

2011

Redbanded stink bug, *Piezodorus guildinii* (Westwood): pest status, control strategies, and management in Louisiana soybean

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REDBANDED STINK BUG, *PIEZODORUS GUILDINII* (WESTWOOD): PEST STATUS,
CONTROL STRATEGIES, AND MANAGEMENT IN LOUISIANA SOYBEAN

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Entomology

by

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December 2011

ACKNOWLEDGEMENTS

I sincerely appreciate the support and encouragement provided by my major professor, Dr. Rogers Leonard. After working with him for ten years, I recognize what it means to put all your energy into doing a job and showing professionalism while doing it. The knowledge I have attained from him will help me throughout my life. I would like to thank the members of my graduate advisory committee including my Dr. Jeffrey A. Davis (Co-advisor), Dr. Fangneng Huang, Dr. Thomas Reagan, Dr. Guy “Boyd” Padgett, and Dr. David Foltz. I would like to thank Steve Micinski, Dr. Jim Griffin, Joey Boudreaux, Karla Emfinger, Colleen Cookson, Art Richter, Darrell Franks, and Trey Price for their assistance with these studies. I would also like to thank Dr. Tim Schowalter for his support and guidance as head of the Department of Entomology at Louisiana State University. I am deeply indebted to my fellow graduate students, Jarrod Hardke, Kyle Fontenot, and Jessica Moore-Parker, for their support during my graduate studies. I would also like to acknowledge the help of the numerous summer interns and the staff at Macon Ridge Research Station and the Department of Entomology. Without the support of the aforementioned people, I could not have completed the requirements for this degree in a timely manner. The LSU Agricultural Center, Louisiana Soybean and Grain Promotion Board, and numerous Agro-Chemical Companies are recognized for their financial contributions to these projects.

Finally and most importantly, I would like to express my sincere appreciation for my family for their guidance and encouragement during this time of study. To my parents, Teresa and Bonner Wooldridge, I thank you for the support you have provided me throughout my life. Many times you put my well-being ahead of your own. You made sacrifices to ensure that my life was as good as it could be. You have always encouraged me to be the best I can be, and

without you, my successes in this life would not have been possible. To my wife Brooke, I thank you for the love and support you have given me during my graduate studies. I appreciate you holding the fort down during all the nights I was away from home working on my graduate degrees. A very special thanks to my sons, Jackson and Andrew, for always keeping me on my toes and reminding me daily of what the most important things in life are.

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ABSTRACT

The redbanded stink bug, *Piezodorus guildinii* (Westwood), was identified as a pest of Louisiana soybean during 2000. Currently, this species has become established throughout Louisiana and into the soybean production regions of bordering states. Seasonal abundance, patterns of oviposition, and population dynamics of this species were surveyed in soybean during 2008-10. The redbanded stink bug comprised the largest percentage (54%) of the total stink bug complex collected at five survey sites followed by the southern green (27%), *Nezara viridula* L. and brown (7%) stink bugs, *Euschistus servus* L. Peak oviposition occurred during the (R5) soybean growth stage. Most (80%) egg clusters were observed on leaves and pods in the lower two-thirds of the soybean plant canopy. Additional field experiments evaluated redbanded stink bug effects on soybean seed yield, as well as susceptibility to selected insecticides. Both native and artificial infestations of redbanded stink bug significantly reduced yield and seed quality, and increased incidence of delayed crop maturity at population levels below the Louisiana recommended action threshold of nine insects per 25 sweeps. Native populations reduced yields up to 55% in the non-insecticide treated control plots. Yield losses (4.7 to 14.1%), stink bug damaged seed (4.1 to 8.1%), and incidence of green stems (4.7 to 32.3%) from artificial infestations occurred during the R4, R5, and R6 growth stages at infestations of 0.5-1 insect/0.3 row m. No significant effects were detected for yield, stink bug damaged seed, or incidence of green stem with infestations at the R7 growth stage. Populations of redbanded stink bug collected from Louisiana soybean fields were less susceptible to neonicotinoid, organophosphate, and pyrethroid insecticides compared to the southern green stink bug in field and laboratory tests. The occurrence of redbanded stink bug in Louisiana soybean has altered pest management

strategies and increased production costs that are now necessary to reduce yield and quality losses.

CHAPTER 1

INTRODUCTION AND REVIEW OF LITERATURE

Soybean, [*Glycine max* (L.) Merr.], is the leading oilseed crop consumed in the world (Wilcox 2004). Soybean is also an important crop in the United States with 30.6 million hectares planted in 2008 with a production of 80.5 billion kg. Louisiana is one of 31 states that produce soybean in the United States and ranked 18th in planted hectares for 2008. Soybean was the most abundantly planted crop in Louisiana during 2008, accounting for 425,000 hectares and producers harvested an average of 2257.6 kg per hectare (NASS 2009).

Several important insect pests such as velvetbean caterpillar, *Anticarsia gemmatalis* (Hübner); soybean looper, *Chrysodexis 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); and a complex of stink bugs infest soybean at various stages of development in the Southern U. S. (Funderburk et al. 1999). Among these, stink bugs are the most economically important soybean pest across the Southern United States (Funderburk et al. 1999). In a comprehensive survey of insect losses in Mississippi soybean during 2008, Musser et al. (2009) stated that stink bugs infested 99% of acreage and caused a loss of 1.5 million bushels. Stink bugs have been the number one soybean pests in Mississippi from 2003-2008 with annual control costs ranging from 11-20 dollars/acre (Musser and Catchot 2008, Musser et al. 2009). A complex of stink bug species including green stink bug, *Acrosternum hilare* (Say), southern green stink bug, *Nezara viridula* L., and the brown stink complex, *Euschistus* spp., are annual pests in

Louisiana soybean fields. In 2002, an emerging stink bug pest, the redbanded stink bug, *Piezodorus guildinii* (Westwood), reached treatable levels (Baldwin 2005). Since then it has become the most devastating stink bug pest in Louisiana soybean production (Paxton et al. 2007).

Redbanded Stink Bug, *Piezodorus guildinii* (Westwood), Geographical Distribution

The redbanded stink bug (with other common names such as Neotropical green stink bug and small green stink bug) is a Neotropical stink bug species that ranges from Argentina to the Southern United States (Panizzi and Slansky 1985). In the United States this species was first reported during the 1960's and since has been reported in several states including South Carolina, Florida, Georgia, and New Mexico (McPherson and McPherson 2000a). The redbanded stink bug is a significant annual soybean pest in South America. A considerable amount of work has been done on this species in Brazil where it is particularly damaging to soybean (McPherson and McPherson 2000a). Since the late 1970's, the redbanded stink bug has replaced the southern green stink bug as the principle stink bug pest in portions of Brazil (Turnipseed and Kogan 1976). The redbanded stink bug feeds on a wide range of cultivated and non-cultivated plant hosts, but has a particular fondness for legumes. It is capable of causing severe economic damage in soybean, alfalfa, and other bean crops (Panizzi and Slansky 1985). McPherson et al. (1993) has reported the redbanded stink bug on soybean in the United States, but it was not considered an economical pest. Redbanded stink bugs were first reported in South Louisiana during 2000, but it was initially misidentified as the redshouldered stink bug, *Thyanta accerra* McAtee. In 2002, the redbanded stink bug

reached treatable levels and required insecticide sprays in South Louisiana soybean (Baldwin 2005).

Redbanded Stink Bug in Louisiana

Since 2002, the redbanded stink bug has migrated north and now infests all major soybean producing parishes in Louisiana (Personnel Communication; Jack Baldwin, Blaine Viator, Dwayne Coulon, Roger Carter, Grady Coburn, Richard Costello, Steve Micinski, and Howard Anderson 2007). However, these reports have not been validated with statewide coordinated surveys.

Redbanded stink bugs occurred in sufficient numbers in soybean insecticide screening trials near Winnsboro, LA to warrant treatment during 2005. Further migration has been reported with the pest reaching Southern Arkansas during 2006 and the Delta and Hill regions of Mississippi during 2007 (Personnel Communication Gus Lorenz, Jeff Gore, and Angus Catchot). Over the past few years the redbanded stink bug has become the predominant stink bug pest in Southeastern Texas, as well. Very little is known about the redbanded stink bug in the Louisiana soybean agro-ecosystem. Preliminary research has shown that the redbanded stink bug appears to be less susceptible to labeled insecticides compared to other stink bugs in the complex. This aspect, coupled with limited knowledge of pest biology and wild host range, has hindered satisfactory control of this pest in Louisiana.

Pest Status of Stink Bug in Southern United States Soybean

Stink bugs are key pests in soybean production systems across the Mid-South and Southeastern region of the United States (Funderburk et al. 1999). A complex of stink

bug species infest soybean with the predominant species being the green stink bug, southern green stink bug, and the brown stink bug (Funderburk et al. 1999). McPherson et al. (1993) survey (four years) of stink bug species in Georgia found the southern green stink bug, green stink bug, and brown stink bug comprised 98% of all species found in soybean. McPherson et al. (1979) reported four common species found in Louisiana soybean including the southern green stink bug, green stink bug, brown stink bug, and dusky stink bug, *Euschistus tristigmus* (Say). Boyd et al. (1997) and Peters et al. (2004) reported that the principal stink bug species were southern green stink bug, green stink bug, and brown stink bug, but the southern green stink bug represented the highest proportion of the complex in Louisiana. Gore et al. (2006) reported southern green stink bug, green stink bug, and brown stink bug the most abundant species in Mississippi soybean although *Thyanta* spp. were found in low numbers. During a Southeast Texas survey (three years), the southern green stink bug was the predominant species, but green stink bug, brown stink bug, *Edessa bifida* (Say), *Euschistus crassus* (Dallas), *Euschistus ictericus* L., *Euschistus quadrator* Rolston, *Oebalus pugnax* (F.), *Proxys punctulatus* (Palisot), and redshouldered stink bug were also recorded (Drees and Rice 1990). Smith et al. (2009) found the southern green stink bug and brown stink bug to be the most abundant stink bug species in Arkansas and recorded the presence of redbanded stink bug in southern Arkansas soybean fields during 2006 and 2007.

Stink Bug Injury and Yield Losses in Soybean

Stink bugs feed by inserting their stylet and removing plant nutrients from either vegetative tissue or fruiting structures (Panizzi et al. 2000). This feeding can injure the plant, cause abortion of fruit or seed, and in some instances, vector diseases. Among the

most economically important species are generalists such as the southern green stink bug, several *Euschistus spp.* and *Piezodorus spp.* (Panizzi et al. 2000). Stink bugs are primarily attracted to soybean in the reproductive stages of development and prefer to feed on developing seed pods (McPherson and McPherson 2000). Direct feeding by stink bug and indirect disease transmission can reduce yield and seed quality (McPherson and McPherson 2000). Numerous studies with southern green stink bug in soybean have shown that feeding causes yield and quality loss, decreases pod fill and seed weight, delays crop maturity, reduces seed oil content, increases seed protein levels, and reduces germination of harvested seed (Miner 1966, Duncan and Walker 1968, Jensen and Newsom 1972, Cherry 1973, Thomas et al. 1974, Todd and Turnipseed 1974, Todd 1976, Miller et al. 1977, McPherson et al. 1979, Russin et al. 1987, Brier and Rogers 1991). Stink bug feeding punctures on seed appear as minute dark spots. Seed that have been heavily damaged may be shriveled or distorted and have little to no value for oil, meal, or plant seed (Miner 1966, Todd 1976, Todd 1981). Prices of soybean seed damaged by stink bug may be reduced (Todd 1976, 1981) or if damage is persistent in large quantities seed may not be marketable (McPherson et al. 1994). Other studies have shown that stink bug feeding on soybean can influence damage by secondary pests. Todd and Womack (1973) showed that the cigarette beetle, *Lasioderma serricornis* (F.), preferred to feed on soybean seed damaged by stink bugs over non-damaged seed. Russin et al. (1988) also reported a higher incidence of the seedborne pathogen, *Fusarium spp.*, as stink bug damage to seed increased.

Miner (1966) noted in cage studies that seed damage caused by green stink bug was most severe on soybean plants during the R6 growth stage (full seed, Fehr et al.

1971). Green stink bug caused 10% damage to seed and reduced yields by 8.4%. From these studies, Miner developed an action threshold to initiate insecticide applications when stink bug levels reach one insect/row ft. Studies with the southern green stink bug by Thomas et al. (1974) and Todd and Turnipseed (1974) showed significant yield losses occurred when infestations were \geq one insects/row ft. Russin et al. (1987) used four natural population levels of stink bugs to evaluate action thresholds in Louisiana. Differences were detected in raw yields with means of 0.09, 0.46, 0.91, and 1.16 stink bugs/row ft infested during R4 (full pod) to R8 (physiological maturity) growth stages. However, significant yield losses were observed at 0.46 and 1.16 stink bugs/row ft when yields were adjusted for seed damage discounts. Brier and Rogers (1991) infested 1.34 southern green stink bugs/row ft during the growth stage intervals of R3 (beginning pod)-R5 (beginning seed), R5-R6.5 (full seed), and R6.5-R8. Soybean received the most damage and experienced significant yield loss only during the R5-R6.5 infestation period. Lingren (1995) infested 0, 0.5, 1, 3, 6, and 10 southern green stink bugs/row ft during the R2 (full bloom), R4, R5, R5.5, R6, and R7 growth stages for seven days. Seed yield from stink bugs were reduced as infestation levels increased during R5. Maturity was significantly delayed by stink bug infestations greater than 1.0 stink bug/row ft when infested during R4 and R5 growth stages. Lingren (1995) also noted that as stink bug populations increased there was an increase in soybean seed protein but a decrease in oil.

Duncan and Walker (1968) caged southern green stink bugs on one raceme of a soybean plant for three days during the R5, R6 and R7 (beginning maturity) growth stages. Significantly more seed were damaged during the R5 stage. Miller et al. (1977) caged one southern green stink bug on 10 pods for seven days at the R5, R6, R7, and R8

growth stages. Results from this study showed that stink bugs decreased seed weight and damaged more seed during both the R5 and R6 growth stages.

Studies in Argentina have shown that the redbanded stink bug has the greatest capacity to damage soybean among all South American phytophagous stink bugs (Vicentini and Jimenez 1977). Correa-Ferreira and Azevedo (2002) directly compared yield losses and seed damage of redbanded, southern green, and *Euchistus heros* (F.) in field cages and individual plant infestations in the greenhouse. In the field trials (four stink bugs/three row ft), there was no difference detected in yield among the species but the redbanded stink bug damaged more seed compared to the southern green and *E. heros*. In the greenhouse infestations (two stink bugs/plant), lower yields were recorded in plants infested with redbanded stink bug. Plants infested with redbanded stink bug also had less full pods (all three seed present) and significantly more empty pods compared to the other species (Correa-Ferreira and Azevedo 2002). In the Southern U. S., southern green and green stink bug are considered the most damaging species in soybean compared to other species (Miner 1961, McPherson et al. 1979). However, no literature is currently available in the United States comparing redbanded stink bug damage potential in soybean to that of other phytophagous stink bugs.

Delayed Crop Maturity Associated with Stink Bugs

Delayed soybean maturity can be defined as whole-fields or portions of fields that retain leaves, green stems, and/or green pods after the normal harvest date (Boethel et al. 2000). Delayed maturity of soybean plants in response to infestations of stink bug have been reported for southern green 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). Boethel et al. (2000) showed that southern green stink bug infestations between the R3-R5.5 growth stages resulted in delayed maturity.

In Brazil, delayed soybean maturity has been associated with redbanded stink bug infestations in numerous reports. Panizzi et al. (1979) reported that soybean retained green leaves when redbanded stink bugs infested soybean during pod development and pod fill stages (R3-R6). Heinrichs (1976) reported excessive green foliage retention in Brazilian soybean when stink bug populations were \geq eight insects/three row ft during early seed development, but similar populations at R6 did not delay maturity. Populations of two to five redbanded stink bug adults/plant caused excessive green foliage retention when infested at the R4 stage (Costa and Link 1977). Continuous infestations (six to ten bugs/three row ft) of redbanded stink bug during seed development caused green foliage retention, but did not occur in soybean infested prior to seed development (R1-R3) (Galileo and Heinrichs 1978a). Sosa-Gomez and Moscardi (1995) investigated differences in leaf retention among different stink bug species and found that redbanded stink bug caused greater leaf retention than the southern green stink bug and *E. heros*.

Current Stink Bug Action Thresholds and Economic Importance

The action threshold (Table 1) for green, southern green, and brown stink bugs is nine insects/25 sweeps or one insect/row ft in Louisiana, Texas, and Arkansas (Gouge et al. 1999, Lorenz et al. 2006, Baldwin et al. 2009). In the Southeastern United States (Alabama, Georgia, North Carolina, and South Carolina), the action threshold for green, southern green, and brown stink bugs is 6 insects/25 sweeps. In Alabama, Georgia, Mississippi, and Tennessee recommendations are to treat for stink bugs when three

insects/25 sweeps are detected in R1-R5.5 soybean and six (AL, GA) to nine (MS, TN) insects/25 sweeps for the rest of the season (Catchot 2007, Stewart et al. 2008).

Table 1.1. Current stink bug thresholds in the Southern United States.

State	Species*	Growth Stage	Threshold
Alabama	SGSB, GSB, BSB	R1-R5.5	3/25
Alabama	SGSB, GSB, BSB	R5.5-R8	6/25
Arkansas	SGSB, GSB, BSB, RBSB, RSSB	R3-R8	9/25
Georgia	SGSB, GSB, BSB	R1-R5.5	3/25
Georgia	SGSB, GSB, BSB	R5.5-R8	6/25
Louisiana	SGSB, GSB, BSB	R3-R8	9/25
Louisiana	RBSB	R3-R8	6/25
Mississippi	SGSB, GSB, BSB	R1-R5.5	3/25
Mississippi	SGSB, GSB, BSB	R5.5-R8	9/25
North Carolina	SGSB, GSB, BSB	R3-R8	6/25
South Carolina	SGSB, GSB, BSB	R3-R8	5/25
Tennessee	SGSB, GSB, BSB	R1-R5.5	3/25
Tennessee	SGSB, GSB, BSB	R5.5-R8	9/25
Texas	SGSB, BSB	R3-R8	9/25

*Southern green stink bug (SGSB), green stink bug (GSB), brown stink bug (BSB), redbanded stink bug (RBSB), and redshouldered stink bug (RSSB). The threshold for redbanded stink bug in Louisiana is six insects/25 sweeps (Baldwin et al. 2009).

The threshold is slightly lower than the other stink bug species because of lower insecticide efficacy compared to that for other species. Currently Louisiana and Arkansas are the only states reporting redbanded stink bug as an economically important

soybean pest. In Brazil, the current threshold for stink bugs is 0.67 insects/row ft (Anonymous 2007). Principle pests in Brazil soybean are *E. heros*, redbanded stink bug, and southern green stink bug. There currently are no data available to support the action thresholds used to initiate insecticide applications for control of the redbanded stink bug in the United States.

Insecticide Susceptibility of Stink Bug Species

Recent studies have reported differences in susceptibility of stink bugs to several classes of chemistry. Snodgrass et al. (2005) used an adult vial test (AVT) to establish pyrethroid and organophosphate toxicity levels for southern green, green, and brown stink bugs in Arkansas and Mississippi. Southern green and green stink bugs had similar susceptibilities to several pyrethroids including bifenthrin, cyfluthrin, cypermethrin, and lambda-cyhalothrin. Those LC₅₀'s ranged from 0.12 to 0.82 µg/vial (Snodgrass et al. 2005). LC₅₀'s for the brown stink bug ranged from 0.72 to 3.06 µg/vial and were significantly higher for each pyrethroid compared to those values for the green and southern green stink bug (Snodgrass et al. 2005). No differences were detected between the susceptibility of southern green and green stink bugs to the organophosphate insecticides (dicrotophos, acephate, and methyl parathion) with LC₅₀'s ranging from 0.26 to 9.38 µg/vial (Snodgrass et al. 2005). Brown stink bug expressed similar dicrotophos and acephate susceptibility levels compared to the southern green and green stink bugs, but was less susceptible to methyl parathion (Snodgrass et al. 2005).

Similar results were reported by Willrich et al. (2003) and Emfinger et al. (2001) in Louisiana. Southern green stink bug was significantly more susceptible to pyrethroids (bifenthrin, cyfluthrin, cypermethrin, and lambda-cyhalothrin) compared to the brown

stink bug. In Arkansas, Greene (2007) demonstrated high levels of southern green stink bug mortality using topical applications of pyrethroids. Mortalities ranged from 87 to 92% with bifenthrin, cyfluthrin, cypermethrin, and lambda-cyhalothrin. Brown stink bug mortality was much lower for the same insecticides and ranged from 12 to 60%. Mortalities were similar for both species when subjected to the organophosphates (methyl parathion and acephate).

Field studies also have noted variability in insecticide performance between the southern green stink bug and the brown stink bug. Emfinger et al. (2001) noted that brown stink bug was more difficult to control with pyrethroids than the southern green stink bug. In a Texas study 93% control was achieved of the southern green stink bug compared to 38% control on the brown stink bug using the pyrethroid zeta-cypermethrin (Way et al. 2005). Other field studies also have shown that brown stink bugs are more difficult to control than southern green stink bug with pyrethroids (Gable et al. 2004, Greene et al. 2004).

In a summary of Louisiana insecticide efficacy trials, southern green stink bugs appeared to be more susceptible to both pyrethroids and organophosphates compared to redbanded stink bugs (Temple et al. 2009). Significant differences in laboratory susceptibility of southern green stink bug and redbanded stink bugs were also noted in preliminary AVT tests using acephate and cyfluthrin. Currently, very little literature is available for baseline toxicity of redbanded stink bug to insecticides in South America. Stadler et al. (2006) compared redbanded stink bugs from several different regions of Argentina for susceptibility to endosulfan, but made no direct comparisons of toxicity with other compounds or species.

Guillen and Foerster (1978) reported that methyl parathion and endosulfan provided high levels of mortality to southern green and redbanded stink bugs in Brazil. In Brazil field studies, several compounds including deltamethrin, phosphamidon, monocrotophos, chlorthiophos, and formothion were effective on southern green and redbanded stink bugs. Insecticides currently labeled in Brazil include monocrotophos, endosulfan, thiomethoxam + lambda-cyhalothrin, acephate, methamidophos, carbaryl, and trichlorform (Anonymous 2007). In Louisiana, only five insecticides are currently labeled for control or suppression of redbanded stink bugs including acephate, bifenthrin, bifenthrin + zeta-cypermethrin (Hero), thiomethoxam + lambda-cyhalothrin (Endigo), and cyfluthrin (Baldwin et al. 2009). Producers and consultants have reported in Louisiana that the redbanded stink bug is harder to control with these insecticides than southern green stink bug (Louisiana Agricultural Consultants Association, personnel communication). Fewer insecticides in Louisiana are available to control redbanded stink bug compared to the green and southern green stink bug (Table 1.2).

Redbanded Stink Bug Identification, Biology, and Damage to Soybean

The redbanded stink bug is a neotropical insect species that ranges from Argentina to the Southern United States (Panizzi and Slansky 1985). The redbanded stink bug has five generations per year in South America (Panizzi and Slansky 1985). The list of economically important host of this species includes soybean, other legumes such as common bean, *Phaseolus vulgaris* L., pea, *Pisum sativum* L., and alfalfa, *Medicago sativa* L.

Table 1.2. Insecticide recommendations for controlling stink bugs in soybean in the Southern United States.

Chemical	Trade Name	Species*	State
acephate	Orthene 90S	SGSB, GSB, BSB, RBSB ^{1,2}	AR, GA, LA, MS, NC, SC, TN
beta-cyfluthrin	Baythroid XL 1EC	SGSB, GSB, BSB	AL, AR, GA, LA, MS, SC, TN
bifenthrin	Brigade 2EC	SGSB, GSB, BSB, RBSB ¹	AL, AR, LA, TN
carbaryl	Sevin 4F	SGSB, GSB, BSB	AL, TX
esfenvalerate	Asana XL 0.66EC	SGSB, GSB	AR, SC, TX
gamma-cyhalothrin	Prolex, Declare 1.25 SC	SGSB, GSB, BSB	AL, AR, GA, LA, SC, TN
lambda-cyhalothrin	Karate-Z 2.08 SC	SGSB, GSB, BSB	AL, AR, GA, LA, MS, NC, SC, TN, TX
methyl parathion	Methyl Parathion 4EC	SGSB, GSB, BSB	AL, AR, GA, LA, MS, NC, SC, TN, TX
zeta-cypermethrin	Mustang Max 0.8EC	SGSB, GSB, BSB	AL, AR, GA, LA, MS, NC, SC, TN
Pre/Tank Mix			
Chemicals	Trade Name	Species*	State
cyfluthrin + imidacloprid	Leverage 2.7F	SGSB, GSB, BSB	AR, TN
gamma-cyfluthrin + chlorpyrifos	Cobalt 2.54EC	SGSB, GSB, BSB	AR, TN
Lambda-cyhalothrin + thiamethoxam	Endigo 2.06SC	SGSB, GSB, BSB, RBSB ^{1,2}	AR, TN, LA
zeta-cypermethrin + bifenthrin	Hero 1.24EC	SGSB, GSB, BSB, RBSB ¹	LA, TN
Beta-cyfluthrin + acephate	Baythroid XL + Orthene	RBSB ¹	LA

*Southern green stink bug (SGSB), green stink bug (GSB), brown stink bug (BSB), redbanded stink bug (RBSB), and redshouldered stink bug (RSSB).

