infested pods.

Mature seed weights were categorized by treatment (infested vs. non-infested) and the presence of injured seed (Figure 12). Significant differences ($P \leq 0.0001$) were detected between the weights of injured and non-injured seed. No significant infestation duration effect (*P*=0.3684), or treatment by infestation duration interaction (*P*=0.6072) was observed. Weights for non-injured seed in non-infested pods ranged from 141 to 202mg/seed. Weights of injured seed from non-infested pods ranged from 37 to 79mg/seed. Weights of non-injured seed from infested pods ranged from 203 to 142mg/seed. Weights of injured seed from infested pods ranged from 53 to 76mg/seed. Weights of non-injured seed and weights of injured seed were similar when comparing infested pods to non-infested pods.

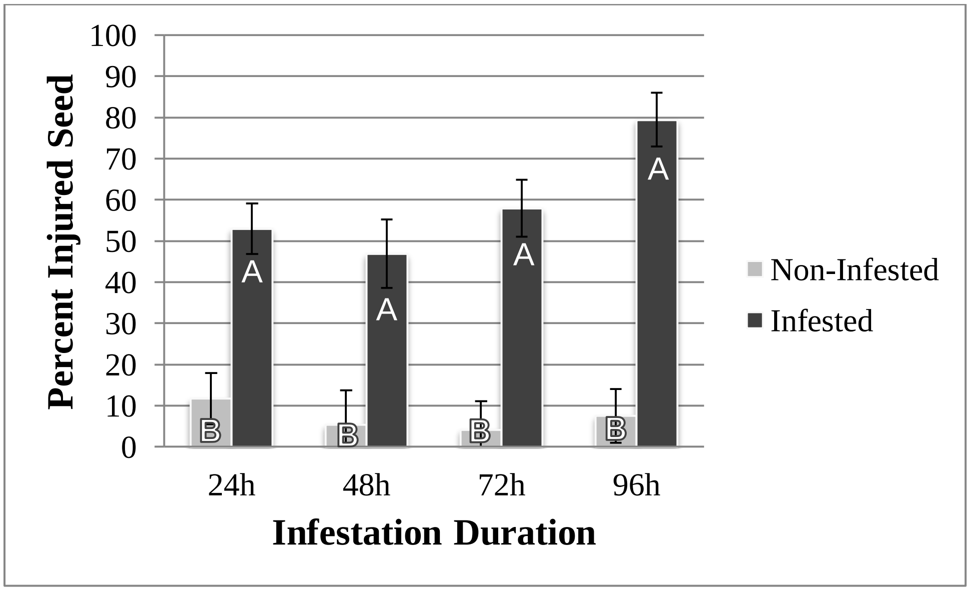

Figure 10. Immature seed injury (mean ± S.E.) from brown stink bug (*E. servus*) adults caged on R5.5 pods. Means with a common letter are not significantly different (LSD, *P*≤ 0.05) for treatment (non-infested vs. infested) effects.

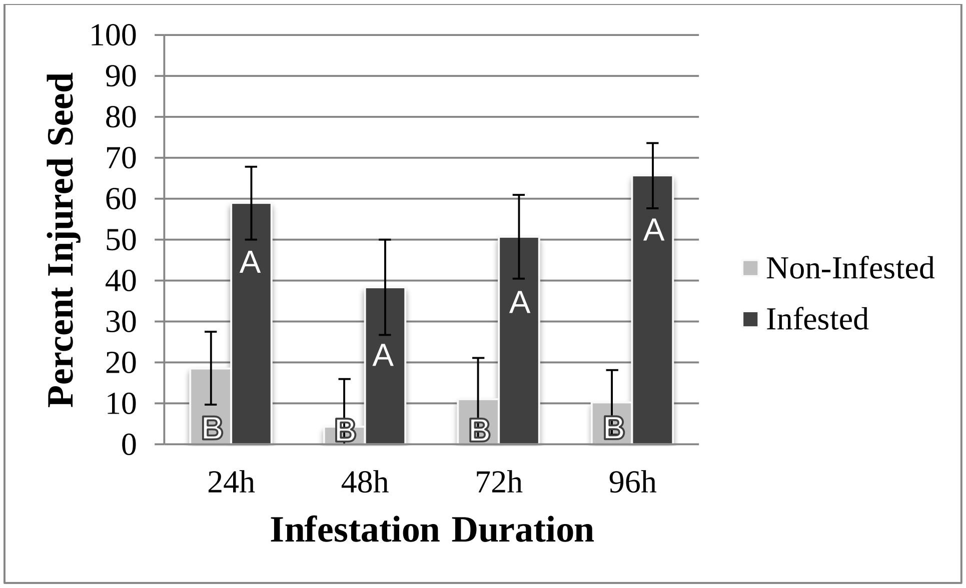

Figure 11. Mature seed injury (mean + S.E.) from brown stink bug (*E. servus*) adults caged on R5.5 pods. Means with a common letter are not significantly different (LSD, $P \leq 0.05$) for treatment (non-infested vs. infested) effects.