¹Recommended for RBSB in Louisiana

²Recommended for RBSB in Arkansas

The redbanded stink bug has occasionally been reported on sunflower, *Helianthus annuus* L.; cotton, *Gossypium hirsutum* L.; 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 U. S., Columbia, and Brazil. It also feeds on legumes in the genera *Sesbania* and *Crotalaria* (Panizzi et al. 2000).

The adults of the species have been described as light green to yellowish in color, with a yellowish, reddish, or brownish band at the base of the scutellum (Fraga and Ochoa 1972, Galileo et al. 1977, Grazia et al. 1980). The eggs are black and laid in two distinct parallel rows (Panizzi et al. 2000). Grazia et al. (1980) described the nymphs for first to fifth instars as 1.30, 2.25, 2.58, 4.60, and 7.87 mm long, respectively. The redbanded stink bug can be distinguished from other small green stink bugs (i.e. *Thyanta* spp.) by a long ventral abdominal spine that reaches the mesothoracic coxae and nearly to the probocis (Greene et al. 2006).

The biology of the redbanded stink bug has been well-studied in South America. These studies include nymphal development and survivorship, adult longevity and reproduction, and life stage dispersal on host plants (Panizzi et al. 2000). Longevity and reproductive capacity of this species varies tremendously depending on host plant. Even though redbanded stink bug is highly injurious to soybean, it reproduces relatively poorly and has shorter longevity on soybean compared to that on wild hosts (Panizzi et al. 2000). The number of egg clusters per female varies greatly depending on the host plant and may range from as few as three clusters on soybean and to many as 37 on *Indigo* spp.

(Panizzi et al. 2000). Total egg production per female is approximately 28 on soybean and ranges from 200-500 on *Indigo spp.* (Panizzi et al. 2000).

Link and Concatto (1979) studied the oviposition habits of the redbanded stink bug on soybean in Brazil. The mean number of eggs recorded from 542 egg masses was 17.5, and number of eggs ranged from 4-39 per mass. Redbanded stink bugs oviposited eggs on the soybean pods, stems, and upper and lower leaf surfaces. Oviposition preference was on soybean pods (80%). Redbanded stink bug did not oviposit lower than 10cm from the soil. Redbanded stink bug nymphs emerged from 46% of the eggs collected. Panizzi and Smith (1977) studied the oviposition, development time, adult sex ratio, and longevity of redbanded stink bug on soybean in Brazil. They evaluated 500 egg masses and reported average number of eggs were 15.1 per mass and 60% of the masses were found on pods. The sex ratio observed in the field studies was 1.4 females for each male (Panizzi and Smith 1977). Eggs in the laboratory (24° C, 80% RH) required a mean of 7.5 d from oviposition to eclosion. Silva and Ruedell (1983) reported that redbanded stink bug oviposited 51% of eggs on pods and 48% on leaves. The average number of eggs per cluster was 14.2. Redbanded nymphs emerged from 42% of eggs; but 34% of the total were parasitized (Silva and Ruedell 1983).

A parasitic wasp of redbanded stink bug eggs was identified in this study, *Telenomus mormideae* Costa Lima (Hymenoptera:Scelionidae). *T. mormideae* emerged from 18% of the eggs collected (Link and Concatto 1979). In a study by Venzon et al. (1999), three parasites including *Telenomus podisi*, *Trissolcus brochymenae*, and *Ooencyrtus sp.* attacked redbanded stink bug eggs and parasitized 31-77% of eggs.

First instar redbanded stink bugs remain in a group near the oviposition site and do not feed on plants (Panizzi and Slansky 1985). Panizzi et al. (1980) found that second and third instars remain gregarious, but the fourth and fifth instars were the principal instars involved in dispersion throughout soybean fields. Costa and Link (1982) found that redbanded stink bug adults are more mobile than southern green stink bug adults.

Kogan and Turnipseed (1987) reported that redbanded stink bug had begun to replace southern green stink bug in some areas of Brazil. Redbanded stink bugs appear to be more adapted to warmer climates. They are smaller and more mobile, are capable of colonizing soybean early in the season, experience a low rate of parasitism, and are less susceptible to common insecticides (Kogan and Turnipseed 1987).

Redbanded stink bugs feed on soybean pods similar to other pentatomids and the cause similar damage as southern green stink bug. Redbanded stink bugs are generally the first species to appear in soybean fields prior to or during flowering in Brazil (Panizzi et al. 2000). This observation is similar to that for other *Piezodorus spp.* in Nigeria (Ezueh and Dina 1980). Redbanded stink bug is adapted to feeding on flowering plants better than other pentatomids, but does require reproductive structures to survive and reproduce (Panizzi et al. 2000). Preliminary data from Louisiana also suggest that redbanded stink bugs appear in soybean fields earlier and in a higher frequency than southern green stink bugs.

Galileo and Heinrichs (1978b,c,d) evaluated redbanded stink bug effects on soybean pod production in Brazil. Redbanded stink bugs were infested at zero, two, four, six, and ten insects/0.5 row m during the growth stages R2-R4, R5, R6-R7, and R2-R7. No significant reduction in yields was detected at two-ten stink bugs/1.5 row ft during

R2-R4 growth stages. During the R5 growth stage, only two stink bugs/1.5 row ft significantly reduced yields. Infestations during R6-R7 growth stages required six stink bugs/1.5 row ft to significantly reduce yields. Significant reductions in seed production only occurred when \geq four redbanded stink bugs/ 1.5 row ft were infested during R2-R7 growth stages. All treatments had significantly more damaged seed compared to the non-infested control during all growth stages except R2-R4. Percent of seed damaged ranged from 39% (two insects/0.5 m row) to 72% (10 insects/1.5 row ft) (Galileo and Heinrichs 1978c). Costa and Link (1977) infested redbanded stink bugs at levels of zero, one, two, three, four, and five insects/plant at the R1 and R4 growth stages. This study did not correlate yield effects or damaged seed to stink bug populations.

The following objectives were proposed:

Project Objectives

- I. To determine the comparative abundance and seasonal occurrence of redbanded stink bug and other stink bug species in Louisiana soybean.
- II. To characterize redbanded stink bug oviposition habits in soybean.
- III. To determine redbanded stink bug effects on soybean yield and seed quality.
- IV. To determine the susceptibility of redbanded stink bugs to selected insecticides in laboratory bioassays and field trials.

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CHAPTER 2

SPECIES COMPOSITION AND SEASONAL ABUNDANCE OF STINK BUGS (HEMIPTERA: PENTATOMIDAE) IN LOUISIANA SOYBEAN

Introduction

Soybean, *Glycine max* (L.) Merr, is the leading oilseed crop consumed in the world (Wilcox 2004). In 2010, 31.3 million hectares were planted in the U.S. with a production of 99 million tons. Louisiana is one of 31 states that produce soybean in the U.S. and was ranked 18th in planted acreage for 2010. Soybean was the most abundantly planted crop in Louisiana during 2010, accounting for >445,000 hectares with an average yield of 2729 kg/ha (NASS 2011).

Soybean grown in the Southern U.S. are infested annually by a diverse complex of insect pests including velvetbean caterpillar, *Anticarsia gemmatilis* (Hübner), soybean looper, *Pseudoplusia 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), and a complex of stink bugs (Funderburk et al. 1999). Among these insects, stink bugs are the most economically important pests infesting soybean in the Southern U.S. The predominant species include the green stink bug, *Acrosternum hilare* (Say), southern green stink bug, *Nezara viridula* L., and the brown stink bug, *Euschistus servus* (Say) (Funderburk et al. 1999). In a comprehensive survey of insect losses in Mid-South soybean, Musser et al. (2010) found that stink bugs, infested ≈80% of soybean acreage, caused losses of 126,000 tons in 2009, and were the primary pests in Arkansas, Mississippi, and Tennessee. Stink bugs have been the primary pest of Mississippi

soybean from 2003 to 2009 with annual control costs ranging from 27 to 49 dollars/ha (Musser and Catchot 2008, Musser et al. 2009, Musser et al. 2010).

Comprehensive surveys of stink bug species in soybean have been done across much of the southern soybean production region. During a four year period in Georgia, McPherson et al. (1993) found that southern green stink bug, green stink bug, and brown stink bug comprised 98% of all species infesting soybean. Gore et al. (2006) reported southern green stink bug, green stink bug, and brown stink bug being the most abundant in Mississippi soybean, although *Thyanta* spp. were found in low numbers. During a Southeast Texas survey (three years), the southern green stink bug was the dominant species, but green stink bug, brown stink bug, *Edessa bifida* (Say), *Euschistus crassus* (Dallas), *Euschistus ictericus* L., *Euschistus quadrator* Rolston, *Oebalus pugnax* (F.), *Proxys punctulatus* (Palisot), and *Thyanta* spp. were also recorded (Drees and Rice 1990). Smith et al. (2009) found the southern green stink bug and brown stink bug to be the most abundant stink bug species in Southern Arkansas soybean fields. In Louisiana, McPherson et al. (1979) reported four common species found in soybean including the southern green stink bug, green stink bug, brown stink bug, and dusky stink bug, *Euschistus tristigmus* (Say). Boyd et al. (1997) and Peters et al. (2004) reported that the principal stink bug species were southern green stink bug, green stink bug, and brown stink bug, but the southern green stink bug represented the highest proportion of the complex. The redbanded stink bug previously has been reported in soybean across Florida, Georgia, New Mexico, and South Carolina, but has not been considered an economic pest in those states (McPherson and McPherson 2000).

None of the previous comprehensive surveys mention finding the redbanded stink bug, *Piezodorus guildinii* (Westwood) in soybean. During the last decade, the species has become more common across the soybean production region in Louisiana and surrounding states. This species was first reported in South Louisiana during 2000 by crop consultants and Louisiana Cooperative Extension personnel (Baldwin 2004). By 2002, the redbanded stink bug exceeded the stink bug action threshold (nine insects/25 sweeps) and justified the application of insecticide applications on much of the soybean area in South Louisiana (Baldwin 2004). Redbanded stink bugs now have been reported in all Louisiana soybean producing parishes at sufficient numbers to justify chemical control strategies (R. Levy, Personal Communication). Prior to 2000, the redbanded stink bug had not been reported on Louisiana soybean, and no voucher specimens collected in Louisiana were present in the Louisiana State Arthropod Museum (C. Carlton, personal communication). Therefore, the objective of this study was to determine the current species composition and seasonal abundance of stink bug species in Louisiana soybean.

Materials and Methods

Large plots (0.1-0.2 ha) of soybean representing three maturity groups (MG) were planted at three locations in 2008 including Baton Rouge, LA (Ben Hur Research Farm; southeast location), Alexandria, LA (Dean Lee Research Station; central location), and Winnsboro, LA (Macon Ridge Research Station; northeast location). In 2009 and 2010, additional locations were added including Jeanerette, LA (Iberia Research Station; southwest location) and Bossier City, LA (Red River Research Station; northwest location) for a total of five sites (Figure 2.1).

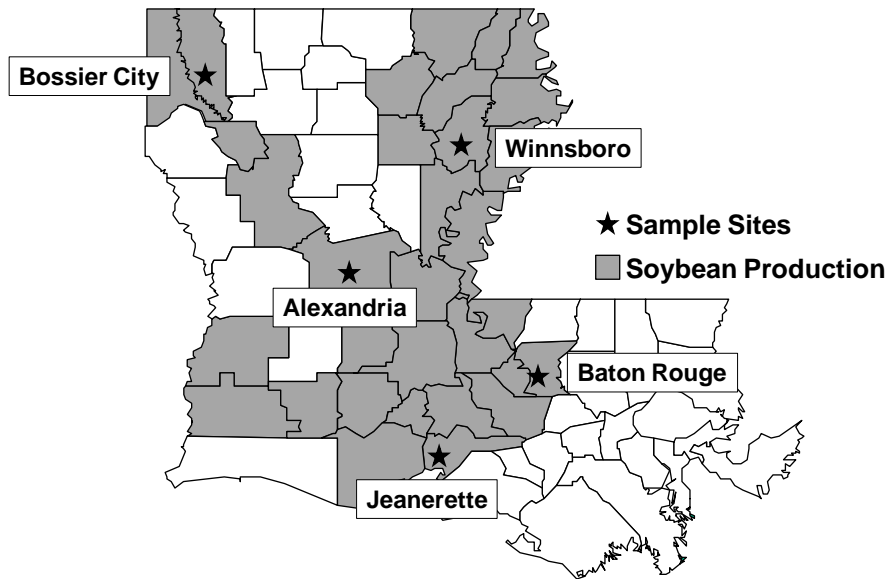


Figure 2.1. Louisiana soybean producing parishes and sample locations for the survey.

Cultivars representing the MG of soybean were planted (Table 2.1) during LSU AgCenter-recommended planting dates and managed according to best agronomic practices at each location (Levy et al. 2011). Soybean representing maturity groups (MGs) IV, V, and VI were planted at Alexandria, Baton Rouge, Bossier City, and Winnsboro. MGs III, IV, and V were planted at the southernmost location, Jeanerette, according to typical production practices in that region.

Native infestations of all species of arthropods were allowed to build within plots which were not treated with insecticides. Each large plot was divided into six equal size blocks. Each of the six blocks within a maturity group was sampled weekly from R1 (beginning bloom) to R8 (physiological maturity) with a standard (38 cm diameter) sweep net by taking six sets of 25 sweep samples (150 total/MG/week). When plants within each plot reached R8, the plot was destroyed (harvested or mowed) and removed

from the test area. Alternating rows were sampled within each block each week such that no single row was sampled more than once within a period of four weeks. On each sampling date, the soybean growth stages for each MG were recorded based on descriptions by Fehr et al. (1971).

Table 2.1. Survey sites, maturity groups (MG) and planting dates in Louisiana soybean stink bug surveys, 2008-2010.

Location	MG	Planting date		
		2008	2009	2010
Alexandria (Central)	IV	May 1	April 27	May 21
	V	May 20	May 11	May 28
	VI	June 11	June 2	June 24
Baton Rouge (Southeast)	IV	April 30	May 11	May 24
	V	May 20	May 11	May 24
	VI	June 24	June 15	June 24
Bossier City (Northwest)	IV	-----	May 15	April 22
	V	-----	May 15	May 14
	VI	-----	June 9	June 14
Jeanerette (Southwest)	III	-----	April 24	April 21*
	IV	-----	April 24	April 21*
	V	-----	May 15	May 19
Winnsboro (Northeast)	IV	April 16	April 16	April 16
	V	May 9	May 18	May 15
	VI	June 1	June 9	June 7

2008 varieties: Asgrow 4404 (MG IV), Asgrow 5905 (MG V), Asgrow 6702 (MG VI).

2009 varieties: Pioneer 93Y90 (MG III), Asgrow 4606 (MG IV), Asgrow 5606 (MG V), Asgrow 6301 (MG VI).

2010 varieties: Pioneer 93Y90 (MG III), Asgrow 4404 (MG IV), Asgrow 5335 (MG V), Asgrow 6303 (MG VI).

*Did not emerge until May 18 due to lack of moisture.

Each individual set of sweep net samples was bagged, labeled, taken to the laboratory, and frozen until it could be evaluated. Upon evaluation, samples were segregated by stink bug species and life stage (adult or $\geq 2^{\text{nd}}$ instar nymph) based on diagnostic keys from McPherson (1982) and McPherson and McPherson (2000). Data were summarized by species within each maturity group and sample location. The total numbers of all stink bugs within the pest complex were summarized by soybean growth

stage within each MG at each sample location. For the purpose of summarizing data, numbers of southern green stink bug and green stink bug were combined and are referred to as the green complex. All *Euschistus* species were combined and are referred to as the brown complex. Other minor pentatomid pests (*Thyanta accera*, *Oebalus pugnax*, *Edessa bifida*, and the beneficial *Podisus maculiventris*), representing <3.0% of all stink bugs sampled, are not summarized by MG or location. Chi-square analysis was used to determine the predominant stink bug species collected by year and location within a year (PROC FREQ, SAS Institute 2003, SAS/STAT user's guide, version 9.1).

Results

From 2008-2010, 3,084 samples from 77,100 sweeps were taken in multiple soybean MGs at five geographical locations across Louisiana. During the study, a total of 13,146 stink bugs were captured and subsequently identified to species (Table 2.2). An average of 1,468, 1,234, and 514 stink bugs were collected from each location in 2008, 2009, and 2010, respectively. Across all years and sample sites, the predominant species (>90%) included the redbanded stink bug (54.2%), southern green stink bug (27.1%), brown stink bug (6.6%), and the green stink bug (5.5%). Other phytophagous pentatomids captured during the surveys included *Euschistus quadrator* (1.6%), *E. tristigma* (1.6%), *Thyanta accera* (0.9%), *Oebalus pugnax* (0.2%), *Edessa bifida* (0.1%), *Euschistus consperus* (0.1%), and *Euschistus ictericus* (0.1%). The spined soldier bug, *Podisus maculiventris*, a beneficial pentatomid, comprised 1.6% of the total stink bug population. The redbanded stink bug was the predominant stink bug species collected in all three years ($P < 0.0001$, $df = 10$).

Table 2.2. Summary of stink bug population data collected from Louisiana sample sites from 2008-2010.

Species	Common Name	Number ²			Total	Percent
		2008	2009	2010		
<i>Acrosternum hilare</i>	Green stink bug	86	415	228	729	5.5
<i>Edessa bifida</i>	Morningglory stink bug	9	5	2	16	0.1
<i>Euschistus conspersus</i>	----- ¹	5	6	3	14	0.1
<i>Euschistus ictericus</i>	----- ¹	2	1	3	6	0.1
<i>Euschistus quadrator</i>	----- ¹	61	98	60	219	1.6
<i>Euschistus servus</i>	Brown stink bug	209	294	370	873	6.6
<i>Euschistus tristigma</i>	Dusky stink bug	37	97	83	217	1.6
<i>Nezara viridula</i>	Southern green stink bug	1195	1907	471	3573	27.1
<i>Oebalus pugnax</i>	Rice stink bug	5	12	9	26	0.2
<i>Piezodorus guildinii</i>	Redbanded stink bug	2775*	3105*	1249*	7129	54.2
<i>Podisus maculiventris</i>	Spined soldier bug	7	126	86	219	1.6
<i>Thyanta accera</i>	Redshouldered stink bug	14	105	6	125	0.9
Total		4405	6171	2570	13146	100

¹ No approved common name.

² Total number collected at all sample sites.

*Predominant species collected that year (P<0.0001).

In 2008, the redbanded stink bug was the primary species ($\chi^2=159.8$, $df=2$, $P<0.001$) in all three MGs (IV, V, and VI) at the Baton Rouge (southeast) sample site (Figure 2.2).

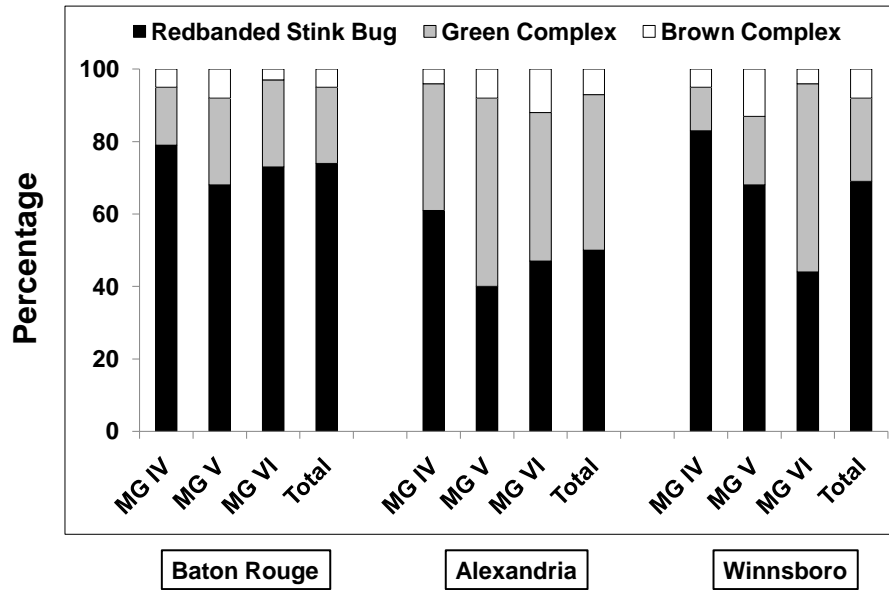


Figure 2.2. Stink bug species composition for three maturity groups (MG) (IV, V, and VI) at selected Louisiana locations, 2008.

Redbanded stink bug levels ranged from 68 to 79% across the three MGs and comprised 74% of total stink bugs collected at the Baton Rouge site during 2008. The green stink bug and brown stink bug complexes made up 21% and 5% of the total population, respectively, collected at Baton Rouge. At the Alexandria (central) location, the redbanded stink bug was the dominant species in MGs, IV (61%) and VI (47%) soybean while the green stink bug complex was most dominant in MG V (52%) soybean. Of the total stink bugs collected at Alexandria that year, the redbanded stink bug green stink bug complex and brown stink bug complex comprised 50%, 43%, 7%, respectively; however, redbanded stink bug numbers were not significantly higher compared to the green complex ($\chi^2=0.1$, $df=2$, $P=0.72$). At Winnsboro (northeast), the most

northern site, the redbanded stink bug was the most abundant species in MG IV (83%) and V (68%) soybean while the green stink bug complex was most prevalent (52%) in MG VI soybean. Of the total stink bugs collected at Winnsboro the redbanded stink bug, green stink bug complex, and brown stink bug complex represented 69%, 23%, and 8%, respectively, of the total collections. Redbanded populations significantly higher ($\chi^2=546.1$, $df=2$, $P<0.001$) than other species.

In 2009, two additional sites were added and included Jeanerette located in southwestern Louisiana, and Bossier City, located in northwestern Louisiana. At Jeanerette (southwest), the redbanded stink bug was the dominant ($\chi^2=341.2$, $df=2$, $P<0.001$) species (60-81%) collected in all MGs (III, IV, and V) (Figure 2.3). The redbanded stink bug, green stink bug complex, and brown stink bug complex comprised 70%, 22%, and 8%, respectively, of the population collected at Jeanerette. At Baton Rouge, the redbanded stink bug was found to be the most abundant ($\chi^2=78.6$, $df=2$, $P<0.001$) species (63-81%) of the stink bug complex in all MGs (IV, V, and VI). The redbanded stink bug, green stink bug complex, and brown stink bug complex made up 73%, 9%, and 18% respectively, of the population at Baton Rouge. At Alexandria, the green stink bug complex (48%) was predominant in the MG IV soybean while the redbanded stink bug was most dominant in MG V (58%) and MG VI (55%) soybean. The redbanded stink bug (50%) was the primary ($\chi^2=20.8$, $df=2$, $P<0.001$) stink bug species collected at Alexandria, followed by the green stink bug complex (40%), and the brown stink bug complex (8%). In Winnsboro, redbanded stink bug numbers ranged from 53-67% of the total population across MGs VI, V, and VI. The redbanded stink bug (57%) was the predominant ($\chi^2=56.1$, $df=2$, $P<0.001$) stink bug collected in Winnsboro in 2009 followed by the green stink bug complex (36%), and brown stink bug complex (7%).

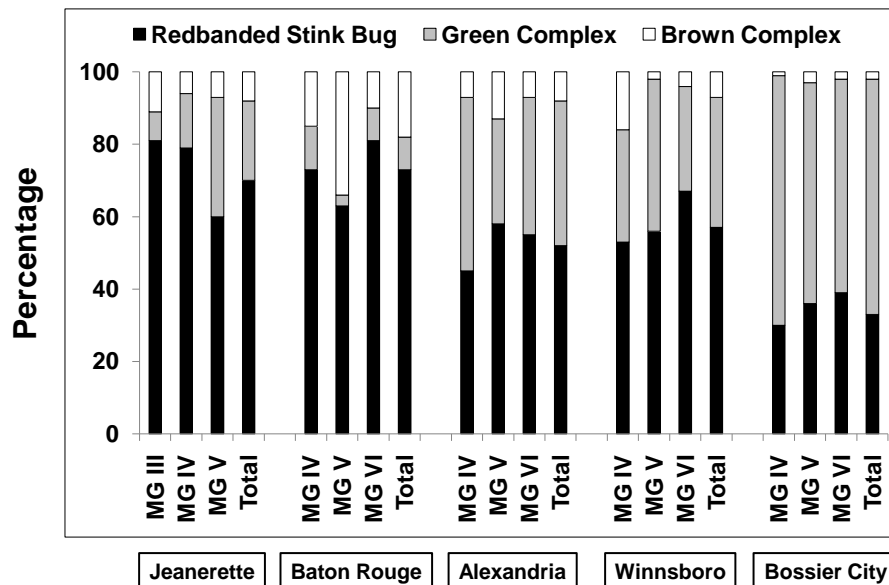


Figure 2.3. Stink bug species composition for four maturity groups (MG) (III, IV, V, and VI) at selected Louisiana locations, 2009.

During 2009, Bossier City (northwest) was the northern-most site, and the green stink bug complex was most abundant in all MGs (MG IV [69%], V [61%], and VI [59%]). The green stink bug complex (65%) were the most prevalent ($\chi^2=150.6$, $df=2$, $P<0.001$) stink bugs collected at Bossier City, followed by the redbanded stink bug (33%) and the brown stink bug complex (2%).

In 2010 at Jeanerette, the redbanded stink bug was the most abundant species ($\chi^2=341.2$, $df=2$, $P<0.001$), ranging from 76-92% of the total collected stink bug population in all MGs (III, IV, and V) (Figure 2.4). The redbanded stink bug, green stink bug complex, and brown stink bug complex made up 86%, 13%, and 1%, of the population, respectively at Jeanerette. At the Baton Rouge site, the green stink bug complex was most prevalent in MG IV (52%) soybean, while the redbanded stink bug was most abundant in MG V (49%) and VI (63%) soybean.

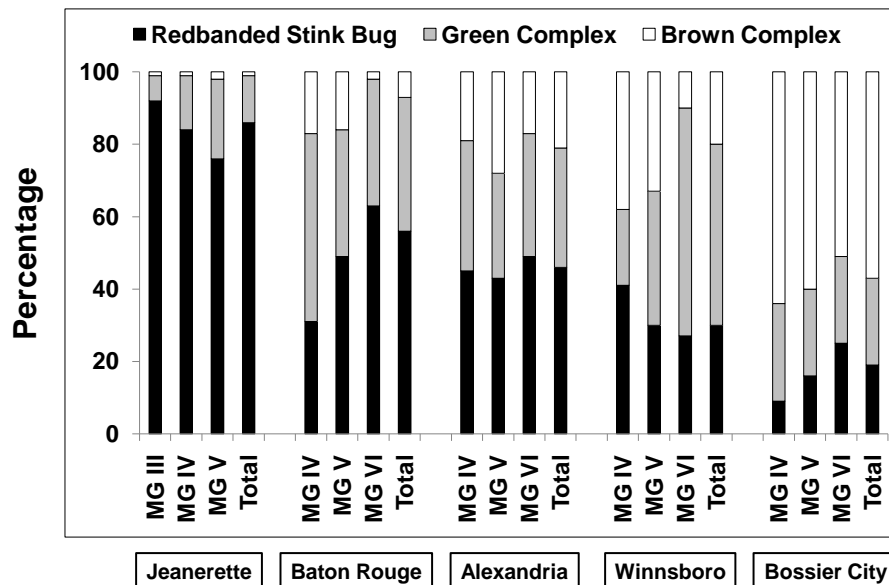


Figure 2.4. Stink bug species composition for four maturity groups (MG) (III, IV, V, and VI) at selected Louisiana locations, 2010.

The redbanded stink bug (56%) was the most abundant ($\chi^2=15.3$, $df=2$, $P<0.001$) stink bug species at Baton Rouge in 2010, followed by the green (37%) and brown (7%) stink bug complexes. At Alexandria, the red banded stink bug accounted for the majority ($\chi^2=11.4$, $df=2$, $P<0.001$) of stink bugs sampled (43-49%) in all MGs (IV, V, and VI). At Winnsboro, the redbanded stink bug was the predominant species collected in MG IV (41%) soybean, while the green complex was most abundant in MG V (37%) and MG VI (63%) soybean. The green stink bug complex (50%) was most abundant ($\chi^2=16.6$, $df=2$, $P<0.001$) at Winnsboro, followed by the redbanded stink bug (30%) and the brown stink bug complex (20%). At the northern-most survey site (Bossier City), the brown stink bug complex was dominant ($\chi^2=43.5$, $df=2$, $P<0.001$) in all three MGs (IV, V, and VI), ranging from 51-64% of the total population. The species frequency in Bossier City consisted of the brown stink bug complex (57%), the green stink bug

complex (24%) and the redbanded stink bug (19%). Redbanded stink bugs made up the largest percentage of the stink bug complex in MG III (86%) and IV (60%), MG V (54%), and VI (50%) during the complete survey period (Figure 2.5).

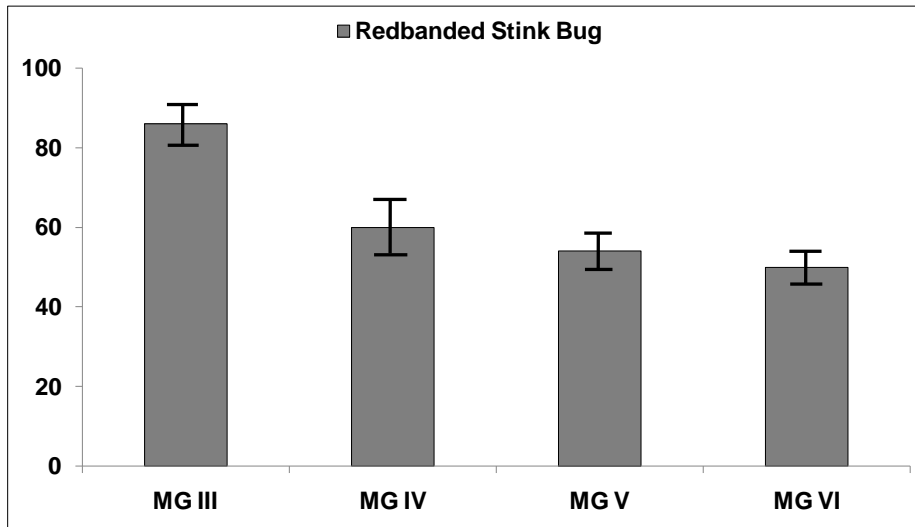


Figure 2.5. Frequency of redbanded stink bug within the entire stink bug complex across several soybean maturity groups (MG), in Louisiana during 2008-2010.

In 2008, stink bug infestations did not reach an action threshold level (9/25 sweeps) in MG IV or V soybean at Baton Rouge (Figure 2.6). Highest numbers were recorded during R5 to R6 growth stages (3.5 to 5.7 insects per sample). In the MG VI soybean, the action threshold was exceeded during the R5 (13.7 insects per sample) growth stage and numbers remained above threshold through R6 (11.3 insects per sample).

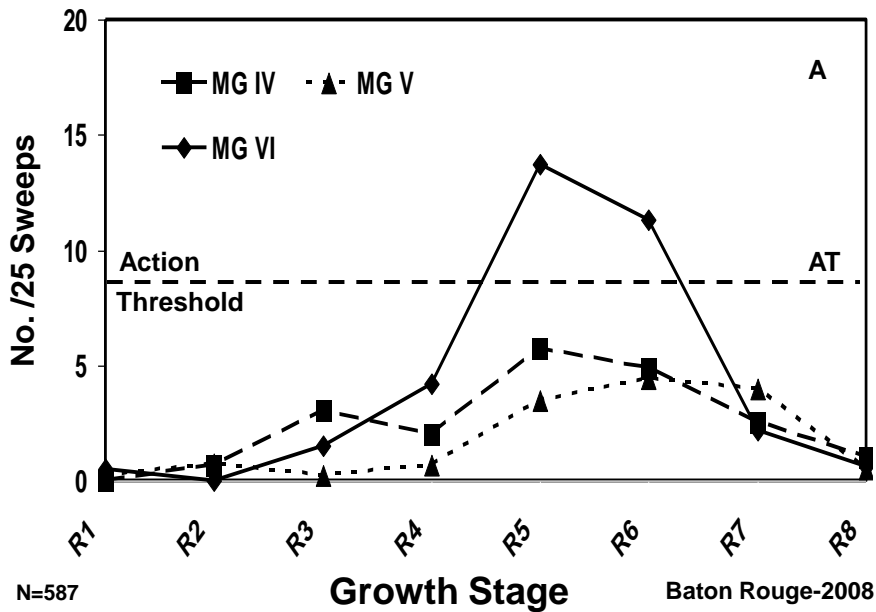


Figure 2.6. Average number of stink bugs collected at Baton Rouge during each soybean phenological growth stage in multiple maturity groups, 2008 (Action threshold of 9 insects/25 sweeps).

At the Alexandria site, the stink bug complex reached action threshold levels during R5 (8.5-15.0 insects per sample) and R6 (18.6-19.3 insects per sample), but declined during R7 (4.3-6.8 insects per sample) in MG IV and V (Figure 2.7). In MG VI soybean, stink bug numbers (10.5 insects per sample) were above the action threshold only during R6. At Winnsboro, stink bugs exceeded action thresholds (Figure 2.8) in all MGs (IV, V, and VI). Highest numbers were recorded during R6 (45.0 insects per sample) and R7 (10.3 insects per sample) in MG IV soybean and R5 (19.8-20.2 insects per sample) and R6 (9.2-15.1 insects per sample) in MGs V and VI.

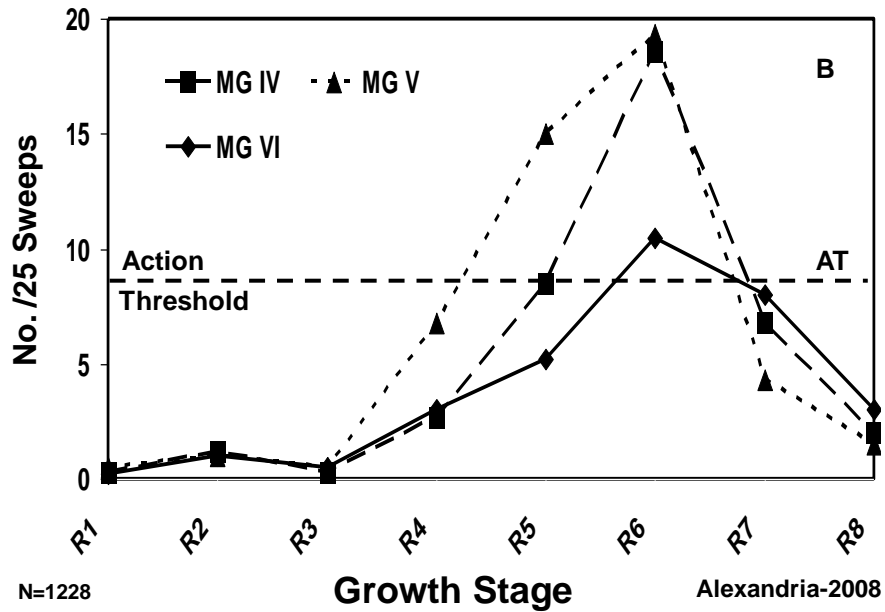


Figure 2.7. Average number of stink bugs collected at Alexandria during each soybean phenological growth stage in multiple maturity groups, 2008 (Action threshold of 9 insects/25 sweeps).

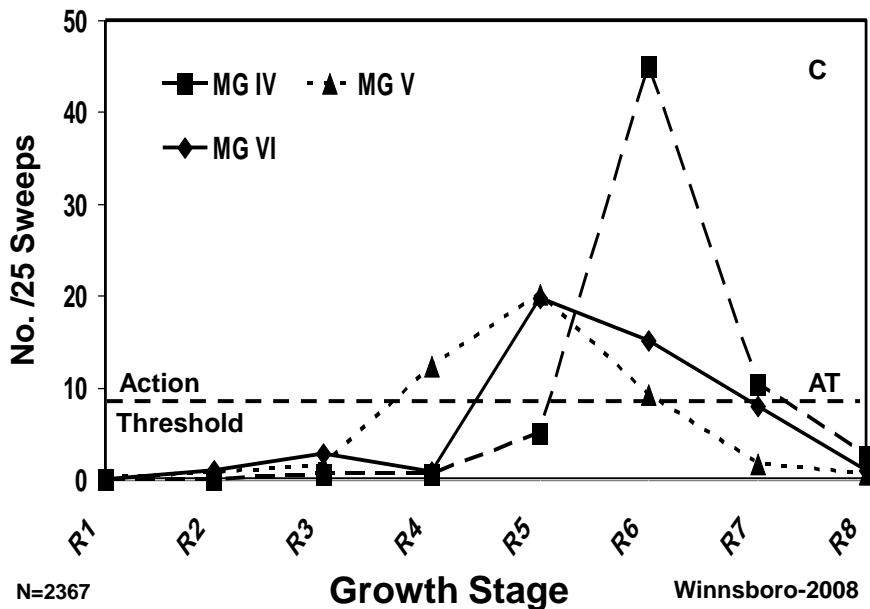


Figure 2.8. Average number of stink bugs collected at Winnsboro during each soybean phenological growth stage in multiple maturity groups, 2008 (Action threshold of 9 insects/25 sweeps).

In 2009, stink bugs did not reach the action threshold in MG III soybean, but exceeded action threshold levels during R6 (9.9 insects per sample) in MG IV and R6 (15.4 insects per sample) and R7 (11.3 insects per sample) in MG V soybean at Jeanerette (Figure 2.9).

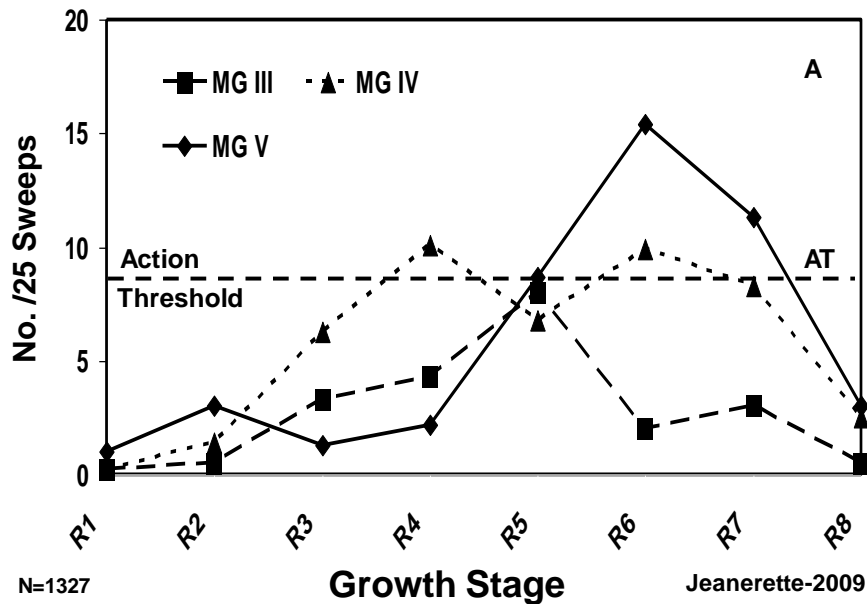


Figure 2.9. Average number of stink bugs collected at Jeanerette during each soybean phenological growth stage in multiple maturity groups, 2009 (Action threshold of 9 insects/25 sweeps).

At Baton Rouge, stink bugs in each MG remained below the action threshold level throughout the sampling period, and peak numbers ranged from 3.8-4.4 insects per sample (Figure 2.10). At Alexandria, peak levels (8.1-18.8 insects per sample) were recorded during R6 in all MGs. Action threshold levels were reached in MG IV and VI soybean (Figure 2.11).

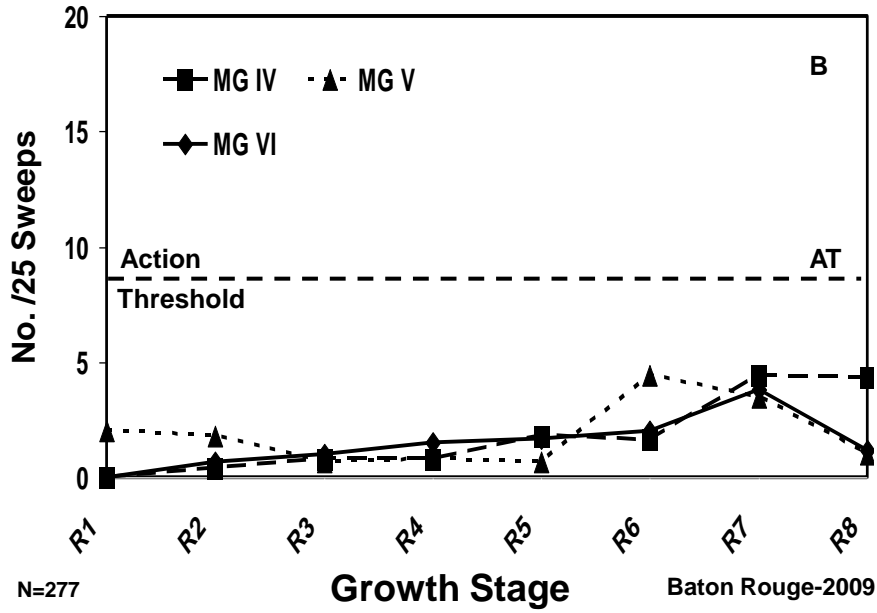


Figure 2.10. Average number of stink bugs collected at Baton Rouge during each soybean phenological growth stage in multiple maturity groups, 2009 (Action threshold of 9 insects/25 sweeps).

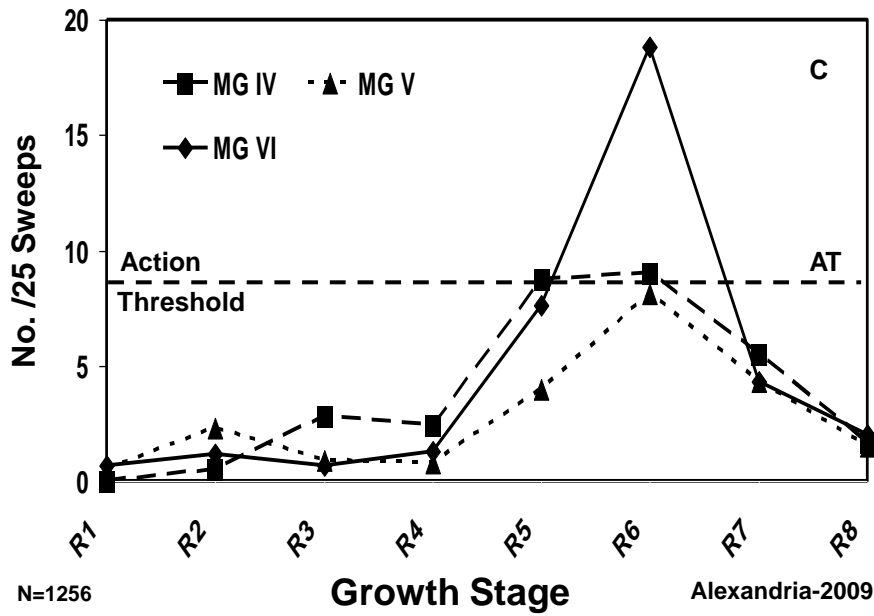


Figure 2.11. Average number of stink bugs collected at Alexandria during each soybean phenological growth stage in multiple maturity groups, 2009 (Action threshold of 9 insects/25 sweeps).

At Winnsboro, only stink bugs in MG V soybean exceeded the action threshold (32.0 insects per sample) (Figure 2.12). Peak numbers of stink bugs were detected at R7 in MGs IV and V and at R5 in MG VI. In Bossier City, stink bug numbers were highest during R6 (8.8-32.7 insects per sample) with action threshold levels exceeded in MG IV and VI soybean (Figure 2.13).

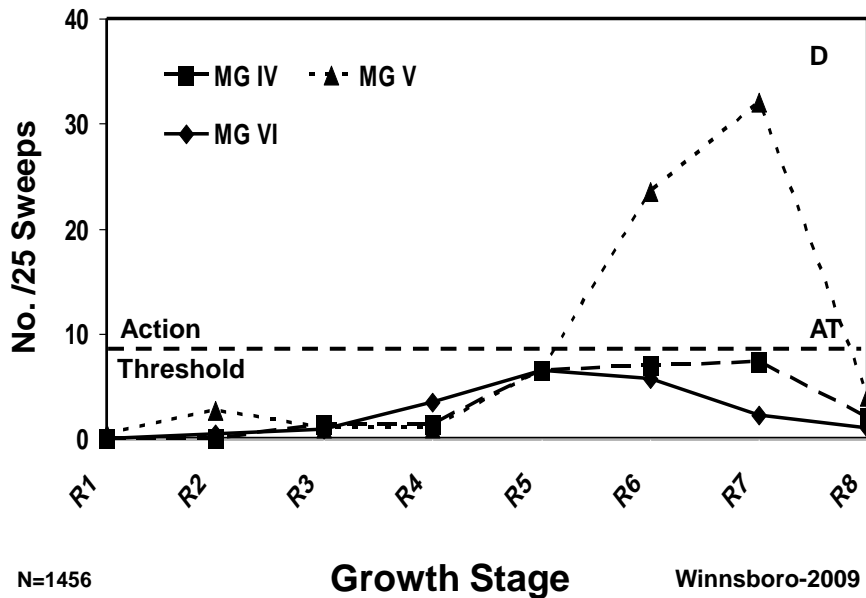


Figure 2.12. Average number of stink bugs collected at Winnsboro during each soybean phenological growth stage in multiple maturity groups, 2009 (Action threshold of 9 insects/25 sweeps).

In 2010, action thresholds were exceeded in MG IV and V soybean with peak stink bugs collected during R6 in MG III (7.4 insects per sample) and MG IV (14.5 insects per sample) soybean and during R7 (17.3 insects per sample) in MG V soybean at Jeanerette (Figure 2.14). At Baton Rouge, stink bugs remained relatively low (0-5 insects per sample) in MG IV and V soybean but were above the action threshold during R6 (13.7 insects per sample) and R7 (10.5 insects per sample) in MG VI soybean (Figure 2.15).

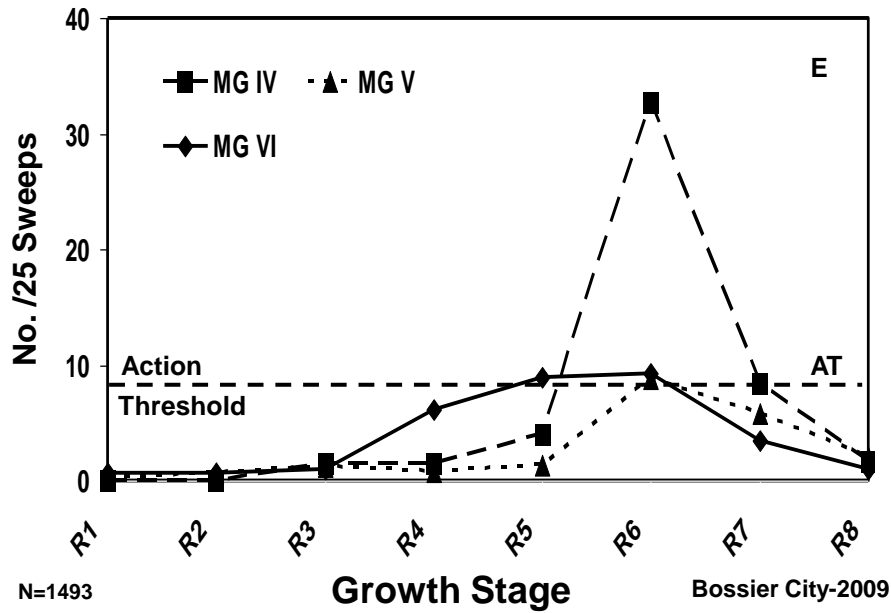


Figure 2.13. Average number of stink bugs collected at Bossier City during each soybean phenological growth stage in multiple maturity groups, 2009 (Action threshold of 9 insects/25 sweeps).

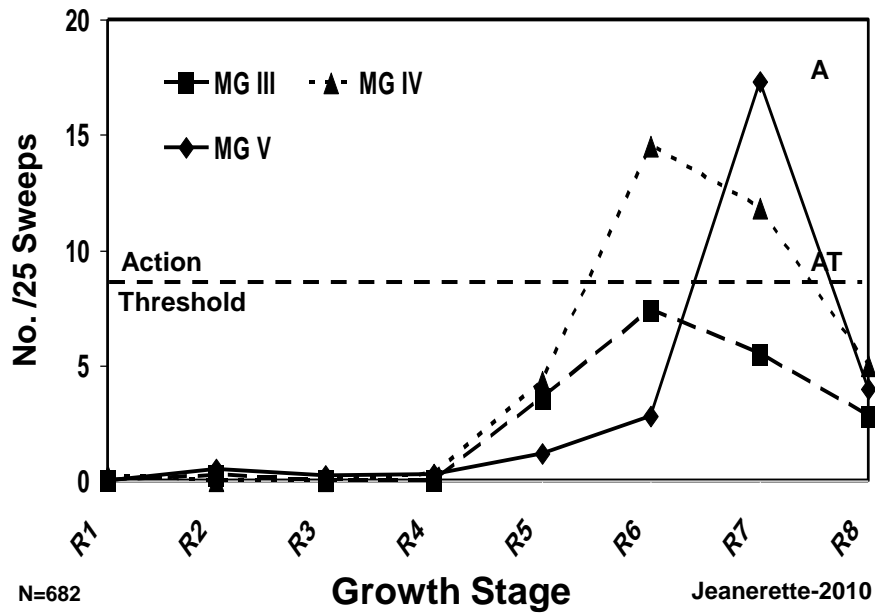


Figure 2.14. Average number of stink bugs collected at Jeanerette during each soybean phenological growth stage in multiple maturity groups, 2010 (Action threshold of 9 insects/25 sweeps).