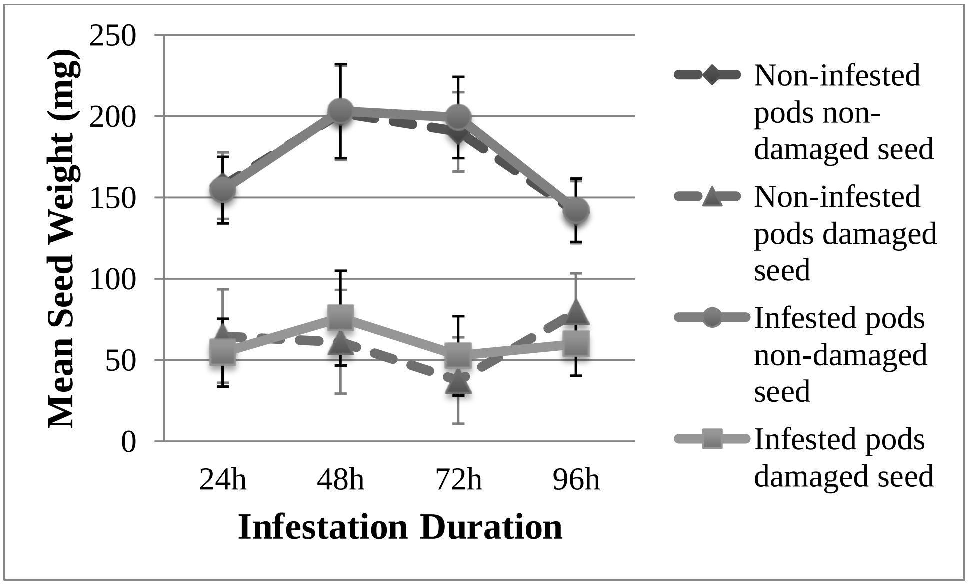

Figure 12. Mature seed weights (mean + S.E.) from brown stink bug (*E. servus*) adults caged on R5.5 pods.

For *Euschistus quadrator* (Rolston) caged on R5.5 stage pods, there was a significant difference (*P*<0.0001) detected between the infested and non-infested treatments for immature injury at all intervals (Figure 13). No significant infestation duration effect (*P*=0.1977) or treatment by infestation duration interaction (*P*=0.1583) was detected. There were no significant differences in seed injury for non-infested pods and seed injury for infested pods across all duration periods (*P*>0.05).

Mature seed injury from stink bug-infested pods was significantly higher (*P*<0.0001) than injury in the non-infested pods (Figure 14). No significant effect was detected for infestation duration (*P*=0.2499) or treatment by infestation duration interactions (*P*=0.8795). There were no significant differences in mature seed injury among infestation durations for non-infested and infested pods (*P*>0.05).

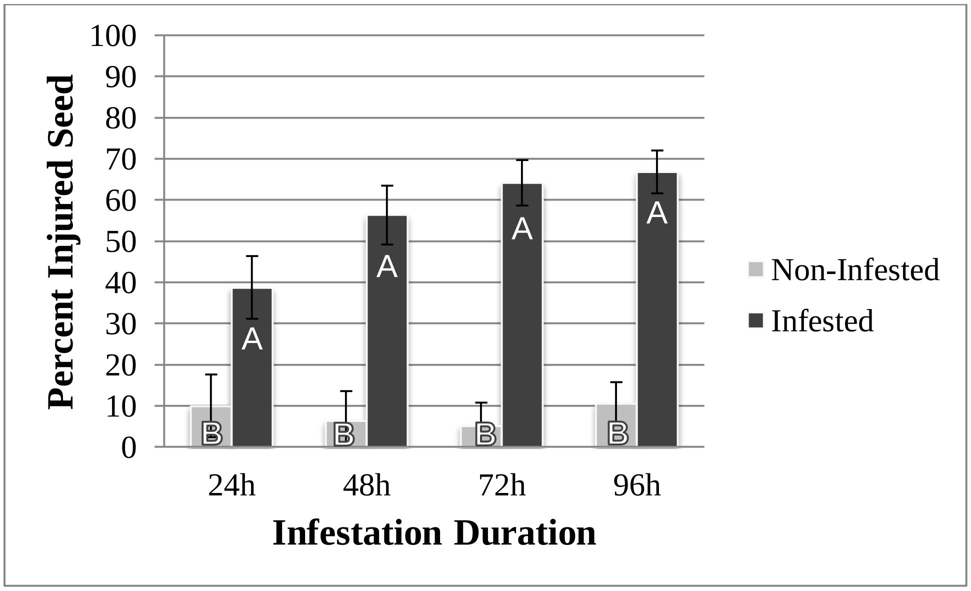

Figure 13. Immature seed injury (mean + S.E.) from *Euschistus quadrator* adults caged on R5.5 pod. Means with a common letter are not significantly different (LSD, *P*≤ 0.05) for treatment (non-infested vs. infested) effects.

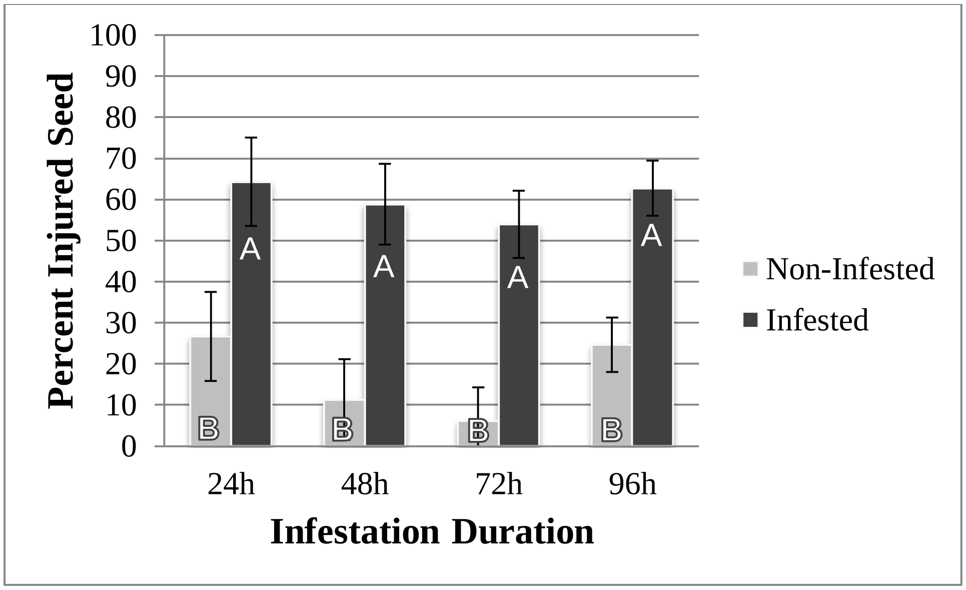

Figure 14. Mature seed injury (mean + S.E.) from *Euschistus quadrator* adults caged on R5.5 pod. Means with a common letter are not significantly different (LSD, *P*≤ 0.05) for treatment (non-infested vs. infested) effects.