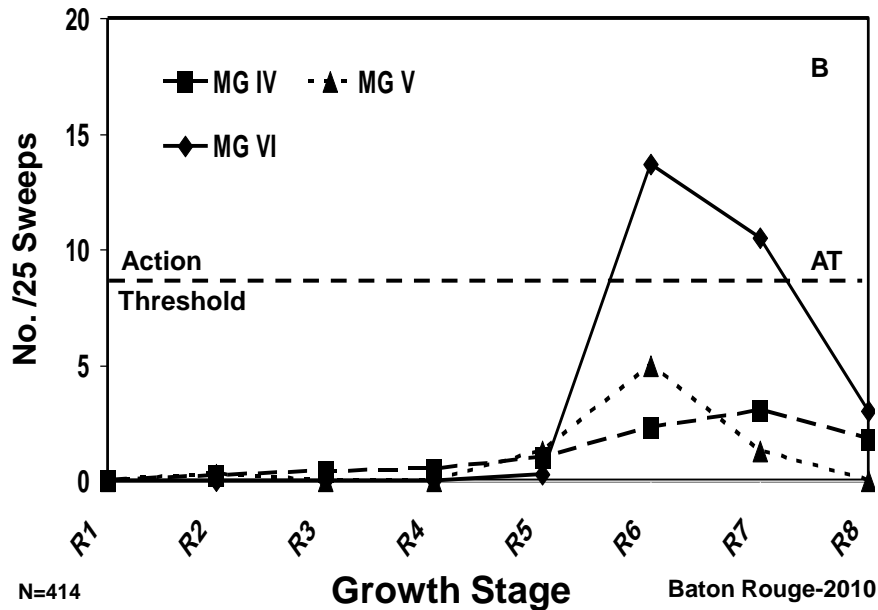


Figure 2.15. Average number of stink bugs collected at Baton Rouge during each soybean phenological growth stage in multiple maturity groups, 2010 (Action threshold of 9 insects/25 sweeps).

At Alexandria, peak stink bug numbers (6.8-12.0 insects per sample) were recorded at R6 in all MGs (IV, V, and VI) with populations exceeding action thresholds in MG IV and VI soybean (Figure 2.16). At Winnsboro, stink bugs were low in MG IV and V soybean, ranging from 0-2.1 insects per sample (Figure 2.17). Stink bug populations were slightly higher in MG VI soybean (0-7.3 insects per sample), peaking at R6, but remained below the action threshold. At Bossier City, stink bug numbers peaked at R7 in all MGs (IV, V, and VI) but only reached the action threshold in the MG V soybean (Figure 2.18). Total stink bugs collected at the five sample sites decreased $\approx 58\%$ from 2009 to 2010.

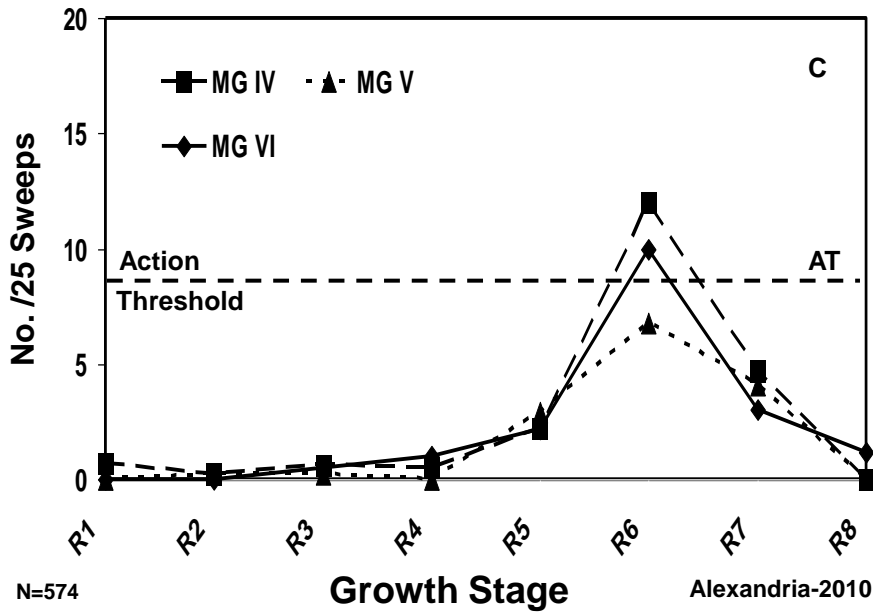


Figure 2.16. Average number of stink bugs collected at Alexandria during each soybean phenological growth stage in multiple maturity groups, 2010 (Action threshold of 9 insects/25 sweeps).

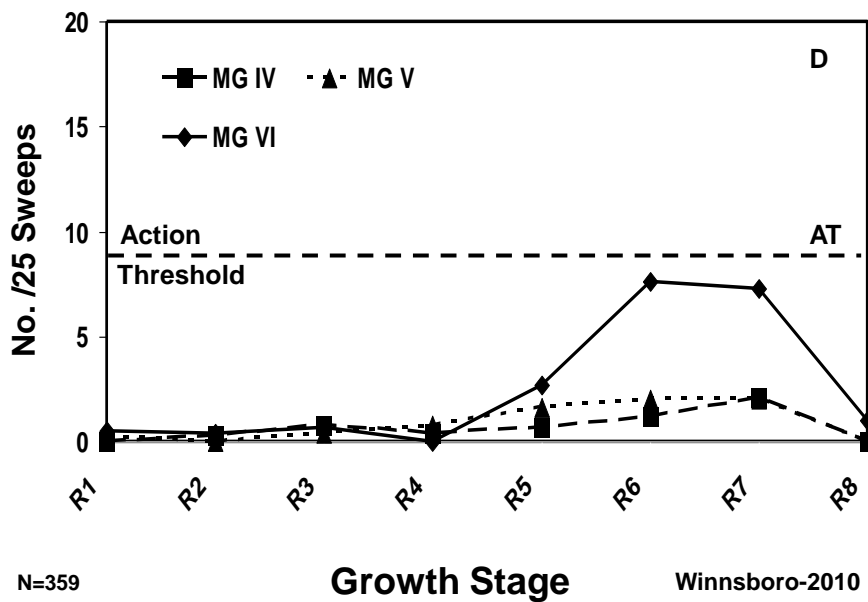


Figure 2.17. Average number of stink bugs collected at Winnsboro during each soybean phenological growth stage in multiple maturity groups, 2010 (Action threshold of 9 insects/25 sweeps).

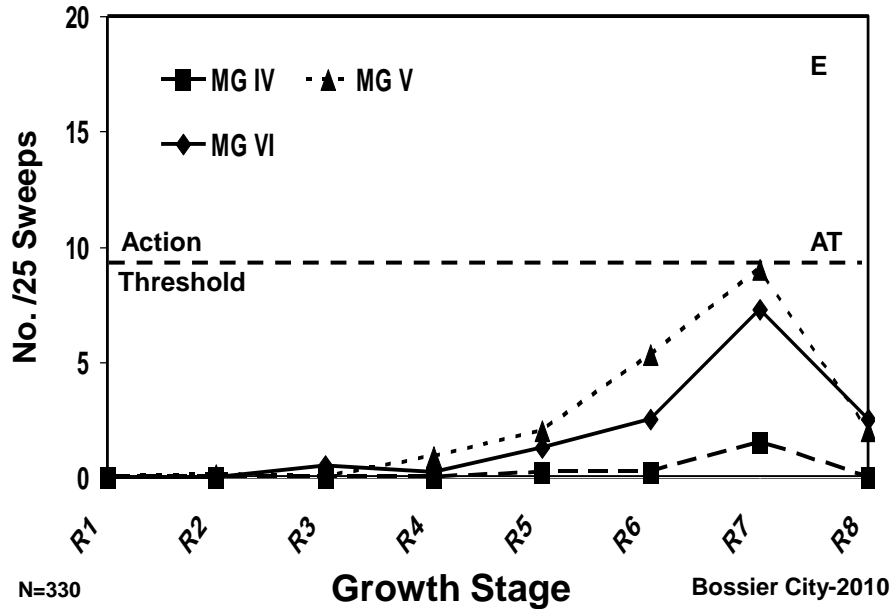
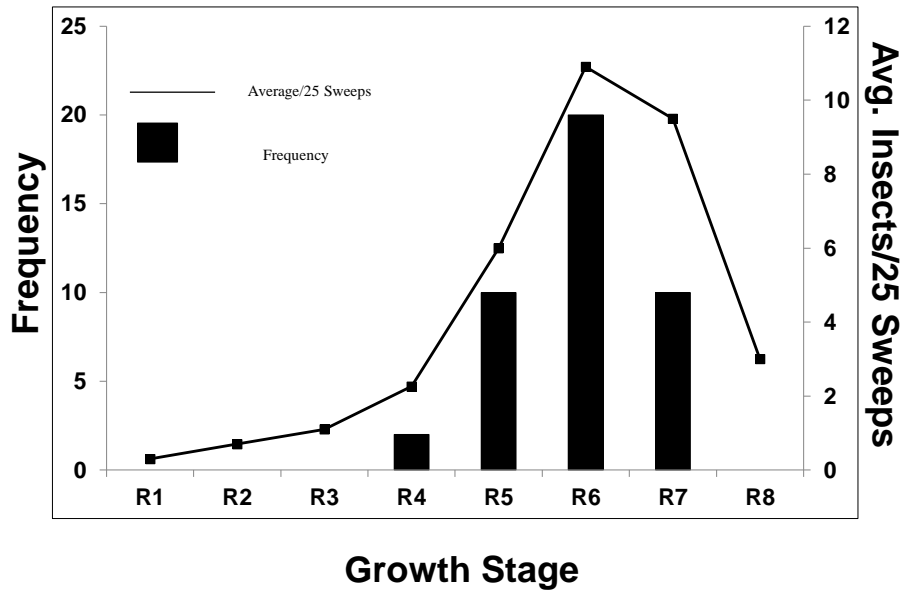


Figure 2.18. Average number of stink bugs collected at Bossier City during each soybean phenological growth stage in multiple maturity groups, 2010 (Action threshold of 9 insects/25 sweeps).

Summarized across all MGs and survey sites, action thresholds for the stink bug complex were detected during the R4 (pod elongation) to the R7 (beginning maturity) soybean growth stages (Figure 2.19). Low levels of stink bugs detected during R1 (first flower) to R3 (pod initiation) or during R8 (full maturity) soybean growth stages but action thresholds were not exceeded during those periods. Only 5% of the samples collected at R4 had stink bug numbers above an action threshold, while 25%, 51%, and 25%, of samples collected at R5, R6, and R7, respectively, had stink bug numbers above the action threshold. Mean numbers of the stink bugs ranged from a low of 0.3 per sample during R1 to a high of 10.9 per sample during R6.



*Locations x maturity groups=39

Figure 2.19. Frequency of the samples above an action threshold and mean stink bug density per sample (25 sweeps) within a soybean growth stage.

Discussion

The redbanded stink bug was the dominant stink bug species sampled in Louisiana soybean during this study. This is the first report of the redbanded stink bug as a primary pentatomid pest of soybean at locations within the U.S. Previously, this insect was not considered to be an economically important pest within the U.S., despite being reported in Florida, Georgia, New Mexico, and South Carolina (McPherson and McPherson 2000).

Redbanded stink bug populations have been found above action thresholds in all Louisiana soybean-producing parishes (Figure 2.20). In this study, the redbanded stink bug was the primary stink bug detected at four of the five survey sites during the three year period. This species was found at the highest levels in the southern-most survey sites of Jeanerette and Baton Rouge where it has been established since 2000 (Figure 2.21).

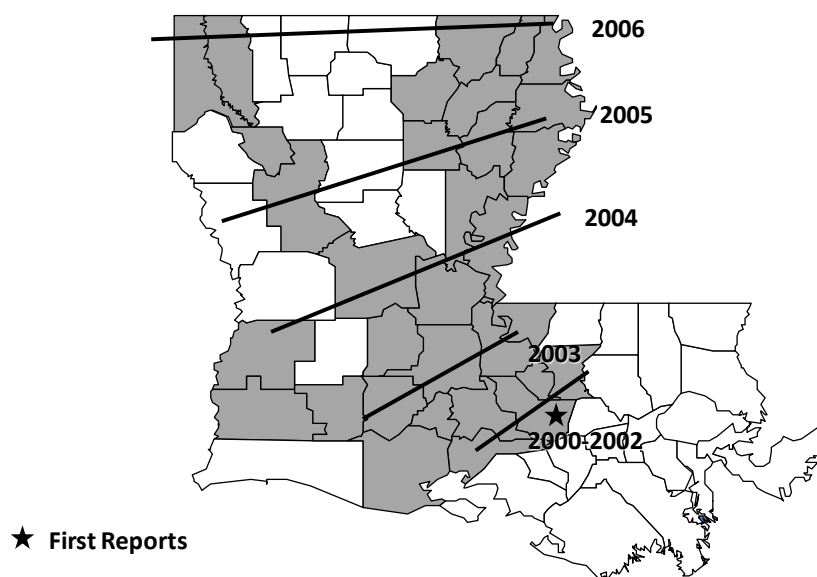


Figure 2.20. Migration of redbanded stink bug from initial infestations throughout the Louisiana soybean-producing parishes. Years refer to northern-most boundary of collection.

Given the current distribution pattern of the redbanded stinkbug, the lowest levels were detected at Bossier City, the northern-most survey site. The insect bug was not detected at Bossier City until the summer of 2006, and the expansion of the range of this insect has not been limited to the Louisiana borders. Redbanded stink bugs have now reached soybean pest status in all states bordering Louisiana and have been reported as far north as Missouri and Tennessee (Stalcup 2007, Bailey 2009, Catchot 2009, Smith 2009, Smith et al. 2009).

Interestingly, the redbanded stink bug has only been a significant pest of soybean and not other Southern row crops such as cotton, field corn, grain sorghum or rice. The insect has been observed in these crops, but only at very low levels and only when soybean is not available in adjacent fields (B. R. Leonard, Unpublished Data). This observation differs from that of other stink bugs typically associated with soybean such as the green, southern green, and brown stink

bugs, which are more generalist and may reach damaging levels in other crops including field corn, cotton, and grain sorghum (McPherson and McPherson 2000, Panizzi et al. 2000). With limited alternative hosts available to redbanded stink bug populations during the summer months, populations remain concentrated in soybean fields where they are capable of quickly building to injurious levels.

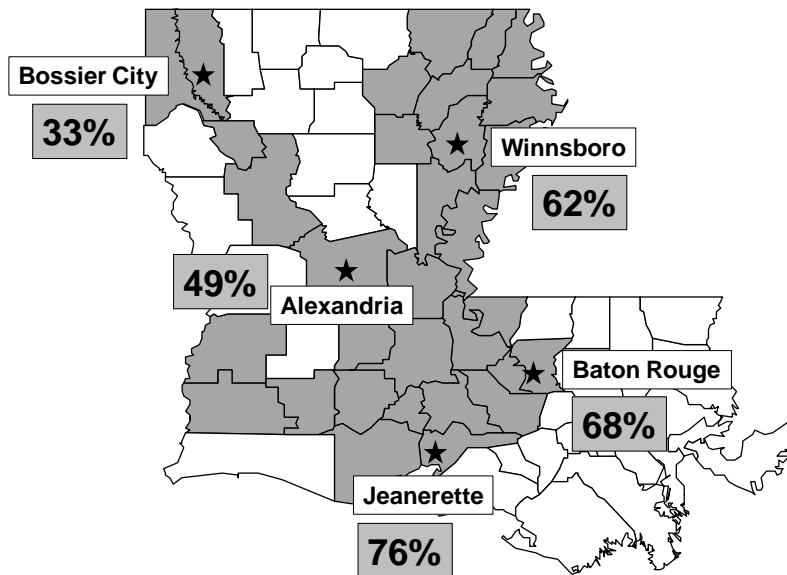


Figure 2.21. Percentage of redbanded stink bugs in the total stink bug complex for each sample site averaged across the 2008-2010 sample period.

In this study, redbanded stink bugs made up the largest percentage of the stink bug complex in the earlier maturing soybean varieties, MGs III (86%) and IV (60%). Numbers declined slightly in the later maturing soybean, MGs V (54%) and VI (50%). This observation may be due to the absence of other suitable hosts for the redbanded stink bug, while the green, southern green, and brown stink bugs have a wider variety of crop and non-crop hosts available during the period MG III and IV soybean are most susceptible. These species may become more

distributed across the general farmscape leaving redbanded stink bug populations concentrated in soybean.

The early soybean production system that is widely utilized on much of the Louisiana acreage may allow the overwintering and first generation populations of redbanded stink bug to have a suitable host with little competition from other stink bug species that inhabit a wide range of alternative hosts. In 2010, a significant portion of the Louisiana soybean acreage was planted to early-maturing MGs III and IV (64%) varieties, while MGs V (35%) and VI (1%) varieties were grown on the remaining acreage (R. Levy personal communication). Historically, Louisiana has planted later-maturing varieties (MGs V, VI, and VII) but began a transition to an early soybean production system during the late 1990's to early 2000's (Honeycutt 1996, Heatherly 1999, Baur et al. 2000). In the Mid-South, the early soybean production system has been adopted to reduce late season drought stress, insect problems, and inclement weather that are often encountered in early-August to mid-September (Heatherly 1999).

In 2010, stink bug numbers in this survey and in many Louisiana production fields were generally lower compared to populations in 2009. This reduction in total stink bug numbers could be attributed to lower-than-normal temperatures during the winter months of 2009 to 2010 and drought conditions during the following spring that reduced the availability and quality of alternative non-crop hosts (Robinson 2011). The proportion of brown stink bugs within the stink bug complex was higher in 2010 (20%) compared to 2008 (7%) and 2009 (8%) which could have been related to overall colder winter from 2009 to 2010. Green and brown stink bugs are more tolerant to cold temperatures, have a wider geographical range, and can successfully overwinter in the northern United States (Greene et al. 2006). Overwintering mortality is the major population limiting factor for southern green stink bug populations. Cold temperatures

during the winter months contribute to the annual variation in southern green stink bug populations (Jones and Sullivan 1981, Elsey 1993, McPherson and McPherson 2000). Although stink bug numbers declined sharply from 2009 to 2010, the redbanded stink bug remained the predominant pest species in Louisiana soybean. Little is known about the northern range of the redbanded stink bug in the United States at this time, and these data indicate that the pest has become established in Louisiana as a perennial pest of soybean with populations fluctuating annually due to environmental conditions.

Since the redbanded stink bug has become more prevalent in Louisiana soybean, the frequency of insecticide applications has increased from one to two per season during the late 1990's to a current average of three to five per season. The bulk of these applications target the redbanded stink bug (Bechtel 2000, Paxton 2005, Paxton 2007, Guidry 2010). The redbanded stink bug is more difficult to control in soybean with currently available insecticides compared to the southern green stink bug, which has historically been the predominant stink bug pest of soybean in Louisiana (Baur et al. 2010; Temple et al. 2011). Difficulty controlling the redbanded stink bug with currently available insecticides, coupled with reports from South America indicating that this species has the ability to cause greater injury to soybean than southern green stink bug, has resulted in a reduction of the action threshold for initiating insecticide applications from nine stink bugs per sample to six stink bugs per sample (Vicentini and Jimenez 1977, Correa-Ferreira and Azevedo 2002, Baur and Baldwin 2006, Pollet et al. 2010). Additional research is needed to fully understand the biology of redbanded stink bug in Louisiana soybean agro-ecosystems and to determine how this species became the dominant pest of soybean in only a decade.

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CHAPTER 3

OVIPOSITION, SEX RATIO, AND POPULATION DYNAMICS OF THE REDBANDED STINK BUG, *PIEZODORUS GUILDINII* (WESTWOOD), IN SOYBEAN

Introduction

Several phytophagous stink bugs including green stink bug, *Acrosternum hilare* (Say), southern green stink bug, *Nezara viridula* L., and the brown stink bug complex, *Euschistus spp.*, are annual pests in many Southern soybean fields. In 2002, an emerging stink bug pest, the redbanded stink bug, *Piezodorus guildinii* (Westwood), exceeded action threshold levels (Baldwin 2005). It has become the most yield-limiting stink bug pest in the Louisiana soybean production system (Paxton et al. 2007; Baur et al. 2010). The redbanded stink bug is a Neotropical stink bug species that ranges from Argentina north to the Southern United States (Panizzi and Slansky 1985). The redbanded stink bug is a significant annual pest in South American soybean, especially in Brazil (McPherson and McPherson 2000). In the United States, this species was first reported during the 1960's and has been reported in several states including South Carolina, Florida, Georgia, and New Mexico (McPherson and McPherson 2000). This stink bug has been previously reported on soybean in the United States, but it was not considered an economic pest (McPherson et al. 1993).

Studies on nymphal development and survivorship, adult longevity and reproduction, and life stage dispersal on South American soybean have provided considerable information in the biology and ecology of the redbanded stink bug (Panizzi et al. 2000). Even though redbanded stink bug is highly injurious to soybean, it reproduces poorly and has shorter longevity on soybean plants compared to that on non-crop hosts (Panizzi et al. 2000). The number of egg clusters per female ranges from three clusters on soybean to as many as 37 on *Indigo spp.* (Panizzi et al. 2000). Panizzi and Smith (1977) characterized the oviposition, development time,

sex ratio, and longevity of redbanded stink bug on soybean in Brazil. This study reported that the average number of eggs were 15.1 per cluster and 60% of the clusters were observed on pods. The sex ratio observed in these field studies was 1.4 females for each male (Panizzi and Smith 1977). Redbanded stink bug eggs maintained in the laboratory at 24° C and 80% RH required a mean of 7.5 d from oviposition to eclosion. In a subsequent study, Link and Concatto (1979) found number of eggs per clusters (mean=17.5) ranged from 4 to 39. Oviposition preference among soybean pods, stems, and leaves indicated the greatest frequency of eggs occurred on pods (80%) in that study. Silva and Ruedell (1983) reported that redbanded stink bug oviposited 51% of eggs on pods and 48% on leaves. No studies have examined the vertical distribution of redbanded stink bug eggs within the soybean plant canopy or oviposition frequency related to soybean phenology.

Since 2002, the redbanded stink bug has become established in all major soybean producing parishes in Louisiana. A comprehensive survey of stink bugs in Louisiana soybean from 2008-2010 showed that the redbanded stink bug was the predominant (>50%) species (Temple et al. 2011a). The expansion of this pest's range has not been limited to Louisiana. Redbanded stink bugs have now reached pest status for soybean in all states bordering Louisiana and have been reported as far north as Missouri and Tennessee (Stalcup 2007, Bailey 2009, Catchot 2009, Smith 2009, Smith et al. 2009).

Limited information is available describing the biology of redbanded stink bug in U. S. soybean agro-ecosystems. Compared to other stink bugs attacking soybean, this pest is less susceptible to insecticides commonly used on soybean for stink bug control (Temple et al. 2011b, Baur et al. 2010). This aspect coupled with limited knowledge of pest biology and wild host range has hindered satisfactory management of this pest. The redbanded stink bug has also

shown a propensity to develop high infestations (five-fold action threshold) within soybean fields over a relatively brief period (Temple et al. 2011a). Knowledge of oviposition characteristics and adult behavior during soybean phenological growth stages is needed to develop effective IPM strategies for controlling this pest. The objectives of these studies were to characterize redbanded stink bug oviposition in relation to soybean phenology, describe adult and nymphal population dynamics, and determine adult sex ratios.

Materials and Methods

Observations were made on non-insecticide treated soybean at several Louisiana State University Agricultural Center Research Stations including Baton Rouge (East Baton Rouge Parish), Jeanerette (Iberia Parish), in 2009 and Winnsboro (Franklin Parish) LA in 2008 and 2009. Four maturity groups (MG III, MG IV, MG V, and MG VI) of soybean were planted in large contiguous blocks (0.1-0.2 ha each) at planting dates recommended by the Louisiana Cooperative Extension Service (Table 3.1).

Oviposition Surveys. On each sampling date, the soybean growth stages for each maturity group were recorded based on descriptions by Fehr et al. (1971). Samples of redbanded stink bug eggs were initiated at the R1 (beginning bloom) stage and continued weekly until R7 (beginning maturity). Soybean fields were segregated into six distinct plots for sampling. Five plants were randomly removed from each of the six field plots for a total of 30 plants per field per week. Whole plants were destructively sampled and examined for the presence of redbanded stink bug egg clusters. Specifically, data were recorded for oviposition site (leaf [abaxial or adaxial], pod, or stem), mainstem node of oviposition site, and number of eggs per cluster (mass).