Mature seed weights were categorized by treatment (infested vs. non-infested) and the presence of injured seed (Figure 15). Significant differences (*P*<0.0001) were detected between the weights of stink bug injured and non-injured seed. No significant difference was detected for infestation duration (*P*=0.1093). However, a significant (*P*=0.0207) treatment by infestation duration interaction was detected. Weights of non-injured seed from non-infested pods ranged from 138 to 209mg/seed. Weights of injured seed from non-infested pods ranged from 28 to 80mg/seed. Weights of non-injured seed from infested pods ranged from 138 to 205mg/seed. Weights of injured seed from infested pods ranged from 46 to 62mg/seed. In direct comparison of infested and non-infested pods, no significant differences were found between the weights of the non-injured seed and weights of injured seed. Considerable variability in seed weights of non-damaged seed occurred regardless of infestation across all intervals, weights of injured seed were similar regardless of infestation interval.

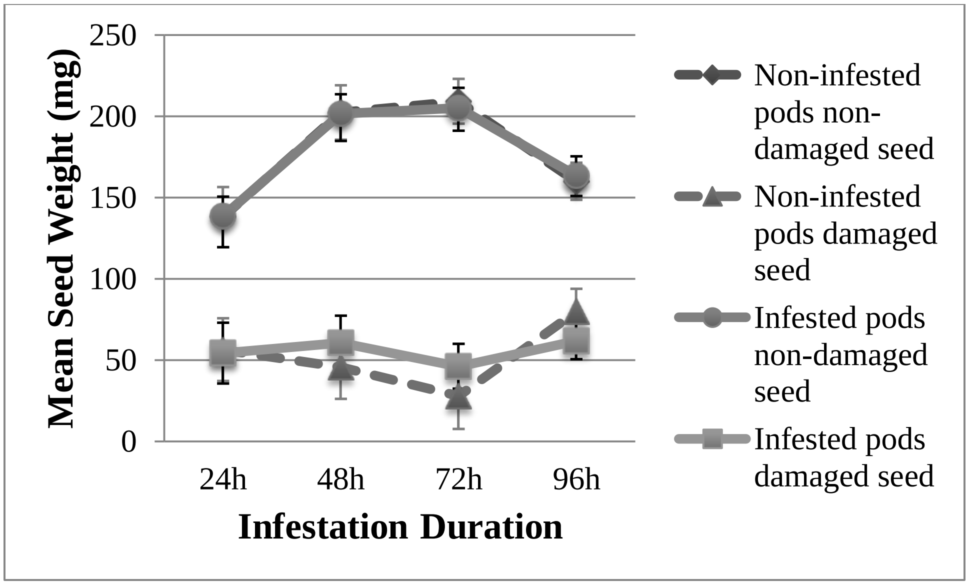

Figure 15. Mature seed weights (mean + S.E.) from *Euschistus quadrator* adults caged on R5.5 pods.

Stink Bug Injury During Selected Pod Development Stages

Redbanded stink bug injury to immature seed was evaluated on R5, R5.5, R6, and R6.5 redbanded stink bug-infested pods 72 HAI (Table 2). Significant differences (*P*<0.0001) were detected between the infested and non-infested treatments at all pod stages. Injured seed from non-infested pods ranged from 0% to 17%, and no significant differences (*P*>0.05) in seed injury among pod stages were detected for non-infested pods (Appendix B). After correcting for seed injury in non-infested pods, no significant differences (*P*=0.1036) were detected for seed injury in the stink bug-infested pods among stages.

Mature seed injury among pod stages also was evaluated at harvest (Table 3). Significant differences (*P*=0.0009) were detected between the stink bug-infested and non-infested treatments. There was no significant difference (*P*>0.05) in seed injury for non-infested pods (1 to 25%) among stages (Appendix B). After correcting for injured seed in the non-infested pods, no significant differences (*P*=0.2259) in seed injury for infested pods were detected among pod stages.

Table 2. Effects of redbanded stink bug (*P. guildinii*) adult injury to immature seed 72 HAI on selected pod stages.

Pod stage	Percent injured immature seed ± S. E.
R5	73.03 ± 12.40 a
R5.5	83.19 ± 8.77 a
R6	43.52 ± 10.13 a
R6.5	52.67 ± 17.54 a
P>F	0.1036

Means corrected using Abbott's formula. Means followed by the same letter are not significantly different (LSD, *P*≤ 0.05). Cages remained on pods for a total of 7d, stink bugs were removed following indicated infestation interval.

Table 3. Effects of redbanded stink bug (*P. guildinii*) adult injury to selected pod stages 72 HAI on mature seed.

Pod stage	Percent injured mature seed ± S. E.
R5	41.20 ± 15.39 a
R5.5	79.57 ± 10.88 a
R6	65.96 ± 10.88 a
R6.5	39.36 ± 21.77 a
P>F	0.2259

Means corrected using Abbott's formula. Means followed by the same letter are not significantly different (LSD, *P*≤ 0.05). Cages remained on pods for a total of 7d, stink bugs were removed following indicated infestation interval.