Table 3.1. Survey sites, maturity groups, and planting dates in Louisiana, 2008-2009.

Location	MG ¹	Planting date	
		2008	2009
Baton Rouge	IV	-----	May 11
Baton Rouge	V	-----	May 11
Baton Rouge	VI	-----	June 15
Jeanerette	III	-----	April 24
Jeanerette	IV	-----	April 24
Jeanerette	V	-----	May 15
Winnsboro	IV	April 16	April 16
Winnsboro	V	May 9	May 18
Winnsboro	VI	June 1	June 9

2008 varieties: Asgrow 4404 (MG IV), Asgrow 5905 (MG V), Asgrow 6702 (MG VI).

2009 varieties: Pioneer 93Y90 (MG III), Asgrow 4606 (MG IV), Asgrow 5606 (MG V), Asgrow 6301 (MG VI).

¹Maturity Group.

Egg clusters were further categorized by their position within the vertical plant strata (upper, middle, or lower plant canopy). Chi-square analysis was used to compare the frequency of oviposition on various plant structures and within plant canopy placement (PROC FREQ, SAS Institute 2003, SAS/STAT user's guide, version 9.1). ANOVA was used to compare the number of eggs within a cluster by field location, soybean maturity group, and year of study (PROC GLM, SAS Institute 2003, SAS/STAT user's guide, version 9.1).

Population Dynamics and Soybean Phenology. During 2009 at Baton Rouge, Jeanerette, and Winnsboro, redbanded stink bug adults and nymphs were sampled in MG IV and MG V soybean. Native infestations were allowed to infest field plots which were not treated with insecticides. Each MG (plot) was divided into six sub-plots. Each of the six sub-plots within a MG was sampled weekly from R1 (beginning bloom) to R8 (physiological maturity) using a standard (38 cm diameter) sweep net and taking 25 sweep samples (150 total/MG/week). When a MG developed to physiological maturity (R8) that plot was either harvested or destroyed by mowing. Individual rows that were sampled within each sub-plot were alternated each week so that a row was not sampled more than once within a period of four weeks. On each sampling

date, the soybean growth stages for each MG were recorded. Each individual set of sweep net samples was bagged, labeled, transported to the laboratory, and frozen until it could be evaluated. The number of redbanded stink bug adults and nymphs was recorded for each sample site and summarized within each maturity group and by soybean phenological stages. The mean (\pm SE) number of redbanded stink bug adults and nymphs from all sampling periods within a phenological growth stage are reported.

Adult Sex Ratio. In 2008 and 2009 at Winnsboro, sampling for redbanded stink bug adults was conducted in a similar manner within each soybean field as previously described in the egg survey and population dynamics experiments. Samples of redbanded stink bug adults were segregated and sex was determined based on diagnostic keys (McPherson and McPherson 2000). Data was summarized within each MG and by soybean phenological stage. Chi-square analysis was used to compare the frequency of males and females collected within a given soybean phenological stage (PROC FREQ, SAS Institute 2003, SAS/STAT user's guide, version 9.1).

Results

Oviposition Surveys. A total of 655 redbanded stink bug egg clusters were characterized from surveys of over 2400 plants during June-September in 2008 and 2009. The mean number of eggs per cluster was 17.6 ± 0.3 (Table 3.2).

Table 3.2. Number of redbanded stink bug egg clusters, range of eggs per cluster, and mean number of eggs per cluster surveyed in each soybean maturity group.

Maturity Group	N	Mean Eggs/Cluster (SE)	Eggs/Cluster (Range)
III	22	16.4 \pm 1.4b	6-33
IV	223	16.2 \pm 0.4b	2-40
V	321	18.6 \pm 0.4a	3-55
VI	89	18.0 \pm 0.7a	6-36
Total	655	17.6 \pm 0.3	2-55

The majority (83%) of egg clusters were found in MG IV and MG V soybean. Most (80%) of the egg clusters were found at the Winnsboro sample site (Table 3.3). The number of eggs within a cluster ranged from 2 to 55. Most egg clusters ranged between 11 to 15 eggs (35.7%) and 16 to 20 eggs (24.5%) (Figure 3.1). Only 4.3% of the egg clusters exceeded 31 eggs per cluster.

Table 3.3 Number of redbanded stink bug egg clusters, range of eggs per cluster, and mean number of eggs per cluster at each location.

Location	N	Mean Eggs/Cluster (SE)	Eggs/Cluster (Range)
Baton Rouge	48	15.6±0.7b	5-32
Jeanerette	82	16.3±0.6b	6-33
Winnsboro	525	18.0±0.3a	2-55
Total	655	17.6±0.3	2-55

Egg cluster distribution on plants showed that 52%, 45%, and 3% were oviposited on leaves, pods, and stems, respectively (Figure 3.2). Significantly more egg clusters were found on leaves and pods compared to that on stems ($\chi^2=279.3$, $df=2$, $P < 0.0001$). Significantly more egg clusters were found on leaves compared to pods ($\chi^2=3.98$, $df=1$, $P= 0.046$). Oviposition occurred on both the abaxial (top) and adaxial (bottom) leaf surfaces. Significantly ($\chi^2=7.54$, $df=1$, $P=0.006$) more egg clusters were found on the abaxial leaf surface (59%) compared to that on the adaxial leaf surface (41%).

Egg clusters were scattered throughout the upper (21%), middle (36%), and lower (43%) vertical strata (Figure 3.3).

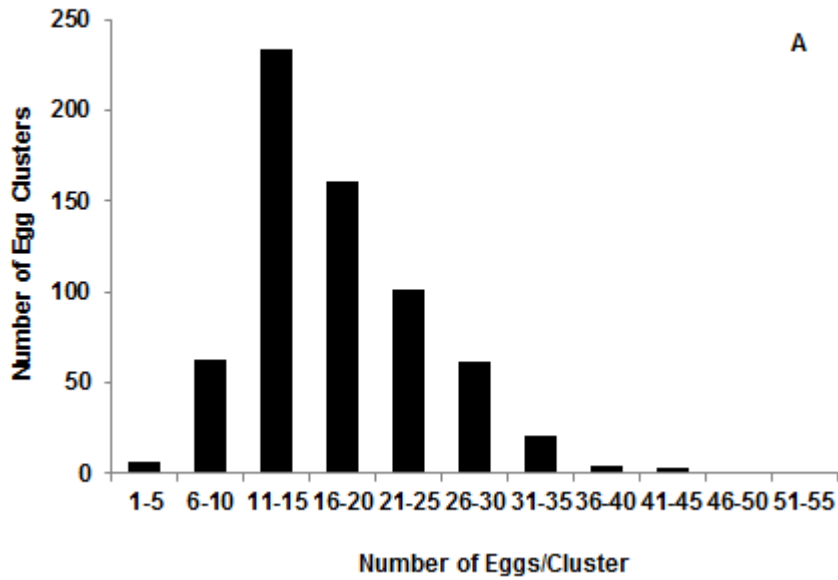


Figure 3.1. The number of eggs per cluster of redbanded stink bug oviposited on soybean.

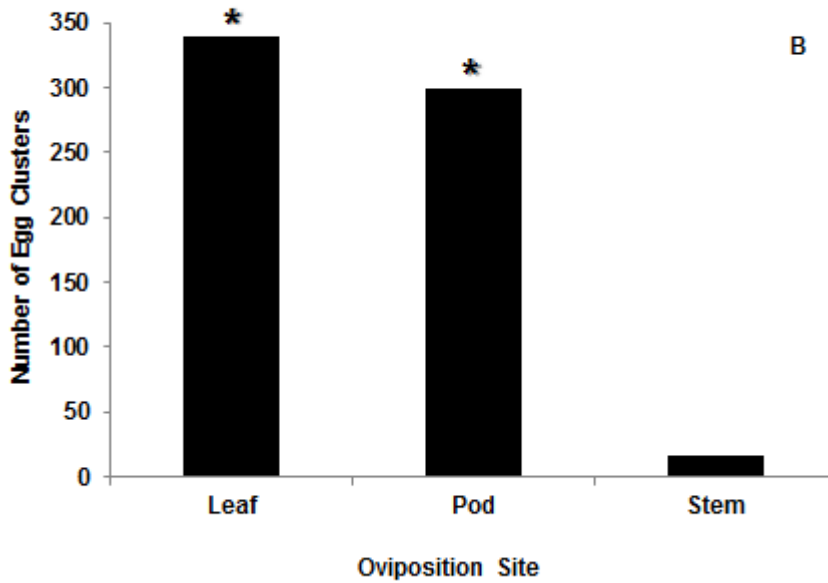


Figure 3.2. Frequency of redbanded stink bug eggs clusters found on soybean plant structures (leaf, pod, stem).

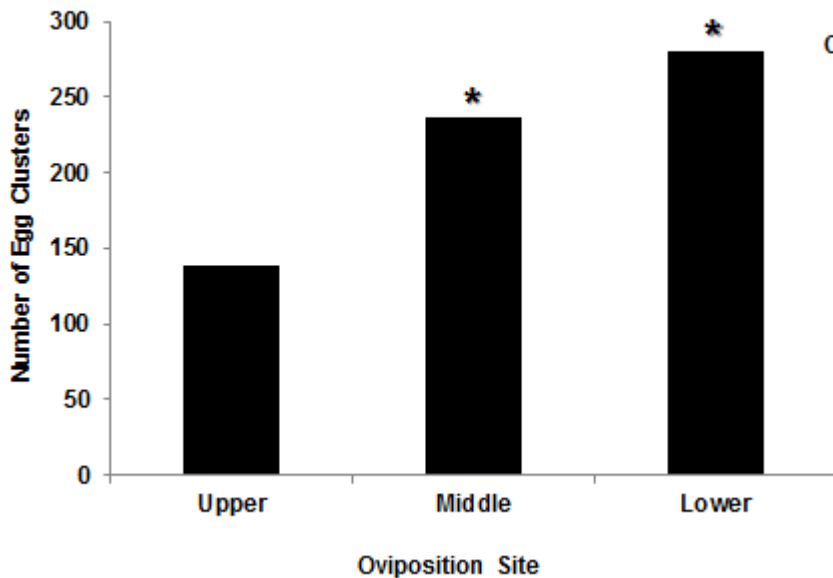


Figure 3.3. Frequency of redbanded stink bug egg clusters found within the vertical strata of soybean plant (upper, middle, lower).

Significantly more egg clusters were found in the middle and lower plant strata compared to that in the upper plant stratum ($\chi^2=48.57$, $df=2$, $P < 0.0001$). There were no significant differences in the number of egg clusters oviposited within lower and middle vertical strata ($\chi^2=3.57$, $df=1$, $P= 0.058$).

Egg clusters were collected during all soybean reproductive stages, R1 (first flower) to R7 (beginning maturity); and were found at lower numbers in R1 to R3 stages (Figure 3.4). Oviposition was highest during the R5 to R6 soybean growth stages, peaking during the R5 stage.

The low number of samples (<90) at Baton Rouge, Jeanerette, and in MG VI soybean at Winnsboro did not allow for additional statistical analyses. At Winnsboro, significant differences ($F=10.25$, $P<0.0001$) were detected in the number of eggs per cluster between MG IV (16.3 ± 0.46) and MG V (19.27 ± 0.50) soybean. No significant differences were detected

within MG IV soybean ($F= 1.03, P=0.35$) and V ($F=1.38, P=0.23$) from 2008 and 2009 at the Winnsboro survey site, so those data were combined for analysis.

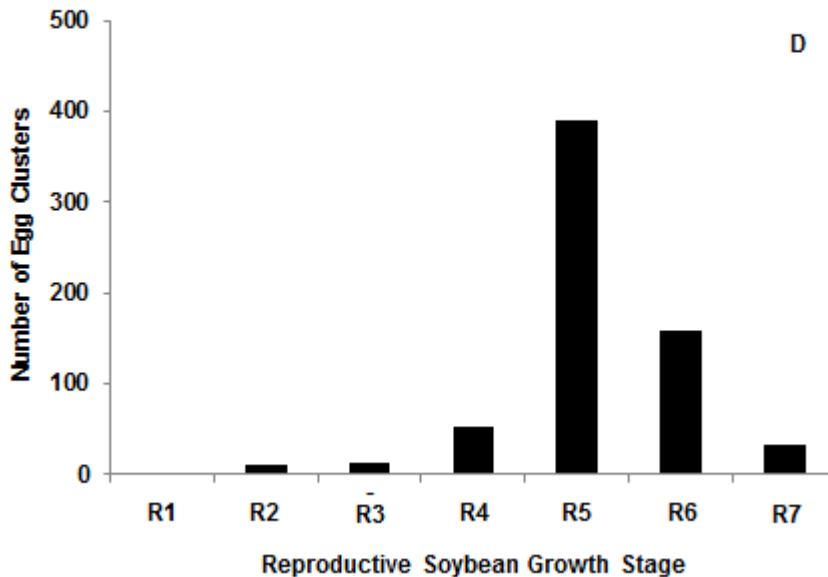


Figure 3.4. Frequency of redbanded stink bug egg clusters found within soybean reproductive stages (R1-R7).

In MG IV soybean at Winnsboro, redbanded stink bugs deposited 100% of egg clusters on leaves during soybean reproductive stages of R1 (first flower) through R4 (pod elongation) (Figure 3.5). During the later reproductive stages of R5 (seed initiation) through R7 (beginning maturity), redbanded stink bugs deposited significantly ($\chi^2=13.07, df=2, P=0.0003$) more egg clusters ($n=169$) on leaves (64%) than on pods (36%). No egg clusters were found on MG IV soybean stems. Redbanded stink bugs deposited 63% and 37% of egg clusters in the upper and middle plant strata, respectively, during R1 through R4 stages (Figure 3.6). During R5 through R7 stages, redbanded stink bugs deposited significantly ($\chi^2=35.3, df=2, P<0.0001$) more egg

clusters in the middle (52%) and lower (34%) plant strata compared to the upper plant stratum (14%).

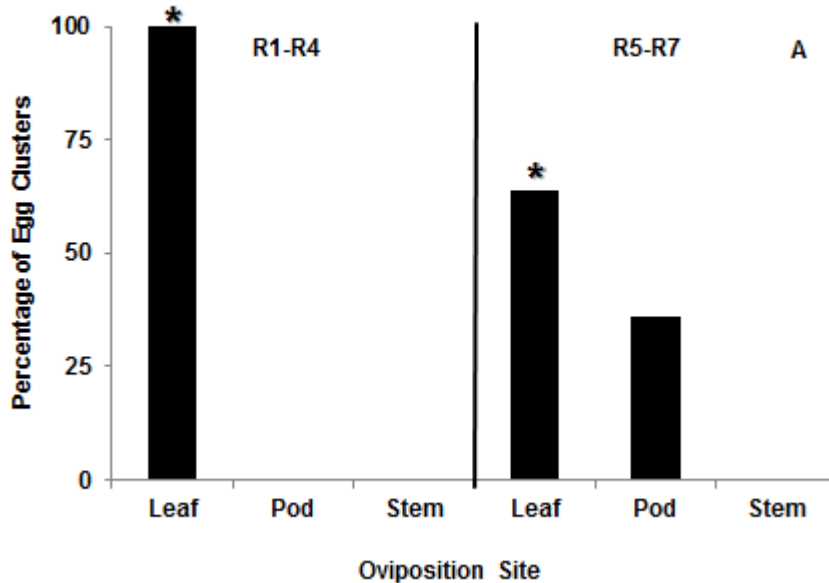


Figure 3.5. Oviposition site preference of redbanded stink bug on plant structures in MG IV soybean.

For MG V soybean at Winnsboro, redbanded stink bugs deposited 57% and 43% of egg clusters (n=28) on leaves and pods, respectively, during the early soybean reproductive stages (R1 through R4) (Figure 3.7). Egg clusters were not detected on R3 stage pods, but were detected on pods during the R4 stage. During the later reproductive stages (R5 through R7), redbanded stink bugs deposited significantly ($\chi^2=30.67$, $df=2$, $P<0.0001$) more egg clusters (n=169) on pods (65%) than on leaves (30%) and stems (5%). Redbanded stink bugs deposited 39%, 36%, and 25% of egg clusters in the lower, middle, and upper plant strata, respectively, from R1 through R4 stages (Figure 3.8). During R5 through R7 stages, redbanded stink bugs deposited significantly ($\chi^2=9.48$, $P=0.0021$) more egg clusters in the lower (50%) plant stratum compared to the middle (32%) and upper (18%) plant strata.

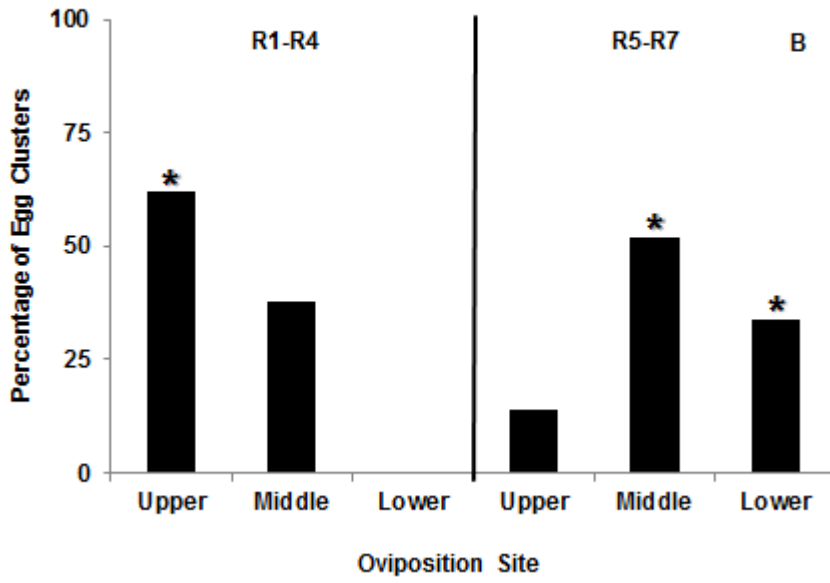


Figure 3.6. Oviposition site preference of redbanded stink bug within vertical strata in MG IV soybean.

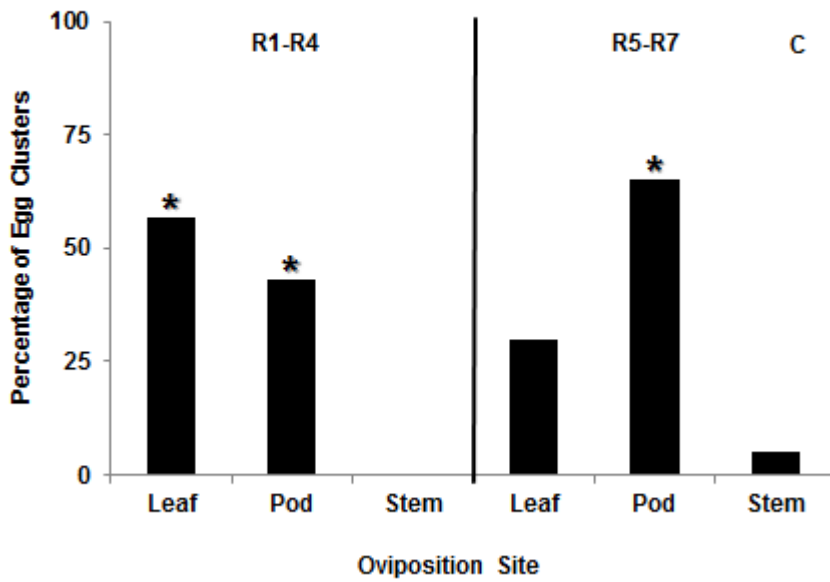


Figure 3.7. Oviposition site preference of redbanded stink bug on plant structures in MG V soybean.

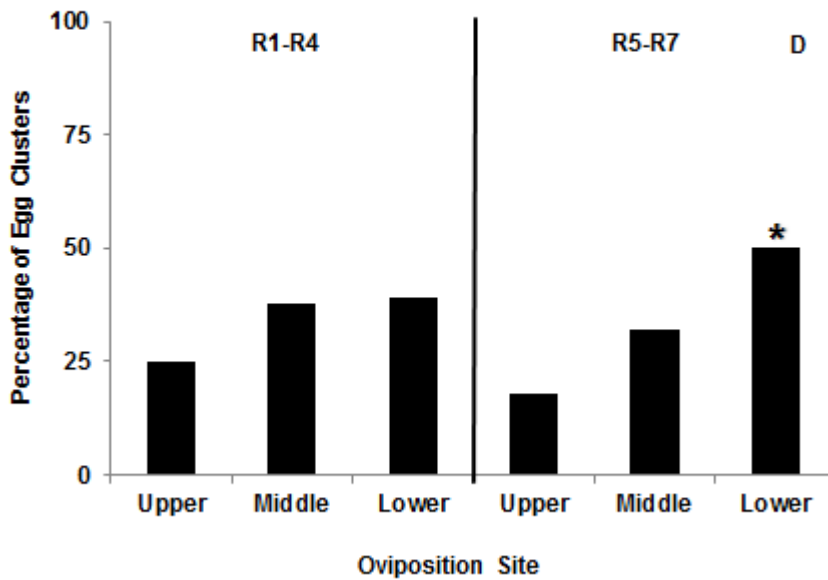


Figure 3.8. Oviposition site preference of redbanded stink bug within vertical strata in MG V soybean.

Population Dynamics and Soybean Phenology. In MG IV soybean at Baton Rouge, redbanded stink bugs adults were first detected in samples during the R2 stage and peaked (1.5/25 sweeps) at R5 stage (Figure 3.9). Redbanded stink bug nymphs were not detected in samples until the R6 stage and peaked (2.2/25 sweeps) during the R7 stage. Redbanded stink bugs began ovipositing during the R2 stage with peak oviposition (0.1 egg cluster/plant) during the R5 stage. In MG V soybean, redbanded stink bug adult populations had two distinct peaks with emigrating adults (1/25 sweeps) at R1 stage that slowly declined through R5 stage and then peaked (2/25 sweeps) again at R7 stage (Figure 3.10). Redbanded stink bug nymphs were not detected in samples until R6 stage which also was when the numbers (0.9/25 sweeps) peaked. Redbanded stink bugs began ovipositing during R3 stage and continued through R7 stage with peak oviposition (0.17 egg cluster/plant) at R4 stage. Total (adult and nymph) redbanded stink bug populations were relatively low (< 3/25 sweeps) at this site.

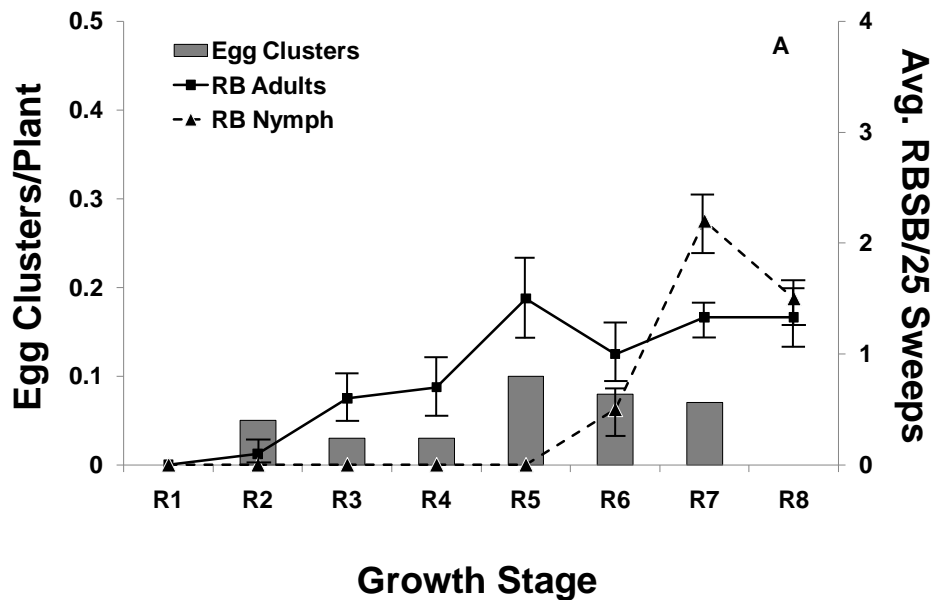


Figure 3.9. Population dynamics of redbanded stink bug adults, nymphs, and egg clusters during reproductive growth stages in MG IV soybean, Baton Rouge.