Mature seed weights were categorized by treatment (infested vs. non-infested) and the presence of injury for pod development stage effects (Figure 16). Significant differences (*P*<0.0001) were detected between weights of injured seed (infested vs. non-infested) and weights of non-injured seed (infested vs. non-infested). There were no significant differences (*P*>0.05) between non-injured seed weights and no significant differences (*P*>0.05) between injured seed weights. Significant differences were detected among pod stages (*P*=0.0001) and for the weight category by pod stage interaction (*P*=0.0177). Weights of non-injured seed in non-infested pods ranged from 123 to 173mg/seed. Weights of injured seed in non-infested pods ranged from 41 to 91mg/seed. Weights of non-injured seed in infested pods ranged from 122 to 171mg/seed. Weights of injured seed in infested pods ranged from 34 to 80mg/seed. For weights of injured seed there were no significant differences between R5 and R5.5 stages. However, injured seed weights from those stages were significantly lower than that in R6 pods. Seed weights of non-injured seed (infested and non-infested) did not follow the same trend as that of injured seed and were no affected by pod stage.

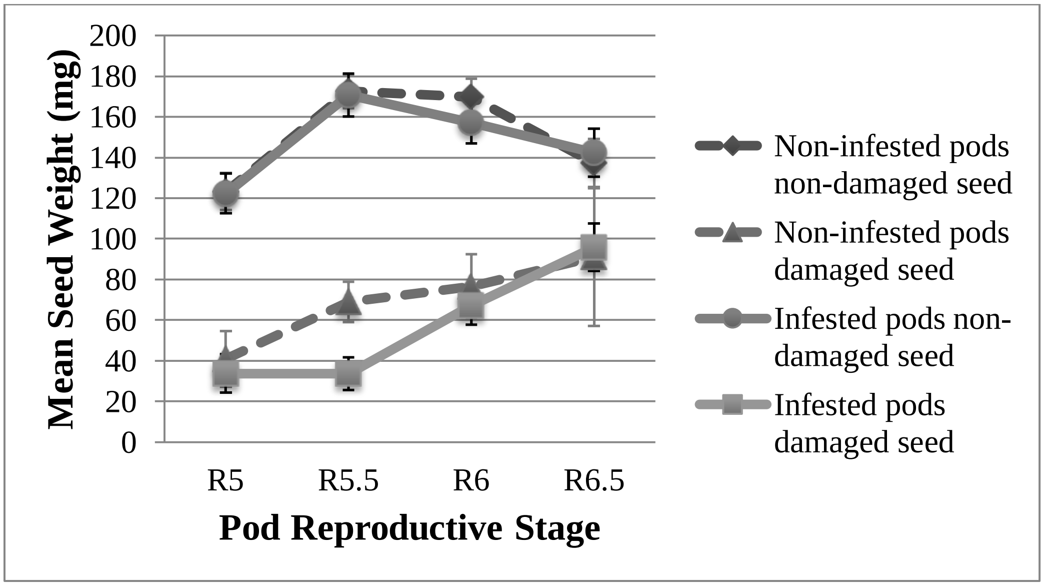

Figure 16. Mature seed weights (mean ± S.E.) for redbanded stink bug (*P. guildinii*) adults caged on R5-R6.5 pods 72 HAI.

Brown stink bug injury to immature seed was evaluated on R5, R5.5, and R6 stage pods 72 HAI (Table 4). Significant differences were detected between the stink bug-infested and non-infested treatments (*P*=0.0001). Injured seed from non-infested pods ranged from 1% to 5%, and no significant differences (*P*>0.05) among pod stages were detected for seed injury in non-infested pods (Appendix B). After correcting for injured seed in non-infested pods, no significant differences (*P*=0.3267) were detected in seed injury among pod stages.

Table 4. Effects of brown stink bug (*E. servus*) adult injury to immature seed 72 HAI on selected pod stages.

Pod stage	Percent injured immature seed ± S. E.
R5	55.58 ± 5.92 a
R5.5	63.04 ± 7.64 a
R6	37.95 ± 13.24 a
P>F	0.3267

Means corrected using Abbott's formula. Means followed by the same letter are not significantly different (*P*≤ 0.05, Fishers Protected LSD). Cages remained on pods for a total of 7d, stink bugs were removed following indicated infestation interval.

Mature seed injury also was evaluated at harvest for pod stage effects (Table 5). Significant differences (*P*=0.0191) were detected between the infested and non-infested treatments. However, there were no significant differences (*P*>0.05) for seed injury (8 to 13%) in non-infested pods (Appendix B). After correcting for injured seed in the non-infested pods, no significant differences (*P*=0.2242) were detected among pod stages.

Table 5. Effects of brown stink bug (*E. servus*) adult injury to selected pod stages 72 HAI on mature seed.

Pod stage	Percent injured mature seed ± S. E.
R5	32.83 ± 8.61 a
R5.5	49.64 ± 11.11 a
R6	7.21 ± 19.24 a
P>F	0.2242

Means corrected using Abbott's formula. Means followed by the same letter are not significantly different (*P*≤ 0.05, Fishers Protected LSD). Cages remained on pods for a total of 7d, stink bugs were removed following indicated infestation interval.

Mature seed weights were categorized by treatment (infested vs. non-infested) and the presence of injury for evaluation of pod development stage effects (Figure 17). Significant differences (*P*<0.0001) were detected between weights of injured seed (non-infested and infested) and of non-injured seed (non-infested and infested). There were no significant differences in non-injured or injured seed weights among stages (*P*=0.4918) and weight category by pod stage interaction (*P*=0.2373). Weights of non-injured seed in non-infested pods ranged from 138 to 191mg/seed. Weights of injured seed in non-infested pods ranged from 37 to 54mg/seed. Weights of non-injured seed in infested pods ranged from 106 to 200mg/seed. Weights of injured seed in infested pods ranged from 49 to 98mg/seed.