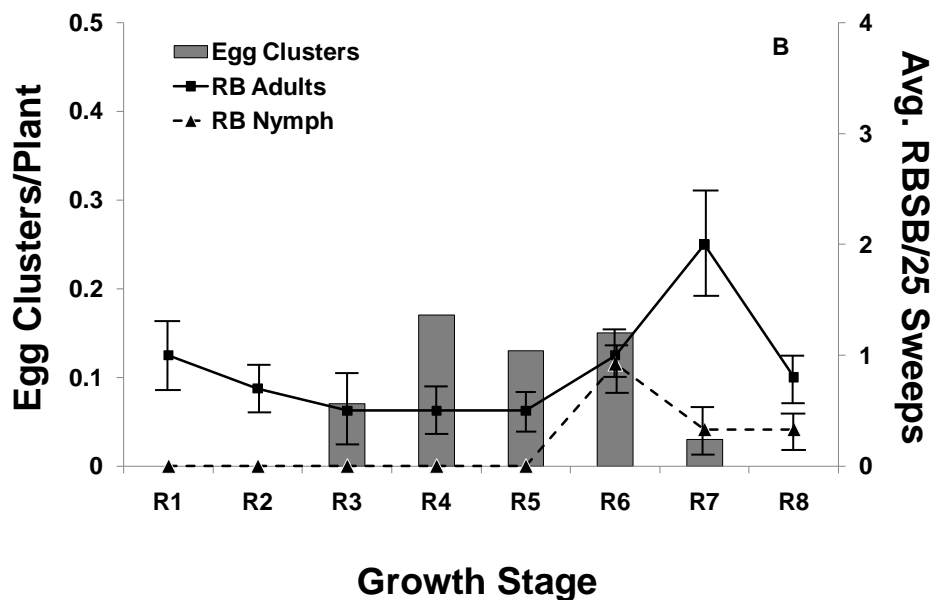


Figure 3.10. Population dynamics of redbanded stink bug adults, nymphs, and egg clusters during reproductive growth stages MG V soybean, Baton Rouge.

In MG IV soybean at Jeanerette, redbanded stink bug adult populations were low (<2/25 sweeps) during R1 to R2 stages, increased to a peak of 9.7/ 25 sweeps at R4 stage and slowly declined through the remainder of soybean growth stages (Figure 3.11). Redbanded stink bug nymphs were first detected during R5 stage and peaked (4.1/25 sweeps) at R6 stage. Oviposition was first recorded during R4 stage with peak oviposition (0.13 egg cluster/plant) at R5 stage through R6 stage. In MG V soybean, redbanded stink bug adults were detected at R1 stage and peaked (3.5/25 sweeps) at R5 stage (Figure 3.12). Redbanded stink bug nymphs were initially detected at R4 stage and the population peaked (6.0/25 sweeps) at R6 stage. Oviposition was first detected at R2 stage and continued through R7 stage with peak oviposition (0.22 egg cluster/plant) at R5 stage. Total (adult and nymph) redbanded stink bug populations exceeded the action threshold (> 6/25 sweeps) in the MG IV and V soybeans at this site.

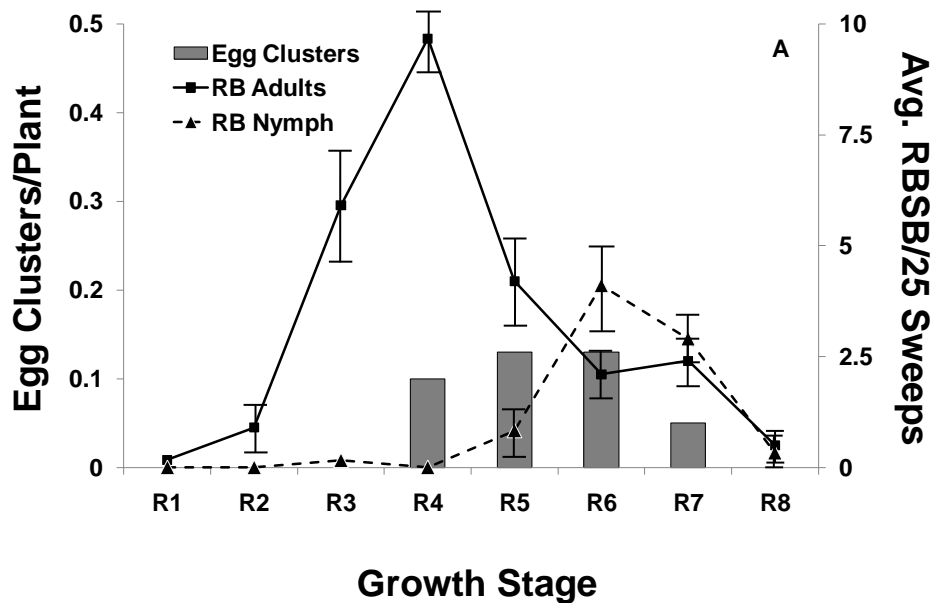


Figure 3.11. Population dynamics of redbanded stink bug adults, nymphs, and egg clusters during reproductive growth stages in MG IV soybean, Jeanerette.

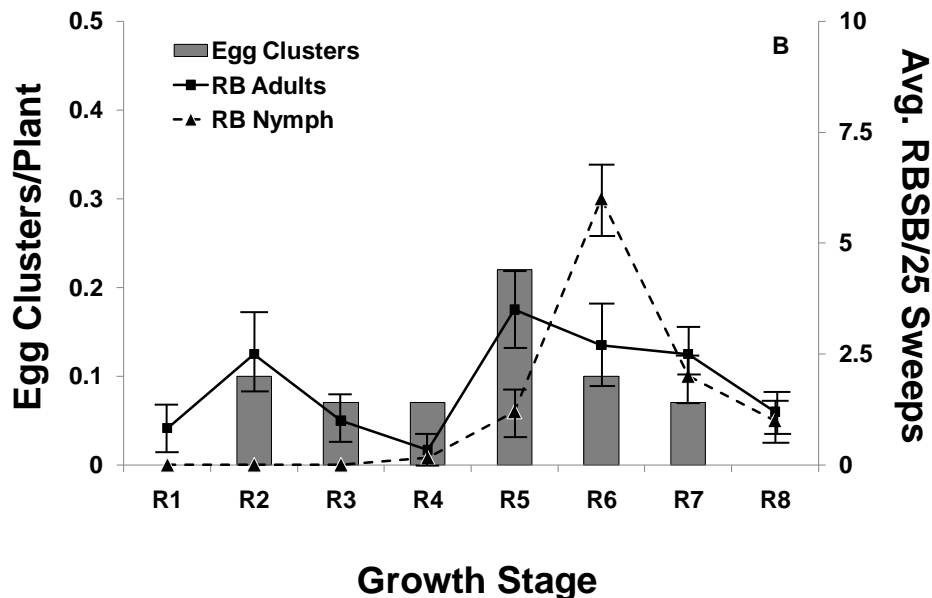


Figure 3.12. Population dynamics of redbanded stink bug adults, nymphs, and egg clusters during reproductive growth stages MG V soybean, Jeanerette.

In MG IV soybean at Winnsboro, redbanded stink bug adults were first detected at R3 stage and populations peaked (3.1/25 sweeps) during R5 stage (Figure 3.13). Redbanded stink bug nymphs were detected at low levels initially during R4 stage and peaked (1.3/25 sweeps) during R7 stage. Oviposition was first detected during R3 stage and peaked (0.68 egg cluster/plant) during R5 stage. In MG V soybean, redbanded stink bug adults were first detected during R2 stage and populations peaked (10.0/25 sweeps) during R7 stage (Figure 3.14). Redbanded stink bug nymphs were initially detected during R5 stage and populations peaked (9.2/25 sweeps) during R7 stage. Oviposition was detected from R2 through R7 stages with peak oviposition (0.58 egg cluster/plant) during the R6 growth stage. Total (adult and nymph) redbanded stink bug populations remained below the action threshold (> 6/25 sweeps) in the MG IV soybean, but peaked at three-fold the action threshold in MG V soybean at this site.

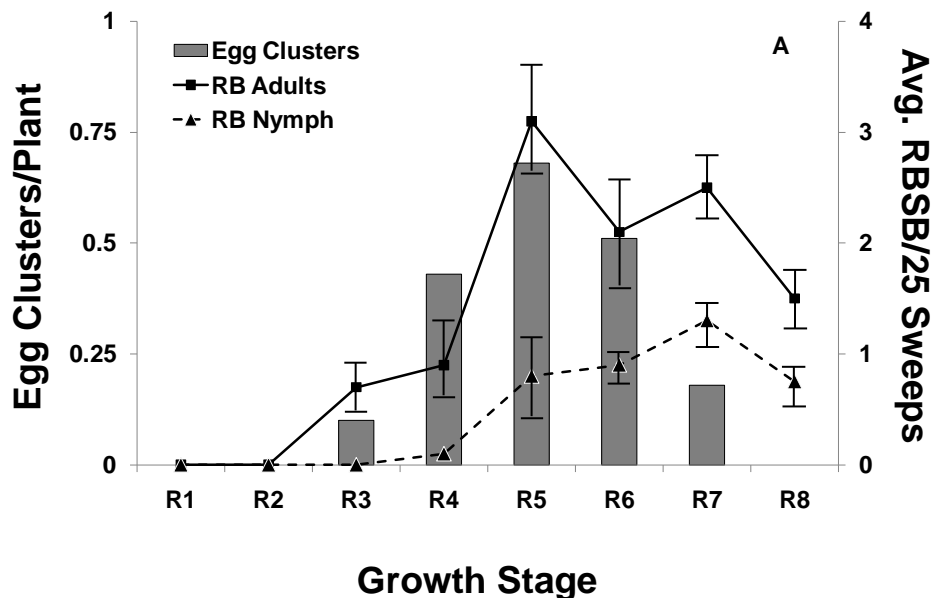


Figure 3.13. Population dynamics of redbanded stink bug adults, nymphs, and egg clusters during reproductive growth stages in MG IV soybean, Winnsboro.

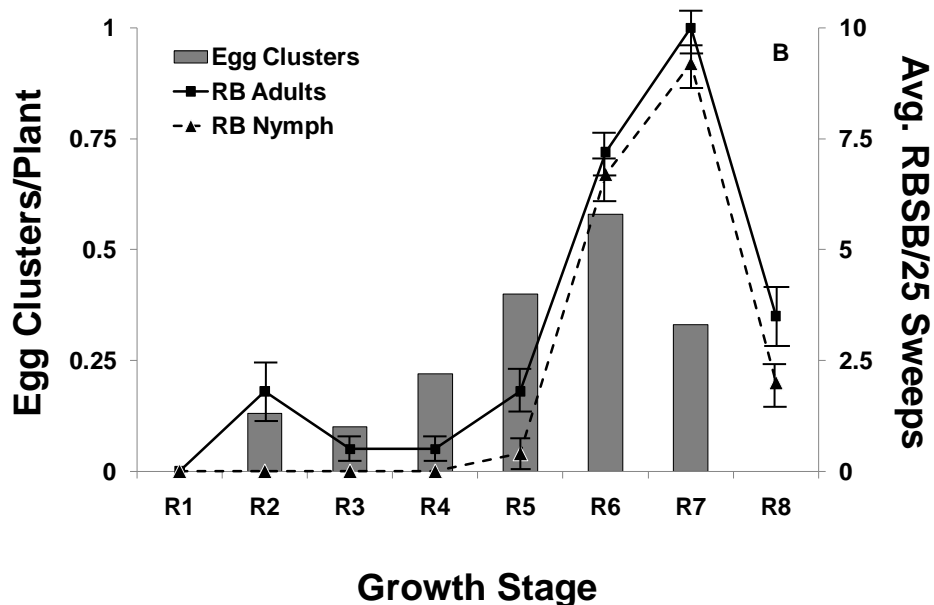


Figure 3.14. Population dynamics of redbanded stink bug adults, nymphs, and egg clusters during reproductive growth stages in MG V soybean, Winnsboro.

Adult Sex Ratio. The sex ratio of 2,147 redbanded stink bugs collected at Winnsboro from 2008-2009 was 1.2 females to 1 male. Across all soybean reproductive growth stages, adult sex ratios ranged from 1.1:1 to 1.4:1 females to male (Figure 3.15). The number of females compared to males was only significantly ($\chi^2=25.32$, $df=1$, $P<0.0001$) higher during the R5 growth stage.

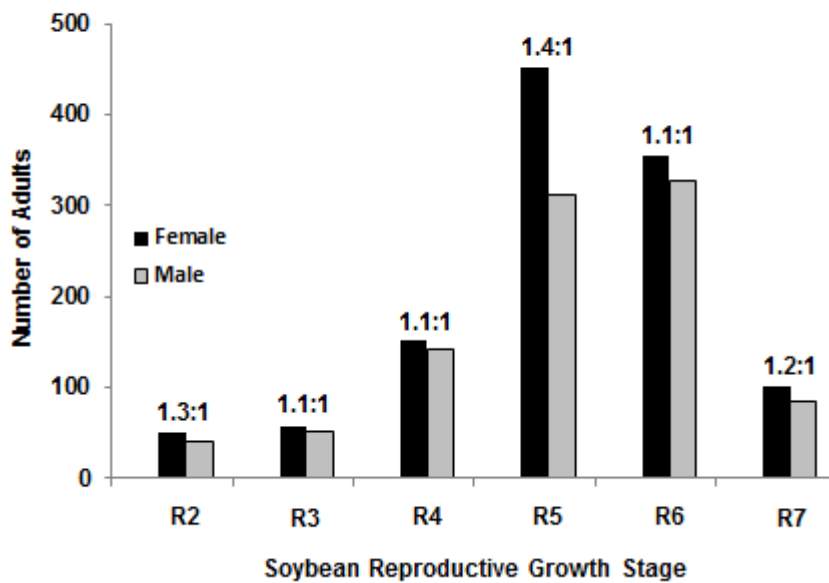


Figure 3.15. Redbanded stink bug sex ratio during soybean growth stages.

Discussion

The number of eggs per cluster from the total ($n=655$) collected in soybean averaged 17.6 across all samples in the current study. These results are similar to that previously reported in Brazil with mean eggs per cluster ranging from 14.2 to 17.5 (Fraga and Ochoa 1972, Panizzi and Smith 1977, Link and Concatto 1979, Silva and Ruedell 1983). Eggs per cluster in the current study ranged from 2 to 55 and was similar to that in other studies (1 to 55 per cluster) (Panizzi and Smith 1977, Link and Concatto 1979, Silva and Ruedell 1983). The range of redbanded stink bug eggs per cluster is generally lower than that reported for the southern green stink bug

(40-116 eggs/cluster), the predominant pest species in Southern U. S. soybean (Jones 1918, Passlow and Waite 1971).

In the current study, the primary oviposition sites for redbanded banded stink bug egg clusters was leaves (52%) and pods (45%). Other soybean surveys, found pods to be the primary oviposition site ranging from 60 to 80% of total egg masses (Panizzi and Smith 1977, Link and Concatto 1979). However, Silva and Ruedell (1983) reported a more even distribution of egg clusters between leaves (51%) and pods (48%). Oviposition on pods is a behavior typically observed for native stink bugs (southern green, green, and brown stink bugs) of Louisiana soybean which prefer to oviposit on the abaxial leaf surface (Jones 1918, Esselbaugh 1946, Passlow and Waite 1971, Dietz et al. 1976). In the present study, redbanded stink bugs oviposited exclusively on leaves until soybean plants reached the R4 stage (pod elongation) in both MG VI and MG V cultivars. Leaves were still the predominant oviposition site during the later stages of plant development (R5 to R7 stages) in MG IV cultivars, but pods were the predominant oviposition site during the R5 to R7 stages of development in MG V cultivars. This difference could be related to growth habits between MG IV (indeterminate) and MG V (determinate) cultivars.

Within the soybean plant canopy, 79% of redbanded stink bug egg clusters were oviposited within the lower and middle plant strata. A substantial portion (43%) of those egg clusters were found in the lower plant stratum. Previous redbanded stink bug oviposition surveys have not reported a preference within the soybean plant canopy. This behavior may be unique among the stink bugs in Louisiana soybean. Southern green, green, and brown stink bugs prefer to oviposit in the upper canopy of soybean (Jones 1918, Esselbaugh 1946, Passlow and Waite 1971, Dietz et al. 1976). Redbanded stink bugs oviposited predominantly in the upper canopy (63%) until

soybean plants reached the R5 stage in MG IV soybean and deposited the bulk of eggs during the later reproductive stages in the middle (52%) and lower portion (34%) of the plant canopy. In MG V soybean, redbanded stink bugs oviposited primarily in the lower plant canopy during both the early (39%) and late (50%) reproductive stages.

Russin et al. (1987) demonstrated that a complex of stink bugs (southern green, green, and brown) preferred to feed on pods in the upper half of the plant canopy. Stink bugs only fed on pods in the lower plant canopy when high infestation levels forced insects into the lower canopy. The preference of oviposition and feeding in the upper plant canopy makes sampling protocols for the southern green, green, and brown stink bug more efficient and increases the likelihood of contact with insecticide residues when using chemical control strategies.

The oviposition behavior of redbanded stink bug could have implications on IPM approaches used to manage this pest. Sampling for stink bugs in soybean has been primarily accomplished with a sweep net because of sampling efficiency (Kogan and Pitre 1980). Sweeping with a net in the upper portion of the plant canopy is considered an appropriate sampling method for estimating phytophagous stink bug populations in soybean (Todd and Herzog 1980). The current study showed the redbanded stink bug adults and nymphs were distributed in the middle and lower portions of the canopy. Sweep samples exclusively in the upper canopy may underestimate the population of redbanded stink bug adults and nymphs, especially during the later soybean reproductive stages (R5 to R7).

With a high frequency of egg clusters found in the lower two-thirds of the plant canopy, redbanded stink bug nymphs could have less exposure to insecticide residues. The effectiveness of any chemical control measure is contingent on delivery of pesticide droplets at or near the target site. Hutchins and Pitre (1984) reported that insecticide droplet deposition significantly

decreased in the middle (37%) and lower plant canopy (82%) in conventional wide-row (96.5 cm spacing) soybean compared to that in the upper plant canopy. The introduction of glyphosate-tolerant soybean cultivars has resulted in an increase in conservation tillage soybean and adoption of narrow row (< 76 cm row spacing) soybean systems. In Louisiana during 2010, 25% of the total soybean acreage was planted to narrow row soybean (R. Levy; LSU AgCenter Soybean Extension Specialist personal communication). In narrow row (17.8 cm spacing) soybean, droplet depositions decreased more sharply in the middle (73%) and lower (84%) plant canopies when compared to conventional row soybean (Hutchins and Pitre 1984).

The adult sex ratio of redbanded stink bug observed in the current study was 1.2 females to 1 male. In Brazilian studies by Silva and Ruedell (1983) and Panizzi and Smith (1977), sex ratios for redbanded stink bug were 1.2:1 and 1.4:1, respectively. However, these were not season-long surveys. In the current study, the ratio of females to males was similar in all soybean growth stages except R5, where the sex ratio increased to 1.4:1, which coincided with peak oviposition.

Redbanded stink bug adults were generally first detected in soybean during the early reproductive stages (R1 to R3). Populations generally increased during the production season, peaking during the later reproductive stages (R5 to R7). Oviposition was generally initiated during R2 to R3 and peaked during R4 to R6 growth stages. Redbanded stink bug nymphs usually were not detected until R4 to R5 stages, and nymph populations peaked during R6 to R7 growth stages. Southern green stink bug adult populations in soybean began to increase during R3 stage, and peaked during R6 stage (Schumann and Todd 1982). Oviposition by the southern green stink bug was initiated prior to R1 and peaked between R3 and R5 stages (Schumann and Todd 1982). Southern green stink bug nymph populations usually peak during R6 to R7 stages.

This study is the first to report oviposition behavior of redbanded stink bug populations and the relationship to soybean phenology in the United States. The redbanded stink bug has become the predominant pest in Louisiana soybean within a decade after its first detection (Temple et al. 2011a). This pest appears to have a narrow non-crop host range, and soybean is one of the few available food sources for this pest after spring alternate host senesce. The redbanded stink bug has the propensity to develop large populations (3-5X action thresholds) very quickly in soybean (Temple et al. 2011a).

Redbanded stink bug oviposit fewer eggs per cluster in soybean than the southern green, green, and brown stink bug, so oviposition frequency is not likely the sole factor creating these large populations (Panizzi et al. 2000). One possibility for the large reproductive capacity of redbanded stink bugs in Louisiana soybean could be due to a lack of natural enemies. Future studies should evaluate predation and parasitism levels on redbanded stink bug adults, nymphs, and egg clusters. Since oviposition is more frequent in the lower plant canopy, nymphs are less likely to be collected in sweep net samples taken from the upper plant canopy. Future studies should evaluate other sampling methods such as the shake sheet (beat cloth) protocol to determine the optimal sampling strategy for this pest. If a sweep net is used, samples should be taken throughout the canopy and especially in the lower plant canopy of indeterminate soybean varieties which initially set pods in the lower portions of the canopy.

Redbanded stink bug adults and nymphs appear to prefer the lower plant canopy where they are less likely to encounter insecticide residues. To compound this issue, many producers, in the interest of saving time and fuel costs, currently apply lower volumes of water with insecticide applications to increase treatable areas. Studies in Brazil suggest that chemical

control of redbanded stink bug is greatly enhanced when insecticide application volumes are increased (Maziero et al 2009).

Currently, the action threshold for redbanded stink bug in Louisiana is six insects/25 sweeps (Baldwin 2009). This trigger is based on reports from Brazil where the current action threshold for this pest is two insects/one row m, which is lower than three insects/one row m currently used in Louisiana for native stink bugs (Anonymous 2011, Baldwin 2009). Redbanded stink bugs can build high population levels during a relatively short period in soybean and the tolerance of redbanded stinkbug to many recommended insecticides are also taken into consideration in the Louisiana action threshold (Temple et al. 2011 a, b). Currently, only a limited number of insecticides recommended at the highest use rates are used for control of this pest.

The redbanded stink bug has become a serious pest of soybean in a relatively short time in Louisiana. With limited alternative hosts available to redbanded stink bug populations during the summer months, populations remain concentrated in soybean fields and are capable of quickly building to economically important levels. This pest appears to favor early soybean production systems. In surveys of Louisiana soybean, redbanded stink bugs comprised the largest percentage of the stink bug complex in MG III (86%) and IV (60%) soybean cultivars. This may be due to the absence of other suitable hosts for the redbanded stink bug, while the green, southern green, and brown stink bugs have a wider availability of crop and non-crop hosts during the period when MG III and IV soybean are most susceptible. Additionally, these species may become more evenly distributed across the landscape leaving redbanded stink bug populations concentrated in soybean. Historically, Louisiana has planted later-maturing varieties (MG's V, VI, and VII) but began a transition to more of an early soybean production system

during the late 1990's to early 2000's (Heatherly 1999, Baur et al. 2000). Interestingly, this transition to early soybean production systems has coincided with the increase in pest status of the redbanded stink bug. Additional research is needed to fully understand the biology of redbanded stink bug in Louisiana soybean agro-ecosystems and to determine how this species became the dominant pest of soybean within a decade.

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CHAPTER 4

REDBANDED STINK BUG (*PIEZODORUS GUILDINII* (WESTWOOD) EFFECTS ON SOYBEAN YIELD, SEED QUALITY, AND DELAYED MATURITY

Introduction

Several important insect pests such as the velvetbean caterpillar, *Anticarsia gemmatalis* (Hübner), soybean looper, *Pseudoplusia 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); and a complex of stink bugs infest soybeans annually in the Southern United States (Funderburk et al. 1999). Among these, A complex of stink bug species including green stink bug, *Acrosternum hilare* (Say); southern green stink bug, *Nezara viridula* L.; and the brown stink complex, *Euschistus spp.*; infest soybean at various stages of development in the Southern U. S. and are the most economically important soybean pests in that region (Funderburk et al. 1999). In 2002, the redbanded stink bug, reached treatable levels (Baldwin 2005), and since has become the primary stink bug pest in Louisiana soybean (Paxton et al. 2007). The redbanded stink bug feeds on a wide range of cultivated and non-cultivated plant hosts, but has a particular affinity for legumes (Panizzi et al. 2000). In other countries, the redbanded stink bug causes severe economic damage in soybean, alfalfa, and other bean crops (Panizzi and Slansky 1985). McPherson et al. (1993) previously had reported the redbanded on soybean in the United States, but it was not considered an economical pest until recently.

Stink bugs feed by inserting their stylet and removing plant nutrients from either vegetative tissue or fruiting structures (Panizzi et al. 2000). This feeding may injure the plant, cause abortion of fruit or seed, and in some instances, vector diseases. Stink bugs are primarily attracted to soybean in the reproductive stages of development and prefer to feed on developing

seed pods (McPherson and McPherson 2000). Direct feeding by stink bugs and indirect disease transmission reduces yield and seed quality (McPherson and McPherson 2000). Stink bug feeding punctures on seed appear as minute dark spots, and seed that have been heavily damaged may be shriveled or distorted (Miner 1966, Todd 1976). Soybean seed with heavy to severe stink bug feeding have little to no value for oil, meal, or plant seed (Todd 1976, 1981). Prices for soybean seed damaged by stink bugs may be reduced (Todd 1976, 1981) or heavy damage may prevent sale of the seed (McPherson et al. 1994). Numerous studies with the southern green stink bug have shown that feeding causes soybean yield and quality losses, decreases seed number, and seed weight, delays crop maturity, reduces seed oil content, increases seed protein levels, and reduces germination of harvested seed (Miner 1966, Duncan and Walker 1968, Jensen and Newsom 1972, Cherry 1973, Thomas et al. 1974, Todd and Turnipseed 1974, Todd 1976, Miller et al. 1977, McPherson et al. 1979, Russin et al. 1987, Brier and Rogers 1991).

Studies in Argentina have shown that the redbanded stink bug is their most damaging stink bug species (Vicentini and Jimenez 1977). Correa-Ferreira and Azevedo (2002) directly compared yield losses and seed damage among redbanded, southern green stink bug, and *Euchistus heros* (F.). In field trials (four stink bugs/three row ft), differences were not detected in yield among the species, but the redbanded stink bug damaged more seed compared to the southern green stink bug and *E. heros*. In greenhouse studies (two stink bugs/plant), lower yields were recorded in plants infested with redbanded stink bug compared to the southern green stink bug and *E. heros*. Plants infested with redbanded stink bugs also had fewer full pods (all three seed present) and significantly more empty pods compared to the other species (Correa-Ferreira and Azevedo 2002). Historically across the Southern U. S., southern green and green stink bugs were considered the most damaging species in soybean compared to other species (Miner 1961,

McPherson et al. 1979). However, no information is currently available comparing redbanded stink bug damage potential to southern green or green stink bugs in the United States.

An indirect effect of stink bug injury to soybean is an increase in the variability of plants developing to normal maturity with mature leaves and senescence. This delay in soybean maturity has been characterized as whole-fields or portions of fields that retain leaves, green stems, and/or green pods long after normal harvest date (Boethel et al. 2000). Delayed maturity of soybean plants in response to infestations of stink bug have been reported for southern green 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). Boethel et al. (2000) showed that southern green stink bug infestations between pod initiation (R3) and seed fill (R5) resulted in delayed maturity.

In Brazil, delayed maturity of soybean has been associated with redbanded stink bug. Panizzi et al. (1979) reported that soybean retained leaves when redbanded stink bugs infested soybean during pod initiation (R3) and pod fill stages (R6). Heinrichs (1976) reported excessive green foliage retention in Brazilian soybean when stink bug populations were \geq eight insects/three row ft during early seed development, but similar infestations at R6 (full seed) did not delay maturity. Two-to-five redbanded stink bug adults/plant caused excessive green leaf retention when infested at the R4 stage (full pod) (Costa and Link 1977). Continuous infestations (six to ten bugs/three row ft) of redbanded stink bug during seed development (R5) caused abnormal green leaf retention, but did not occur in soybean infested prior to seed development (R1-R3) (Galileo and Heinrichs 1978a). Sosa-Gomez and Moscardi (1995) investigated differences in leaf retention among different stink bug species and found that redbanded stink bug caused greater leaf retention than the southern green stink bug and *E. heros*.

The objective of this study was to characterize redbanded stink bug effects on soybean yield, seed quality, and delayed crop maturity.

Materials and Methods

These studies were conducted at the Louisiana State University Agricultural Center's Macon Ridge Research Station near Winnsboro, LA (Franklin Parish) during 2007 to 2009. Normal cultural practices and integrated pest management strategies recommended by the Louisiana Cooperative Extension Service were used to optimize plant development at the test sites (Levy et al. 2011).

Artificial Infestation Study. Soybean seed (Asgrow 6702 RR, Monsanto Co. St. Louis MO) were planted into a Gigger silt loam soil on 3 Jun (2007) and 1 Jun (2008). Plot size was one row (0.6 m) on 1-m centers. Translucent cages, each measuring 0.66 by 0.66 by 1.42 m and covered with a nylon screen (32 mesh / 2.54 linear cm), were placed over soybean plants (0.6 row m/10-12 plants) and infested with redbanded stink bug adults at selected soybean growth stages. Insects were collected from soybean fields with a standard (38 cm diameter) sweep net, held for 24h in insect cages (Bug Dorm, Megaview Co. Taiwan), and provided with a food source and distilled water to reduce mortality from physical injury and stress. One or two stink bugs were placed into a 20 ml scintillation vials and transported to the test site in a chilled cooler to alleviate any heat stress. Plots were treated with insecticides to reduce insect injury throughout the growing season except during infestation events. In 2007, plots were sprayed weekly with methyl parathion (1120 g AI/hectare: Methyl 4EC Cheminova Inc. Research Triangle Park, NC) and were not infested for 24-48 h after an insecticide application. In 2008, to further reduce the amount of native stink bug damage to plots, two insecticides were alternated every 7 d. These included lambda-cyhalothrin + thiamethoxam (81 g AI/hectare: Endigo 2.06

SC, Syngenta Crop Protection Greensboro, NC) and beta-cyfluthrin (25 g AI/hectare: Baythroid XL Bayer CropScience Research Triangle Park, NC) + acephate (840 g AI/hectare: Orthene 90S Valent USA Corp. Walnut Grove, CA). In 2008, plots were not infested until at least 7 d after an insecticide application.

Treatments (infestation level and soybean growth stage) were arranged in a factorial RCBD with five replications. Infestation levels included 0, 1, 2, and 4 stink bugs/0.6 row m. Stink bugs were infested for 7 d in each of the following soybean growth stages: R4 (full pod), R5 (beginning seed), R6 (full seed), and R7 (beginning maturity) (Fehr et al. 1971). When the non-infested field plots reached physiological maturity (R8), a harvest aid (paraquat, Gramoxone Inteon, Syngenta Crop Protection Greensboro, NC) was applied. After 7d, all plots were evaluated for presence of plants with green stems, pods, and abnormal green leaf retention. Plots were hand harvested and threshed with a small bundle thresher (Almaco, Nevada, IA). Stink bug effects on yield (g), weight of 100 seed (g), percent pathogen infected seed (purple seed stain, *Cercospera kikuchii*, or white mold, *Phomopsis spp.*), and stink bug damaged seed. Samples of seed were graded for quality according to methods used by a local grain elevator (Raley Bros. Inc. Crowville, LA). Yields were discounted based on the USDA recommended damage assessments which included 1% for pathogen infected seed and 0.25% for stink bug damaged seed. Stink bug damaged seed, pathogen infested seed, green stem, pod, and leaf retention data for infested plots were corrected for injury within non-infested plots using Abbott's formula (Abbott 1925): $\{[(\% \text{ injury in infested treatment}) - (\% \text{ injury in non-infested treatment})] / [100 - \% \text{ injury in non-infested treatment}]\} \times 100$ (Willrich et al 2004). Seed yield data were normalized to standardize for differences between years. Stink bug infested plot yields were normalized to percent yield of the non-infested control plot (Ring et al. 1993). Data were analyzed using

PROC GLM and means separated according to Tukey's Studentized Range Test (SAS Institute 2003). Incidence of green pods and leaf retention in the experiments were low (<5%) in 2007 and 2008, and excluded from the analysis. All parameters were combined across both years of the studies for analysis.

Action Threshold Verification. A threshold verification study was conducted in 2009 using the currently recommended action threshold for stink bugs in Louisiana soybean. Recommended insecticide use strategies were used for full season control of stink bugs (Baldwin et al. 2009). Soybean seed (Asgrow 6702 RR, Monsanto Co., St. Louis, MO) were planted into a Gigger silt loam soil on 1 Jun (2009). Plots were sampled weekly from R1-R8 for stink bug infestations. Insecticide treatments were initiated when the number of stink bugs in the trial reached an action threshold six to nine insects per 25 sweeps depending on species (Baldwin et al. 2009). An action threshold of six stink bugs per 25 sweeps was used if > 50% of stink bugs sampled were redbanded stink bugs. An action threshold of nine stink bugs per 25 sweeps was used if > 50% was population was green, southern green or brown stink bugs. Stink bug species and life stage (adult or $\geq 2^{\text{nd}}$ instar nymph) were differentiated in the field for each sweep sample. Treatments (Table 4.1) consisted of single products, or a combination of insecticides each time an action threshold was reached. A non-treated control was also included. Plot size was 12 rows (1 m centers) by 15.3 m and arranged in a RCB with five replications. When field plots reached physiological maturity (R8), a harvest aid (paraquat, Gramoxone Inteon, Syngenta Crop Protection Greensboro, NC) was applied, and one week later plots were evaluated for presence of plants with green stems, pods, and abnormal green leaf retention.

Table 4.1. Threshold verification insecticide treatments, rates, and application timings, 2009.

Treatment	Form. ¹	Common Name	Rate ²	Application
Organophosphate	90SP	acephate ³	840	1 st , 2 nd , 3 rd
Pyrethroid	1EC	beta-cyfluthrin ⁴	5	1 st , 2 nd , 3 rd
Combination	2.06SC	*thiamethoxam+lambda-cyhalothrin ⁵	81	1 st
Combination	1EC	*beta-cyfluthrin ⁴ +	25	2 nd
	90SP	acephate ³	840	2 nd
Combination	2.06SC	*thiamethoxam+lambda-cyhalothrin ⁵	81	3 rd
Non-treated			-----	-----

¹Formulation: SP (soluble powder), EC (emulsifiable concentrate), SC (soluble concentrate).

²Grams active ingredient per hectare.

*Co-applications of two insecticides.

³Orthene (Amvac Corp.)

⁴Baythroid XL (Bayer CropScience)

⁵Endigo (Syngenta Crop Protection)

Plots were mechanically harvested (two samples/plot) with a small plot combine (Massey Ferguson/AGCO Corp. Duluth, GA) at maturity. Harvest data included: yield (kg/ha), 100 seed weight (g), stink bug-damaged seed (percent), and purple-stained seed (percent). Seed were graded for quality according to methods used by a local grain elevator (Raley Bros.Inc. Crowley, LA). Yields were discounted based on the USDA recommended damage assessments which included 1% for purple-stained seed and 0.25% for stink bug-damaged seed. Data were analyzed using PROC GLM and means separated according to Tukey's Studentized Range Test (SAS Institute 2003).

Results

Artificial Infestation Study. Differences were not detected between years for any of the results; therefore data were combined and analyzed across years. No significant interactions were detected with year, bugs infested, and growth stage for any of the parameters measured ($F=0.19-1.59$; $P=0.12-0.99$). No significant interactions were detected with year and bugs

infested for any of the parameters measured ($F=0.16-1.74$; $P=0.14-0.92$). No significant interactions were detected with year and growth stages for any of the parameters measured ($F=0.37-1.94$; $P=0.10-0.77$). No significant interactions were detected with bugs infested and growth stage for any of the parameters measured ($F=0.44-0.90$; $P=0.55-0.91$). Significant infestation level effects were detected for yield, weight of 100 seed, green stems, and damaged seed (Table 4.1). Significant infestation level effects were not detected for purple-stained seed or seed with white mold. Significant growth stage effects were detected for yield, weight of 100 seed, and green stems (Table 4.2). Significant growth stage effects were not detected for stink bug-damaged seed, purple-stained seed, or seed with white mold.

Yields ranged from 86.8% to 100.0% of the non-infested control and were significantly higher ($F=11.98$; $P<0.0001$) in the non-infested control (no bugs) compared to those treatments infested with one, two, or four bugs/0.6 row m (Table 4.2). Weights of 100 seed ranged from 15.1-15.7g, and were only significantly lower ($F=2.82$; $P=0.027$) from the non-infested control for the plots infested with 4 bugs/0.6 row m. Green stem frequencies ranged from 0.0% to 17.4% and were significantly higher ($F=11.08$; $P<0.0001$) in the plots infested with one, two, or four bugs compared to the non-infested control. Stink bug-damaged seed ranged from 0.0% to 6.9% and were significantly higher ($F=25.46$; $P<0.0001$) in the plots infested with one, two, or four bugs compared to the non-infested control. Normalized yields ranged from 89.4% to 95.1% for plots infested during the R4 to R7 growth stages. Yields were significantly lower ($F=4.03$; $P=0.009$) for R5 infestations compared to R7 infestations (Table 4.1). Weight of 100 seed ranged from 14.6 to 15.9 g and were significantly lower ($F=27.12$; $P<0.0001$) for plots infested during R6 and R7 growth stages compared to those infested during R4 and R5 growth stages. Green stems ranged from 1.6% to 19.4% and were significantly lower ($F=1.09$; $P=0.36$) for

treatments infested during R6 and R7 growth stages compared to those infested during R4 and R5 growth stages.

Parameters with significant main effect differences were further evaluated within each growth stage. Yield reduction for redbanded stink bug infestations during the R4 growth stage ranged from 4.7% to 10.7%. Reductions were significant ($F=3.12$; $P=0.043$) for plots infested with 4 bugs/0.6 row m (Figure 4.1). During R5, yield reductions in infested plots ranged from 9.3% to 16.1%. The reductions in all infested plots were significantly greater ($F=13.27$; $P<0.0001$). During R6, yield reductions in infested plots ranged from 10.8% to 14.1%. These reductions were only significant ($F=3.98$; $P=0.01$) for plots infested with 4 bugs/0.6 row m. During the R7 growth stage, yield reductions ranged from 2.7% to 10.7% but differences were not detected ($F=1.38$; $P=0.269$). Weight of 100 seed trended downward as redbanded stink bug infestation levels increased, but no differences ($F=0.50-2.55$; $P=0.076-0.68$) were detected within any of the soybean growth stages. Incidence of green stems in redbanded stink bug-infested plots during the R4 growth stage ranged from 22.3% to 24.3%. All infestations produced significant effects ($F=4.56$; $P=0.009$) (Figure 4.2). During R5, green stems ranged from 18.4% to 32.3% in the infested plots. All infestation levels again produced significant effects ($F=8.96$; $P<0.0001$). During R6, the incidence of green stems ranged from 4.7% to 16.3% in the infested plots, but only four bugs/0.6 row m produced a significant effect ($F=2.97$; $P=0.047$). During R7, green stems were low (1.3% to 2.9%) in infested plots and no significant effects were detected ($F=0.52$; $P=0.69$).

Table 4.2. Effects of redbanded stink bug infestation levels and growth stages of infestations on normalized yields, 100 seed weight, percentage of green stems, stink bug-damaged seed, purple-stained seed, and seed with white mold, 2007-2008.

Main Effects	Normalized	100 seed	% Green	% Stink Bug	% Purple	% White
Bugs	Yield¹	Weight²	Stems	Damaged Seed	Seed Stain	Mold Seed
0	100.0a	15.7a	0.0b	0.0b	0.0a	0.0a
1	92.3b	15.4ab	14.0a	5.7a	0.3a	0.3a
2	91.1b	15.3ab	15.8a	6.2a	0.5a	0.3a
4	86.8b	15.1b	17.4a	6.9a	0.5a	0.2a
<i>F; P; df = 3, 140</i>	11.98; <0.0001	2.82; 0.027	11.08; <0.0001	25.46; <0.0001	1.09; 0.36	2.10; 0.08
Growth Stage						
R4	93.9ab	15.9a	19.4a	4.1a	0.6a	0.2a
R5	89.4b	15.7a	18.4a	5.9a	0.5a	0.3a
R6	89.6ab	14.6b	5.9b	5.6a	0.4a	0.2a
R7	95.1a	14.9b	1.6b	4.3a	0.4a	0.2a
<i>F; P; df = 3, 140</i>	4.03; 0.009	27.12; <0.0001	18.54; <0.0001	2.52; 0.06	0.99; 0.40	1.02; 0.38

¹Percent yield as a function of that in the non-treated control.

²Grams.

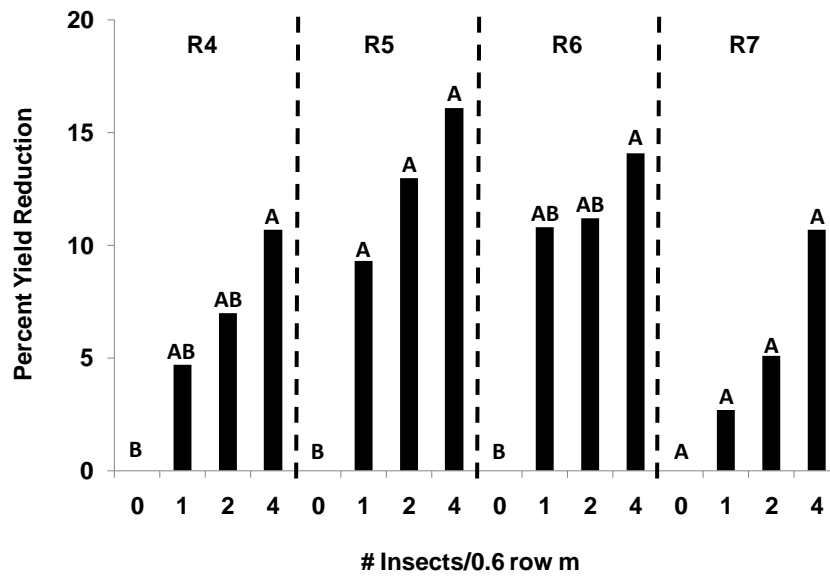


Figure 4.1. Yield reductions from redbanded stink bug infestations during R4 to R7 soybean growth stages, 2007-08.

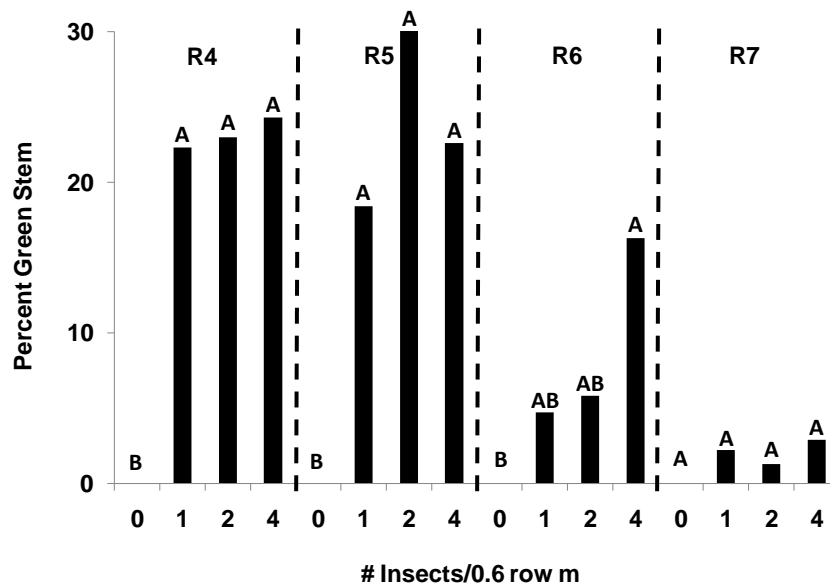


Figure 4.2. Incidence of green stems in redbanded stink bug infested plots during the R4 to R7 soybean growth stages, 2007-08.

Stink bug-damaged seed in the infested plots ranged from 4.1% to 5.8% during R4 but only four bugs/ row m produced a significant effect ($F=5.01$; $P=0.006$) (Figure 4.3). During R5, all infestation levels produced significantly higher ($F=17.24$; $P<0.0001$) frequencies of stink bug-damaged seed (6.3% to 8.1%). For R6, stink bug-damaged seed ranged from 6.0% to 7.8%, and all infestation levels produced significant effects ($F=8.32$; $P<0.0003$). During R7, stink bug-damaged seed ranged from 3.5% to 6.8% in the infested plots but only four bugs/0.6 row m produced significant effects ($F=4.06$; $P=0.015$).

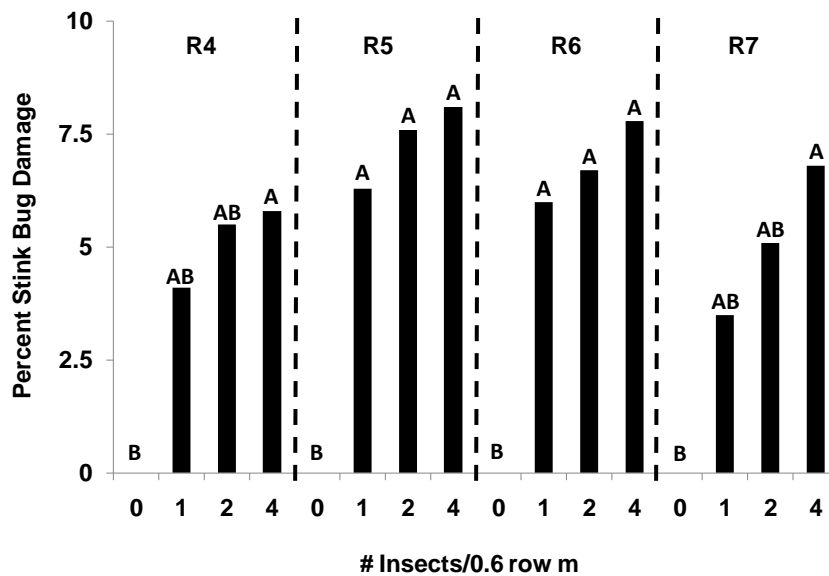


Figure 4.3. Incidence of stink bug damaged seed at selected infestation levels during the R4 to R7 soybean growth stages, 2007-08.

Action Threshold Verification. Stink bug infestations during the study were high with populations peaking at nearly 50 stink bugs/25 sweeps in the non-treated control (Figure 4.4). Three applications were used for all of the insecticide treatments. Stink bugs species in this study included redbanded stink bug, green stink bug, southern green stink bug, and brown stink bug. Prior to the first insecticide application (Aug 21), 72% of the population was redbanded

stink bug and the action threshold of 6 bugs/25 sweeps was used. Insecticide sprays were initiated on Aug 21 when stink bug densities averaged 5.8 to 7.8 insects/25 sweeps (Table 4.3). Post treatment (Aug 27) stink bug numbers after the first application ranged from 0.6 to 3.0 insects/25 sweeps compared to 6.4 insects/25 sweeps in the non-treated control. After the initial application, >90% of the stink bugs remaining during the rest of the trial were redbanded stink bugs. The second applications were made on Sep 3 when stink bug numbers in the insecticide-treated plots exceeded the action threshold (6.6 to 8.8 insects/25 sweeps). The second applications were made on Sep 3 when stink bug numbers in the insecticide-treated plots exceeded the action threshold (6.6 to 8.8 insects/25 sweeps).

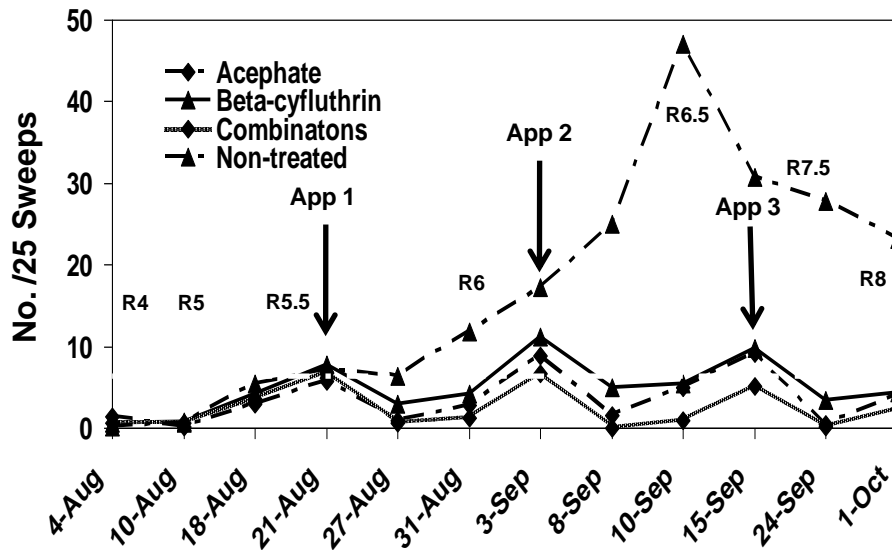


Figure 4.4. Stink bug populations in insecticide-treated and non-treated plots during R4 to R8 growth stages, 2009.

Post-treatment (Sep 8) stink bug numbers after the second treatment ranged from 0.0 to 5.0 insects/25 sweeps in the insecticide-treated plots and were significantly lower than the non-treated control (25.0 insects/25 sweeps). The action threshold was exceeded (5.9 to 9.8) again in all insecticide treatments on Sep 15, and the third insecticide treatment was made. Post-treatment (Sep 24) stink bug numbers after the third application ranged from 0.4 to 3.4 insects/25

sweeps in the insecticide-treated plots and were significantly lower than the non-treated control (27.8 insects/25 sweeps). All insecticide treatments at each application timing initially reduced stink bug numbers below the action threshold. Seasonal mean stink bug numbers ranged from 2.4 to 5.0 insects/25 sweeps in the insecticide-treated plots which were significantly lower than the non-treated control (16.9 insect/25sweeps).

Yields in the insecticide-treated plots ranged from 2284.0 kg/ha to 2760.9 kg/ha and were significantly higher than the non-treated control (1276.6 kg/ha) (Table 4.4). The combination treatment yielded significantly higher than the beta-cyfluthrin treatment. Weights of 100 seed ranged from 19.2 to 20.0 g in the insecticide-treated plots and were significantly higher than the non-treated control (14.9 g). Stink bug-damaged seed ranged from 26.0% to 38.0% in the treated plots and were significantly lower than the non-treated control (82.1%). The combination treatment had a significantly lower percentage of stink bug-damaged seed compared to the other two insecticide regimes. The incidence of purple-stained seed ranged from 0.4% to 2.3% among insecticide treatments and were significantly lower than the non-treated control (4.2%). Purple-stained seed was significantly lower in the combination treatment compared to acephate and beta-cyfluthrin.