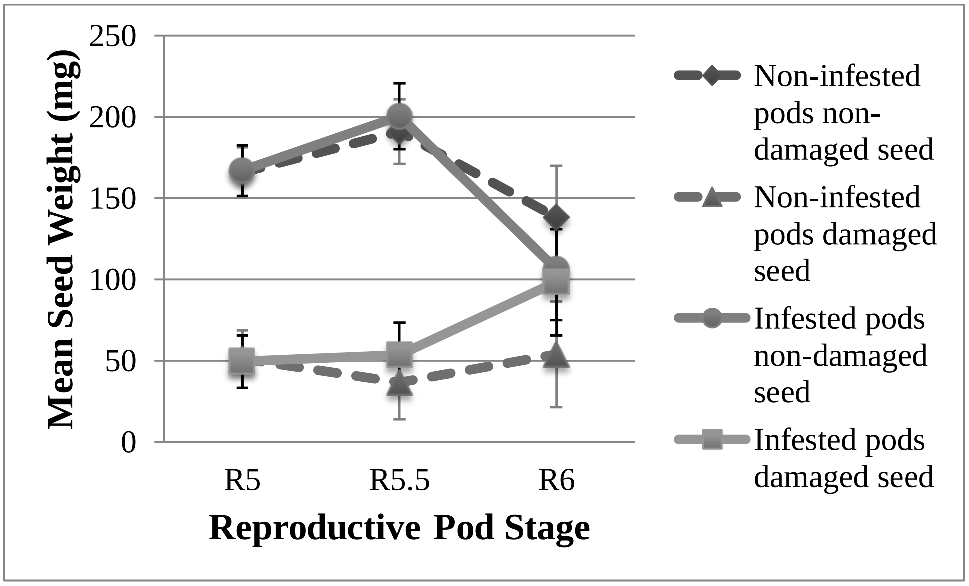

Figure 17. Mature seed weights (mean ± S.E.) for brown stink bug (*E. servus*) adults caged on R5-R6 pods 72 HAI.

Euschistus quadrator injury to immature seed was evaluated on R5, R5.5, and R6 stink bug-infested pods 72 HAI (Table 6). Significant differences were detected between the infested and non-infested treatments (*P*<0.0001). Injured seed from non-infested pods ranged from 2% to 13%, and no significant differences (*P*>0.05) among stages were detected for seed injury in non-infested pods (Appendix B). After correcting for injured seed in non-infested pods, no significant differences (*P*=0.3901) were detected in seed injury among pod stages.

Table 6. Effects of *Euschistus quadrator* adult injury to immature seed 72 HAI on selected pod stages.

Pod stage	Percent injured immature seed ± S. E.
R5	51.32 ± 8.90 a
R5.5	67.53 ± 7.27 a
R6	65.53 ± 12.59 a
P>F	0.3901

Means corrected using Abbott's formula. Means followed by the same letter are not significantly different (LSD, *P*≤ 0.05). Cages remained on pods for a total of 7d, stink bugs were removed following indicated infestation interval.

Mature seed injury also was evaluated at harvest for pod stage effects (Table 7). Significant differences (*P*=<0.0001) were detected between the infested and non-infested treatments. However, there was no significant difference (*P*>0.05) for seed injury (5% to 9%) in non-infested pods (Appendix B). After correcting for injured seed in the non-infested pods, no significant differences (*P*=0.7445) were detected among pod stages.

Table 7. Effects of *Euschistus quadrator* adult injury to selected pod stages 72 HAI on mature seed.

Pod stage	Percent injured mature seed ± S. E.
R5	56.02 ± 6.98 a
R5.5	52.94 ± 4.65 a
R6	64.10 ± 12.10 a
P>F	0.7445

Means corrected using Abbott's formula. Means followed by the same letter are not significantly different (*P*≤ 0.05, Fishers Protected LSD). Cages remained on pods for a total of 7d, stink bugs were removed following indicated infestation interval.

Mature seed weights were categorized by treatment (infested vs. non-infested) and the presence of injury for evaluation of pod development stage effects (Figure 18). Significant differences ($P<0.0001$) were detected between weights of injured seed (non-infested and infested) compared to weights of non-injured seed (non-infested and infested). There were no significant differences ($P>0.05$) between non-injured seed weights and no significant differences between injured seed weights. No significant effect ($P=0.1183$) was detected for pod stages. A significant effect ($P=0.0261$) was detected for the weight category by pod stage interaction. Weights of non-injured seed in non-infested pods ranged from 178 to 209mg/seed. Weights of injured seed in non-infested pods ranged from 28 to 112mg/seed. Weights of injured seed in infested pods ranged from 177 to 205mg/seed. Weights of injured seed in infested pods ranged from 30 to 108mg/seed. For weights of injured seed there were no significant differences between R5 and R5.5 stages. However, injured seed weights from both stages were significantly lower than that in R6 pods. Seed weights of non-injured seed (infested and non-infested) did not follow the same trend as that of injured seed and were not affected by pod stage, with the exception of injury found in non-infested cages on R6 stage pods.

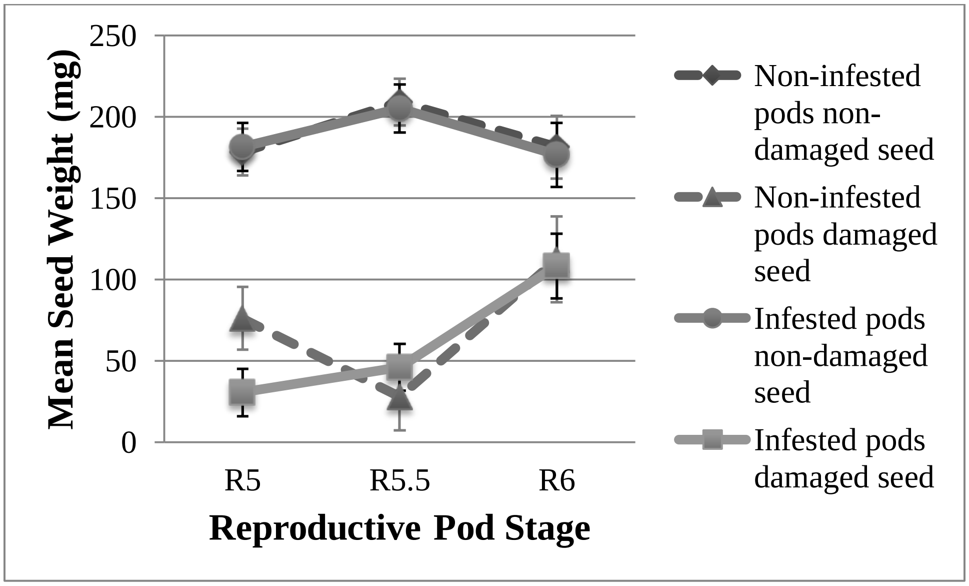

Figure 18. Mature seed weights (mean + S.E.) for *Euschistus quadrator* adults caged on R5-R6 pods 72 HAI.

DISCUSSION

The development of a no-choice feeding protocol to evaluate injury at the soybean pod/seed level creates the opportunity to isolate the effects of stink bug feeding compared to traditional whole plant or plot caging procedures. Focusing on soybean pod stages, as opposed to the whole plant, was of key importance in this study. The value of the soybean crop is contained within harvested seed and this protocol can directly relate stink bug feeding during pod development to yield and quality by reducing the impact of other complicating factors. The results of the work presented herein produced discriminate results across a range of infestation durations for multiple species. The protocol was successful using only a single stink bug adult on a two-pod cohort (3 seed/pod; 6 total seed). The symptoms of pod and seed injury produced by stink bug adults in this work are similar to that described by other research.

The objective of the initial experiment was to determine the minimum infestation period necessary for a single stink bug to produce significant levels of injury to immature and mature soybean seed. This information is necessary to develop baseline infestation duration for future experiments. Stink bugs prefer feeding on soybean pods during the R4-R6 stages (McPherson and McPherson 2000b); therefore infestations for these duration interval experiments were maintained at the R5 or R5.5 pod development stages. The assessment of injury to immature (green) seed was done to determine the initial incidence of feeding by stink bugs and to manage variability in the results by reducing incidental injury produced by native stink bug populations. All three species of stink bugs successfully penetrated pods and injured seed during the infestation duration studies. Some immature seed injury (3% - 17%) was observed in non-infested pods, but this was the result of native infestations of stink bugs feeding prior to the initiation of these tests. Regardless of species (redbanded stink bug, brown stink bug, and *E.*

quadrator) or infestation period (24-96 HAI), pods infested with stink bugs consistently showed significantly higher injury to immature seed compared to that in non-infested pods. Redbanded stink bug injury to immature seed was significantly higher at 72 HAI than that 48 HAI, and both were significantly higher than that 24 HAI. Brown stink bug injury to immature seed was significantly higher 96 HAI than that 72 HAI, and was significantly higher than other infestation durations. There was no other significant infestation duration effect for stink bugs. These results suggest that stink bugs in general can cause visual symptoms of injury to immature seed very rapidly (≤ 72 HAI).

Seed injury from stink bug infestations also was evaluated at plant maturity (R8) for the same 24-96 HAI intervals. On mature seed, injury was observed in non-infested pods (3% - 25%) which was the result of feeding by native populations prior to and after cages were used. All species during each infestation period still caused significant levels of injury that was visible on mature seed. As previously reported for immature seed, redbanded stink bug injury on mature seed was significantly higher at 72 HAI than that ≤ 48 HAI. There was no significant infestation duration effect for other species on mature seed. In addition to visual seed injury, yields (weights of harvested seed) were recorded at harvest. Weights of seed in the stink bug-infested pods were significantly lower than in the non-infested pods for all species at all intervals. Seed weights in the infested pods ranged 22.01 – 60.66 mg per seed compared to 138.0 – 209.1 mg per seed in the non-infested pods. These results are consistent with other reports which have shown phytophogaus stink bugs native to Louisiana soybean can produce significant (63.9 to 78.5 %) injury to pods (Todd and Turnipseed 1974). Feeding from southern green stink bug, green stink bug, brown stink bug, and dusky brown stink bug all caused significant yield losses in Louisiana soybean (McPherson et al. 1979). Todd and Turnipseed

(1974) reported significant reductions in seed yield and significant increases in seed injury from southern green stink bug. Yeargan (1977) reported loses in yield when caging green stink bug adults, which was a result of reduced seed size and reduced seed produced by the soybean plants (Yeargan 1977). Soybean artificially infested with brown stink bugs resulted in significant levels of seed injury and the injured seed were smaller compared to non-injured seed (Daugherty et al. 1964).

A more complete understanding of infestation duration required for stink bugs to injure soybean seed is important for future experiments in order to more efficiently design experiments for detecting significant differences with the briefest infestation period. Significant differences in the percent of injured seed and seed weights can be directly correlated with the intensity of the injury produced by a stink bug (Jensen and Newsom 1972). From a practical perspective, the knowledge of the injury levels and length of infestation can be used to better time field sampling strategies and insecticide application frequency, as well as, refine treatment action thresholds.

In the second experiment examining the effects of stink bug infestations during selected pod development stages (R5-R6.5), similar assessments were made for injury to immature and mature seed. Based upon the results in the infestation duration studies, stink bugs were caged on all pods for 72 HAI. Seed injury in non-infested immature pods ranged from 0% to 17% for all species. No significant differences in immature seed injury were detected among pod development stages within each species. Immature seed injury for all stink bugs ranged from a low of 38% (brown stink bug, R6) to 88% (redbanded stink bug, R5.5). In general, the trend for immature seed injury was highest during R5-R5.5 stages.

Similar results on injury to mature seed were observed at harvest. Seed injury in the non-infested treatment ranged from 1% to 25% on mature pods for all species. No significant differences were detected among pod stages, but seed injury levels ranged from a low of 38% (brown stink bug, R6) to 80% (redbanded stink bug, R5.5). Again trends for the highest levels of mature seed injury generally occurred during the R5 and R5.5 pod stages.

Weights of mature seed collected at harvest were significantly affected by stink bug infestations. With the exception of infestations at R6 (for *E. quadrator*) and R6.5 (for redbanded stink bug), injured seed from infested pods weighed significantly less than non-injured seed from non-infested pods. Weights of stink bug injured seed from infested pods ranged from 30.43 – 108.2 mg per seed and from 123.3 – 209.0 mg per seed, respectively, across all pod development stages. As observed with immature and mature seed injury, trends for stink bug-injured seed indicated the lowest weights to occur during R5 - R5.5 infestations. Overall seed weights of the true control (non-infested non-injured seed) were not significantly different from seed weights of the non-injured seed in stink bug- infested pods. This result suggests that the non-injured seed in a pod that also has had some of the seed injured by stink bugs may not be compensating for the injured seed. In addition, the weights of injured seed in non-infested pods were similar to the weights of injured seed from stink bug-infested pods. Seed injury produced with this protocol appears to be comparable to that injury produced by native populations of stink bugs in a natural environment.

Although no direct comparison was possible in the present study, there was a consistent trend of redbanded stink bug producing higher levels of seed injury than that observed for the other two species observed in both experiments. A Brazilian study evaluating redbanded stink bug, southern green stink bug (*Nezara viridula),* and *E. heros* injury to soybean seed demonstrated that redbanded stink bug injured more seed and significantly lowered seed quality when compared to the other two species (Correa-Ferreira and Azevedo 2002).

Mature seed weights are strong indicators of yield and quality in these types of experiments, but the effects are most apparent in seed with heavy stink bug injury. Todd (1981) described four categories of stink bug injury: (1) Light damage - seed with puncture marks, but no deformity of the seed coat or endosperm; (2) Medium damage- seed with puncture marks and mild deformity but no reduction in size; (3) Heavy damage- seed with puncture marks, gross deformity, and some reduction in size and weight; and (4) Severe damage- seed with puncture marks, gross deformity, and drastically reduced size and weight. The last two categories are sometimes combined because seed in these categories are of no value for oil, meal, or planting (Todd 1981). The last two categories are similar to seed weights because both are characterized by a reduction in size, and weight.

Variability in a soybean plant's susceptibility to stink bug during the plant's reproductive stages could have implications on action thresholds. Temple et al. (2011, Unpublished data) reported that the R5 plant stage is more sensitive to yield effects compared to all other reproductive growth stages. Another study found the presence of southern green stink bug at the beginning of seed fill stages significantly decreased soybean seed yield and quality (McPherson et al. 1979). The recent shift in Louisiana's soybean production in Louisiana has included a transition an early production system (Heatherly 1999, Baur et al. 2000) relying heavily (64% of total acreage) in early maturing varieties (MGs III and IV) (R. Levy personal communication). Many of these varieties express an indeterminate growth pattern and when a soybean plant is in the R4 stage, R5 and R5.5 stage pods can be present on these varieties. Current action thresholds are based on a system that does not account for within plant pod variability on indeterminate varieties.

The results from this work support a no-choice feeding protocol to measure the effects of stink bug feeding at the pod and seed level on soybean plants. The utility of these procedures will allow experiments to focus on comparisons among species, sexes, and life stages, as well as examine the impact of stink bugs as vectors of pathogens and interactions with abiotic yield-limiting factors. Ultimately, the information generated from the use of this protocol can be used to support adjustments in soybean IPM for stink bugs.

SUMMARY AND CONCLUSIONS

An understanding of the period of time required for seed injury to be produced by stink bugs in soybean is essential for understanding the capabilities of these pests. With the redbanded stink bug emerging as a relatively new pest to the Louisiana soybean agro-system, comparing the injury potential of this species to that of native stink bugs may be important in refining stink bug management strategies. The transition to an early production system, with a higher frequency of earlier maturing indeterminate varieties now being planted may further add to these management issues (Heatherly 1999, Baur et al. 2000). Indeterminate varieties may have multiple pod stages on a single plant at the same time. Therefore, knowledge of differences in stink bug injury to multiple pod stages may prove to be beneficial. The current action thresholds in Louisiana are based on a system that does account for within plant variability in pod development on indeterminate varieties.

For immature seed ratings, seed injury was significantly higher in infested pods for all species compared to the non-infested pods. Seed injury peaked at 82% by 72 HAI for redbanded stink bug caged on R5.5 pods, and at 72% by 96 HAI for brown stink bug caged on R5 pods. Injury peaked at a higher intensity and earlier for redbanded stink bug compared to that for the other species. An assessment of injury effects also was evaluated at plant maturity. For redbanded stink bug, seed injury was highest for 72 and 96 HAI compared to the other time periods. Injury to mature seed did not significantly vary across infestation durations for other species. Injury by redbanded stink bug at harvest was higher following a shorter infestation duration than for the two other species.

Injured seed weights from non-infested pods, though more variable, were similar to injured seed weights produced in infested pods using the caging protocol. Native populations damaged seed in the non-infested pods either prior to caging or after cages were removed. In addition, seed weights for the "true control", non-injured seed in non-infested pods, were not significantly different from seed weights of non-injured seed in stink bug -infested pods. Non-injured seed within infested pods did not compensate for injured seed. Injured seed weights were consistently significantly different from non-injured seed weights for all species evaluated.

Stink bug-induced seed injury also was evaluated on multiple pod stages (R5, R5.5, R6, and R6.5) at 72 HAI. Injury for all species did not significantly vary across pod stages for immature or mature seed, though injury was always significantly higher in infested cages. As with the infestation duration experiment, injured seed weights in non-infested pods were not significantly different than injured seed weights produced in infested pods. Seed weights for non-injured seed in non-infested pods, were not significantly different from seed weights of non-injured seed in infested pods. Significant differences were detected among redbanded stink bug injured seed in infested pods, with weights being significantly lower for R5 and R5.5 pods when compared to R6 and R6.5 pods. In addition, *E. quadrator* injured seed in infested pods had weights significantly lower in R5 and R5.5 pods as well when compared to R6 pods. Brown stink bug-injured seed weights did not significantly vary across pod stages.

This protocol produced discriminate results across several infestation durations for all species when caging a single stink bug adult on a two-pod cohort (3 seed/pod; 6 total seed). The protocol produced results comparable with previous descriptions of stink bug injury to soybean. In addition, levels of stink bug-injured seed observed with the protocol were similar to native stink bug populations. Weights of non-injured seed within an injured pod are not compensating for the loss in weight of injured seed within the same pod.

This study is novel in that injury produced by stink bugs was evaluated at the pod/seed level, as opposed to more traditional whole-plant caging methods. This protocol will allow studies to have a more concise focus, providing isolated results. It could be used to compare effects among multiple life stages, between different sexes, identify plant pathogen transmission, or interactions of environmental factors.

Results from these studies suggest that the redbanded stink bug is capable of producing more intense injury when compared to that for brown stink bug and *E. quadrator*. Additional research is needed to fully understand how the redbanded stink bug pest differs from native stink bugs in the Louisiana soybean agro-ecosystem. The Louisiana soybean production system has changed, and pod variability should be considered in the establishment of soybean IPM. A more complete understanding of stink bug injury to soybean and differences in susceptibility of pod stages will allow for improvement of Louisiana IPM recommendations.

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APPENDIX A: SAMPLE SIZES

Table A1. Sample sizes (n) at selected pod stages for the infestation duration experiments.

Infestation duration	Redbanded stink bug	Brown stink bug		*Euschistus quadrator*
	R5.5	R5	R5.5	R5.5
24h	28	28	51	47
48h	34	40	22	21
72h	35	59	28	32
96h	30	29	43	38

Table A2. Sample sizes (n) for the soybean pod stage 72 HAI experiments.

Pod Stage	Redbanded stink bug	Brown stink bug	*Euschistus quadrator*
R5	23	59	29
R5.5	35	28	32
R6	30	23	20
R6.5	25	-	-

APPENDIX B: SUPPLEMENTAL DATA

Data from the experiment evaluating stink bug injury at selected pod development stages prior to being corrected using Abbott's.

Table B1. Effects of redbanded stink bug (*P. guildinii*) adult injury to immature seed 72HAI on selected pod stages prior to Abbott's correction (correlates with Table 2).

	Percent injured immature seed ± S. E.	
Pod stage	**Infested**	**Non-Infested**
R5	75.94 ± 12.35	12.07 ± 12.35
R5.5	85.18 ± 9.18	17.39 ± 9.18
R6	46.76 ± 10.27	12.58 ± 10.27
R6.5	52.67 ± 16.84	0.00 ± 16.84

Table B2. Effects of redbanded stink bug (*P. guildinii*) adult injury to selected pod stages 72 HAI on mature seed prior to Abbott's correction (correlates with Table 3).

	Percent injured immature seed ± S. E.	
Pod stage	**Infested**	**Non-Infested**
R5	46.16 ± 13.95	6.45 ± 13.95
R5.5	88.38 ± 10.63	31.67 ± 10.63
R6	63.46 ± 11.08	11.43 ± 11.08
R6.5	40.47 ± 19.37	0.79 ± 19.37

Table B3. Effects of brown stink bug (*E. servus*) adult injury to immature seed 72HAI on selected pod stages prior to Abbott's correction (correlates with Table 4).

	Percent injured immature seed ± S. E.	
Pod stage	**Infested**	**Non-Infested**
R5	56.46 ± 5.28	3.61 ± 5.28
R5.5	57.75 ± 6.18	4.17 ± 6.18
R6	39.13 ± 9.70	1.45 ± 9.70

Table B4. Effects of brown stink bug (*E. servus*) adult injury to selected pod stages 72 HAI on mature seed prior to Abbott's correction (correlates with Table 5).

	Percent injured immature seed ± S. E.	
Pod stage	**Infested**	**Non-Infested**
R5	44.14 ± 5.84	12.94 ± 5.84
R5.5	48.94 ± 7.35	10.06 ± 7.35
R6	19.13 ± 10.09	12.67 ± 10.09

Table B5. Effects of *Euschistus quadrator* adult injury to immature seed 72HAI on selected pod stages prior to Abbott’s correction (correlates with Table 6).

	Percent injured immature seed ± S. E.	
Pod stage	**Infested**	**Non-Infested**
R5	54.49 ± 5.49	1.20 ± 6.80
R5.5	63.99 ± 5.79	5.12 ± 5.79
R6	68.70 ± 8.78	15.45 ± 8.78

Table B6. Effects of *Euschistus quadrator* adult injury to selected pod stages 72 HAI on mature seed prior to Abbott’s correction (correlates with Table 7).

	Percent injured immature seed ± S. E.	
Pod stage	**Infested**	**Non-Infested**
R5	64.70 ± 5.99	8.63 ± 5.99
R5.5	53.52 ± 5.87	6.27 ± 5.87
R6	58.49 ± 7.69	6.92 ± 7.69

VITA

Jessica Leigh Parker; daughter of Jay and Patsy Moore, was born in 1986, in Eagle Pass, Texas. She was raised in Quemado, Texas. She graduated from Eagle Pass High School at the C.C. Winn Campus in May of 2004. In May of 2008 she received a Bachelor of Science degree in Entomology from Texas Agricultural and Mechanical University. Jessica began her graduate studies in June 2009 at Louisiana State University and Agricultural and Mechanical College under the supervision of Dr. B. Rogers Leonard. She currently resides in Baton Rouge, Louisiana; is married to Dustin Scott, and has a stepson Dakota Scott. Jessica is currently a candidate for the Master of Science degree in the Department of Entomology at Louisiana State University.