The percentage of green stems in insecticide treatments ranged from 20.7 to 35.4, but only the combination treatment was significantly lower than the non-treated control (43.6%). The percentage of plants with green pods in the insecticide treatments ranged from 1.0 to 1.9 and was significantly lower than the non-treated control (12.1%). Green leaf retention in the insecticide-treated plots ranged from 11.7% to 18.9% which was significantly lower than the non-treated control (40.3%).

Table 4.3. Pre- and post-treatment stink bug infestations (no./25 sweeps) in three insecticide regimes, 2009.

Treatments Applications	Pre-Treatment One¹	Post-Treatment One²	Pre-Treatment Two³	Post-Treatment Two⁴	Pre-Treatment Three⁵	Post-Treatment Three⁶	Seasonal Mean⁷
Acephate	5.9a	1.0b	8.8a	1.6b	9.2b	0.4b	3.6b
Beta-cyfluthrin	7.8a	3.0ab	11.2a	5.0b	9.8b	3.4b	5.0b
Combination	6.8a	0.6b	6.6a	0.0b	5.9b	1.0b	2.4b
Non-treated	7.2a	6.4a	17.2a	25.0a	30.8a	27.8a	16.9a
<i>F; P</i>	0.46; 0.71	10.70; 0.001	2.21; 0.13	25.27; <0.001	27.72; <0.001	13.06; 0.004	42.05; <0.001
df	3,12	3,12	3,12	3,12	3,12	3,12	3,12

Sample date: ¹Aug 21, ²Aug 27, ³Sep 3, ⁴Sep 8, ⁵Sep 15, ⁶Sep 24

⁷Seasonal mean number of stink bugs (25 sweeps) from R4 to R8 (12 sampling dates).

Table 4.4. Effects of stink bugs on yield, weight of 100 seed, damaged seed, purple stained seed, green stems, green pod, and green leaf retention in three insecticide regimes, 2009.

Treatments	Discounted yield¹	100 seed weight²	% stink bug-damaged seed	% Purple-stained seed	% Green stem	% Green pod	% Green leaf
Acephate	2475.7ab	19.2a	37.6b	1.9b	35.4ab	1.9b	18.9b
Beta-cyfluthrin	2284.0b	19.4a	38.0b	2.3b	28.6ab	1.1b	13.2b
Combination	2760.9a	20.0a	26.0c	0.4c	20.7b	1.0b	11.7b
Non-treated	1276.6c	14.9b	82.1a	4.2a	43.6a	12.1a	40.3a
<i>F; P</i>	45.73; <0.0001	79.94; <0.0001	63.84; <0.0001	14.97; <0.0001	4.18; 0.03	4.25; 0.03	8.57; 0.002
df	3,27	3,72	3,72	3,72	3,12	3,12	3,12

¹Yields (kg/ha) corrected for dockage from stink bug-damaged seed and purple-stained seed.

²Grams.

Discussion

In the artificial infestation study, redbanded stink bug infestations reduced soybean yield and quality and increased incidence of green stems. Redbanded stink bug infestations during R4-R7 caused a reduction (2.7-16.1%) in yield. The R5 growth stage appeared to be the most sensitive to yield effects. During R5, one and two redbanded stink bug/0.6 row m significantly reduced yields by 9.1% and 13%, respectively. Similar results were observed by Galileo and Heinrichs (1978 b,c,d), during the R5 growth stage, where two stink bugs/0.5 row m significantly reduced yields 14% to 33%. Current action thresholds in Louisiana soybean for southern green, green, and brown are two stink bugs/0.6 row m or nine stink bugs/25 sweeps (Baldwin et al. 2009). Results from the current study suggest the action threshold should be reduced for redbanded stink bug to one to two insects per 0.6 row m depending on growth stage. The higher damage potential of redbanded stink bug compared to southern green stink bug has been substantiated in several studies. Results from Brazil showed that redbanded stink bugs damaged more seed and further reduced yields in artificial infestations compared to southern green stink bug (Correa-Ferreira and Azevado 2002). Moore et al. (2011 Unpublished data) have reported that redbanded stink bug has a higher damage potential when caged on individual pod clusters compared to other native stink bugs in Louisiana soybean.

In the current study, all stink bug infestation levels significantly increased the incidence of green stems during the R4 and R5 growth stage. Significant increases in green stems were also detected during R6 at the highest infestation level (four insects/ 0.6 row m). Incidences of green pods and abnormal green leaf retention were very low in

these experiments and may have been related to a short infestation period (7 d). Leaf retention, presence of green stems, and/or green pods long after normal harvest date in response to stink bug infestations have been reported across the Southern U. S. (Daugherty et al. 1964, Duncan and Walker 1968, Todd and Turnipseed 1974, Boethel et al. 2000). In studies by Boethel et al. (2000), soybean was most sensitive to delayed maturity from southern green stink bug infestations from R3 through R5, which is similar to what was observed in the current study with redbanded stink bug. In Brazil, delayed maturity from redbanded stink bug has been reported in the R3 to R6 growth stages (Heinrichs 1976, Costa and Link 1977, Galileo and Heinrichs 1978a, Panizzi et al. 1979).

The amount of stink bug-damaged seed in infested plots was also highly variable and ranged from 3.5% to 8.1%. All stink bug infestations (one, two, and four insects/ 0.6 row m) had significantly higher stink bug-damaged seed compared to the non-infested control at both R5 and R6 growth stages. During the R4 and R7 growth stages, only the highest infestation level (four insects/0.6 row m) significantly increased the number of stink bug damaged seed compared to the non-infested control. The incidence of purple-stained seed and seed with white mold were low across these trials (< 0.5%) and no significant differences were detected between the non-treated control and the infested treatments. Numerous studies have reported that southern green stink bug feeding in soybean increases the incidence of seedborne pathogens (McPherson and McPherson 2000, Russin et al. 1988). Increases in seedborne pathogens were not detected in the current study from infestations of redbanded stink bug, but several factors could have affected these results including short infestation duration, environmental conditions, and the use of preventative foliar fungicides.

Currently the action threshold for redbanded stink bug in Louisiana is six insects/25 sweeps. The current action threshold in Brazil for this pest is two insects/one row m and is lower than the three insects/one row m currently used in Louisiana for native stink bugs (Anonymous 2011, Baldwin et al. 2009). Also taken into consideration in the Louisiana action threshold are reports that redbanded stink bug can build to high populations in soybean within a relatively short time and that this pest is less susceptible to recommended insecticides used to control stink bugs in soybean (Temple et al. 2011 a, b).

The second experiment evaluated three insecticide regimes including a pyrethroid, organophosphate, and a combination of a pyrethroid with either an organophosphate or a neonicotinoid. Chemical control strategies are the primary tool used to manage stink bugs in soybean. Prior to the redbanded stink bug becoming a pest, it was relatively easy to manage stink bugs in Louisiana soybean with low to mid-rates of pyrethroids or organophosphates (Willrich et al. 2000, Fitzpatrick et al. 2001a, Fitzpatrick et al. 2001b, Fitzpatrick et al. 2002). Nearly 75% of the stink bugs captured prior to the first insecticide application were redbanded stink bugs, while over 90% of those captured after the first insecticide application were redbanded stink bug. Three insecticide applications were necessary in all three spray regimes and an action threshold of six insects/25 sweeps was utilized because the redbanded stink bug was the predominant pest.

Stink bugs significantly reduced yield and seed weights, but increased the frequency of stink bug-damaged seed, purple-stained seed, green pods, and abnormal retention of green leaves in the non-treated control compared to the insecticide treatments. All three insecticide strategies reduced stink bug numbers below the action

threshold after each insecticide application. No significant differences were detected in the number of stink bugs among insecticide treatments for any sample or seasonal mean number of stink bugs. The combination treatment consistently had numerically lower numbers of stink bugs than the other two insecticide regimes. This may explain why the combination treatment yield was statistically higher, and had fewer stink bug damaged seed and purple stained seed compared to the pyrethroid (beta-cyfluthrin) treatment. Although the pyrethroid treatment reduced stink bug numbers below the action threshold after each application, the seasonal mean number of stink bugs was 5.0 insects/25 sweeps. Previous research on redbanded stink bug susceptibility to insecticides in Louisiana soybean has shown that pyrethroids do not provide the same level of control as the organophosphate (acephate) or combinations of products (Temple et al. 2011b).

Further research needs to examine redbanded stink bug effects on soybean yield and quality. That work should focus on the effects of sub-threshold levels (< six/25 sweeps) of red banded stink bug on damage potential of this pest compared to native stink bugs. This is the first study in the United States. to attempt to quantify yield loss, seed injury, and delayed maturity with the redbanded stink bug. These studies support the current action threshold (6 insects/25 sweeps) being used in Louisiana soybean for redbanded stink bug and is similar to the one currently used in Brazil (Anonymous 2011). A second threshold validation experiment was conducted in 2010 at two locations, but stink bug populations never reached an action threshold level. Additional research is needed to fully understand the biology of redbanded stink bug in Louisiana soybean agro-ecosystems and to determine how this species became the dominant pest of soybean in only a decade.

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CHAPTER 5

INSECTICIDE SUSCEPTIBILITY OF SOUTHERN GREEN STINK BUG, *NEZARA VIRIDULA* (L.), AND REDBANDED STINK BUG, *PIEZODORUS GUILDINII* (WESTWOOD) (HEMIPTERA: PENTATOMIDAE), IN SOYBEAN FIELD TRIALS AND LABORATORY BIOASSAYS

Introduction

Soybean, *Glycine max* (L.) Merrill, is the primary oilseed crop consumed in the world (Wilcox 2004). In 2007, the total global planted area and production of soybean were 90.1 million hectares and 220.5 million tons, respectively. The United States, Brazil, Argentina, China, and India are the major soybean-producing countries (Singh 2010). The U.S. is the global leader in soybean production with ≈ 31 million hectares, producing ≈ 99 million metric tons during 2010 (NASS 2011). Louisiana ranked 18th in total production among 31 soybean-producing states with a total grain yield of over 1.25 million metric tons in 2010 (NASS 2011).

In the United States, soybean are attacked by a multitude of insect guilds including those pests that defoliate, or feed on phloem or seed (O'Neal and Johnson 2010). Stink bugs are key soybean pests in production systems across southern United States (Funderburk et al. 1999). The annual impact of the stink bug complex (Hemiptera: Pentatomidae) on soybean yield and quality across the South is a combination of economic losses from reduced seed quality, direct yield losses, and chemical control costs (Funderburk et al. 1999). A complex of stink bugs infests soybean annually, with the predominant species being the green stink bug, *Acrosternum hilare* (Say); southern green stink bug, *Nezara viridula* L.; and the brown stink bug, *Euschistus servus* (Say) (Funderburk et al. 1999). The southern green stink bug historically has been the predominant pentatomid pest within this complex (McPherson et al. 1979, Drees and

Rice 1990, McPherson et al. 1993, Boyd et al. 1997, Gore et al. 2006, and Smith et al. 2009). In 2002, an emerging pest, the redbanded stink bug, *Piezodorus guildinii* (Westwood); reached action threshold levels across South Louisiana (Baldwin 2005). Since then, it has become the predominant stink bug infesting Louisiana soybean (Temple et al. 2011). The redbanded stink bug is a significant annual soybean pest in South America and ranges from Argentina north to the Southern United States (Panizzi and Slansky 1985, McPherson and McPherson 2000). In the United States, redbanded stink bug was first reported in Florida during the 1960's. Later it was reported in several other states including South Carolina, Georgia, and New Mexico, but was never considered an economical and common soybean pest in these states (McPherson and McPherson 2000).

The expansion of this insect's range has not been limited to Louisiana's borders. Since its discovery within the state of Louisiana, the population became more widespread across this state with each succeeding year. By 2006, it was reported in all Louisiana soybean production parishes. Redbanded stink bugs have now reached pest status in soybean in all states bordering Louisiana and have been reported as far north as Missouri and Tennessee (Stalcup 2007, Bailey 2009, Catchot 2009, Smith 2009, Smith et al. 2009). Chemical control strategies are the primary tool used to manage stink bugs in soybean. Prior to the redbanded stink bug becoming a pest; it was relatively easy to manage stink bugs in Louisiana soybean with low to mid-rates of pyrethroids or organophosphates (Willrich et al. 2000, Fitzpatrick et al. 2001a, Fitzpatrick et al. 2001b, Fitzpatrick et al. 2002). In 2000, the budget for Louisiana soybean insect pest management included a single insecticide application at a cost of \$13 /hectare (Bechtel 2000). In 2005, the

frequency for insect control in Louisiana soybean increased to two insecticide applications at a total cost of \$25 /hectare (Paxton 2005). Soybean producers, agricultural consultants, and county extension agents have reported that the redbanded stink bug appears to be more difficult to control with the standard insecticides than southern green stink bug, frequently requiring more insecticide applications for season-long satisfactory management (Louisiana Agricultural Consultants Association, Louisiana Cooperative Extension Service personnel communication). In 2010, due to an increase in the pest status of redbanded stink bug, Guidry (2010) estimated that producers needed to budget three to five insecticide applications at a cost of \$75-112 /hectare in Louisiana soybean.

Differential susceptibility to insecticides is not uncommon among stink bug species. Several studies have reported differences in susceptibility among stink bug species to several classes of chemistry in field and laboratory experiments. For example, field trials conducted in Arkansas, Louisiana, and Texas showed that the southern green stink bug was consistently easier to control with pyrethroids and organophosphates compared to the brown stink bug (Emfinger et al. 2001, Gable et al. 2004, Greene et al. 2004, Way et al. 2005). For populations sampled in Arkansas and Mississippi, Snodgrass et al. (2005) reported that green and southern green stink bugs were more susceptible to several organophosphates and pyrethroids compared to the brown stink bug in laboratory studies. Similar differences for these species and insecticides also were reported in Louisiana (Emfinger et al. 2001, Willrich et al. 2003). In Arkansas, Greene (2007) demonstrated high levels (87 to 92%) of southern green stink bug mortality using topical applications of pyrethroids. However, brown stink bug mortality was much lower (12 to

60%) for the same insecticides. Mortalities between these species were similar when subjected to selected organophosphates.

The available information for baseline insecticide toxicity to redbanded stink bug in laboratory trials from its native range in South America is limited. Stadler et al. (2006) reported differential susceptibility of redbanded stink bugs from several different regions of Argentina to endosulfan, but made no direct comparisons of toxicity with other species or compounds. Guillen and Foerster (1978) reported that methyl parathion and endosulfan caused high mortality to southern green and redbanded stink bugs in Brazil field trials. The current labeled insecticides used to control redbanded stink bugs in Brazilian soybean include organophosphates (acephate, methamidophos, and trichlorform), carbamate (carbaryl), organochlorine (endosulfan), and neonicotinoid + pyrethroid pre-mix (thiomethoxam + lambda-cyhalothrin) (Anonymous 2011). Baur et al. (2010) reported baseline toxicity data for Louisiana redbanded stink bugs to several insecticides including acephate, cypermethrin, and methamidophos in glass vial bioassays. That work also demonstrated initial field efficacy data for control of redbanded stink bug in soybean, but made no direct comparisons to other stink bugs. Control of redbanded stink bugs with all insecticides was estimated at only \approx 50-80% in those Louisiana field trials tested. Therefore, the objective of this study was to determine the susceptibility of Louisiana populations of southern green and redbanded stink bugs to selected insecticides in soybean field trials and laboratory bioassays. These tests represent ongoing trials to further establish species susceptibility to a range of insecticides, validate performance of common products, and identify new insecticides that can be used effectively against redbanded stink bug.

Materials and Methods

Field Trials. Field trials were conducted at the Louisiana State University (LSU) Agricultural Center's Macon Ridge Research Station near Winnsboro, LA (Franklin Parish) from 2005-2009 (Figure 5.1).

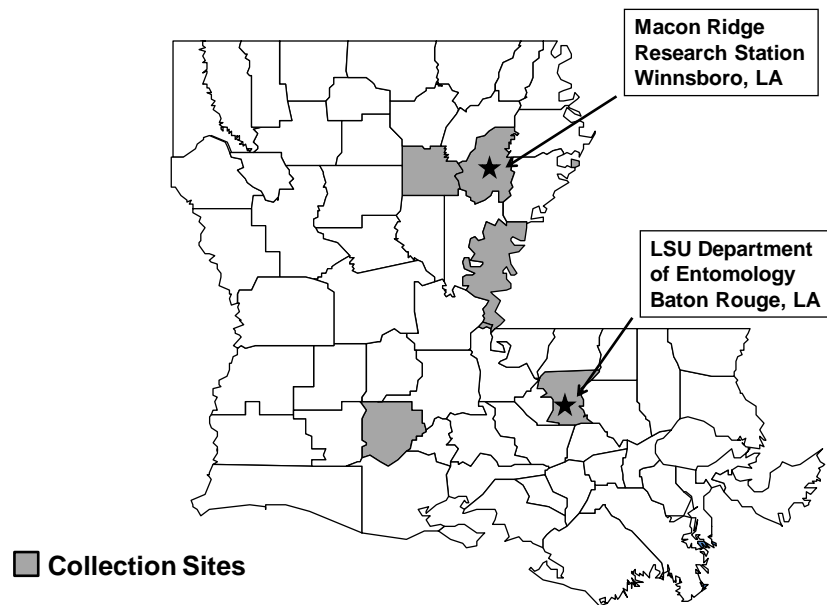


Figure 5.1. Field trial and laboratory locations and sites for insect collections.

Efficacy of 20 insecticides or insecticide combinations in selected formulations and rates were evaluated against redbanded and southern green stink bug in 18 field trials during 2005-2009 (Table 5.1). The sample sizes (replicates and trials) for individual products ranged from eight to fifty-four. A non-treated control was included in all trials to confirm stink bug infestation levels during the sample periods. The general methods and experimental procedures for measuring insecticide efficacy were similar among all trials. Soybean seeds in each trial were planted during the LSU AgCenter recommended planting dates and managed according to best agronomic practices (Levy et al. 2011). Soybean varieties from three maturity groups (IV, V, and VI) were planted in plots that

Table 5.1. Insecticide formulations, rates, and total replicates from field trials, 2005-2009.

Compound	Trade Name	Company	Class (IRAC MOA ^c)	Rate ^a	N ^b
Acephate	Orthene 90S	Amvac Chemical Corp. Los Angeles, CA	Organophosphate (1B)	840.3-1120.4*	54
Beta-cyfluthrin	Baythroid XL 1EC	Bayer CropScience Research Triangle Park, NC	Pyrethroid (3A)	24.6*	36
Bifenthrin	Brigade 2EC	FMC Corp. Philadelphia, PA	Pyrethroid (3A)	89.6-112.0*	49
	Discipline 2EC	Amvac Chemical Corp. Los Angeles, CA			
Bifenthrin + Imidacloprid	Brigadier 2SC	FMC Corp. Philadelphia, PA	Pyrethroid (3A) + Neonicotinoid (4A)	89.6-134.4*	16
Cyfluthrin	Baythroid 2EC	Bayer CropScience Research Triangle Park, NC	Pyrethroid(3A)	49.2*	16
Cyfluthrin + Imidacloprid	Leverage 2.7SC	Bayer CropScience Research Triangle Park, NC	Pyrethroid (3A) + Neonicotinoid (4A)	89.6*	20
Gamma-cyhalothrin	Declare 1.25EC	Cheminova Inc.	Pyrethroid (3A)	14.0-16.8*	16
Imidacloprid	Trimax Pro 4.44F	Bayer CropScience Research Triangle Park, NC	Neonicotinoid (4A)	52.3*	8
Lambda-cyhalothrin	Karate-Z 2.08SC	Syngenta Crop Protection Greensboro, NC	Pyrethroid (3A)	33.6*	24
Lambda-cyhalothrin + Thiamethoxam	Endigo 2.06SC	Syngenta Crop Protection Greensboro, NC	Pyrethroid (3A) + Neonicotinoid (4A)	81.1*	36
Methyl Parathion	Methyl 4EC	Cheminova Inc. Durham, NC	Organophosphate(1B)	1120.4*	12
Thiamethoxam	Centric 40WG	Syngenta Crop Protection Greensboro, NC	Neonicotinoid (4A)	70.0*	12
Zeta-cypermethrin	Mustang Max 0.8EC	FMC Corp. Philadelphia, PA	Pyrethroid (3A)	28*	20
Zeta-cypermethrin + Bifenthrin	Hero 1.24EC	FMC Corp. Philadelphia, PA	Pyrethroid (3A)	84.1-112.0*	12

^aGrams Active Ingredient/Hectare

^bSample size (Trials x Replicates)

* Highest currently allowable use rates in soybean for a single application

S (soluble powder), EC (emulsifiable concentrate), SC (soluble concentrate), F (flowable), WG (wetable granule).

^cInsecticide Resistance Action Committee Mode of Action.

consisted of four to eight rows (centered on 1 m) and 12-16 m in length. Treatments were placed in a RCB design with four to five replications. IPM strategies recommended by the Louisiana Cooperative Extension Service were used to optimize plant development and manage non-target insects across the test sites. Treatments were applied when stink bug levels exceeded the action threshold (9/25 sweeps) (Baldwin et al. 2009). All treatments were applied with either a high clearance sprayer with a CO₂-charged spray system calibrated to deliver 56.8 liters/hectare through TeeJet® TX-8 hollow cone nozzles (2/row) at 31.6 kg/mm² or a tractor mounted CO₂-charged spray system calibrated to deliver 94.6 liters/hectare through TeeJet® TX-8 hollow cone nozzles (2/row) at 33.7 kg/mm². Insecticide efficacy against stink bugs was determined using a standard sweep net (38 cm diameter) and taking 25 sweeps in each plot. Depending on the trials, plots were sampled at two-three and six-eight days after treatment (DAT). The results for each treatment in a specific trial were converted to percent control relative to post-treatment infestations remaining in the non-treated control using Abbott's formula (Abbott 1925). Means (\pm standard error) across all trials along with the lowest and highest relative control levels were combined from the two sampling dates and are reported for each insecticide treatment. Insecticide treatments that provided $\geq 80\%$ control were considered acceptable. This efficacy level was considered as the minimum level that would be provide satisfactory control for a given insecticide.

Laboratory Bioassays. Laboratory bioassays were either conducted at the Macon Ridge location or at the LSU Agricultural Center's Department of Entomology in Baton Rouge, LA (East Baton Rouge Parish) from 2004-2010 (Figure 1). Adult vial test (AVT) bioassays similar to those described by Plapp et al. (1987), Willrich et al. (2003),

and Snodgrass et al. (2005) were used to determine the susceptibility of southern green and redbanded stink bug adults to six selected insecticides. The insecticides included four pyrethroids (bifenthrin, cyfluthrin, cypermethrin, and lambda-cyhalothrin) and two organophosphates (acephate and methamidophos). Stock solutions of insecticides were prepared by dissolving technical grade insecticides (Chem Services, West Chester, PA) in a solution of acetone. Serial dilutions were generated from the stock solution to yield desired concentrations. Concentrations used in the AVT procedure ranged from 0.01 µg/vial to 20 µg/vial depending on insecticide. The interior surface of 20 ml glass scintillation vials were coated with 0.5 ml of the appropriate insecticide solution. Uncapped vials were then rotated on a modified hot dog roller (Star Manufacturing International, St. Louis, MO) (heating element disconnected) until all acetone solution had evaporated leaving only the insecticide residue. Pyrethroid-treated vials were stored in a dark environment at room temperature and used within one month (Willrich et al. 2003). Organophosphate-treated vials were stored in the freezer and used within one month. Insects were collected (Figure 1) from crimson clover, *Trifolium incarnatum* L., and soybean with a sweep net. Insects were held for 24h in bug dorms (Megaview Science Co., Taiwan) and were provided with a food source (soybean pods or green beans) and distilled water to reduce mortality from physical injury and disease. One stink bug was placed into an insecticide-treated or non-treated (control) vial. Mortality was determined at four hours after exposure (HAE) and stink bugs were considered dead if they were unable to right themselves or there was no coordinated movement when prodded with a metal probe (Snodgrass et al. 2005). Two to three replicates (10-20 stink bugs per dose) were used for each species and insecticide. Data were corrected for

control mortality (Abbott, 1925) and analyzed with probit analysis using Polo-Plus (LeOra Software, 2007) to obtain dose mortality (LC₅₀) values. Non-overlapping confidence limits (95%) were used to detect significant differences between species and insecticides.

Results

Field Trials. The efficacies of all insecticides ranged from 40 to 100% (mean=93.6 ± 1.0%) control for southern green stink bug and 22 to 100% (mean= 79.2 ± 1.3%) control for redbanded stink bug. Neonicotinoids demonstrated 78.0 ± 5.6% and 63.2 ± 6.0% mean control of southern green and redbanded stink bugs, respectively (Figure 5.2).

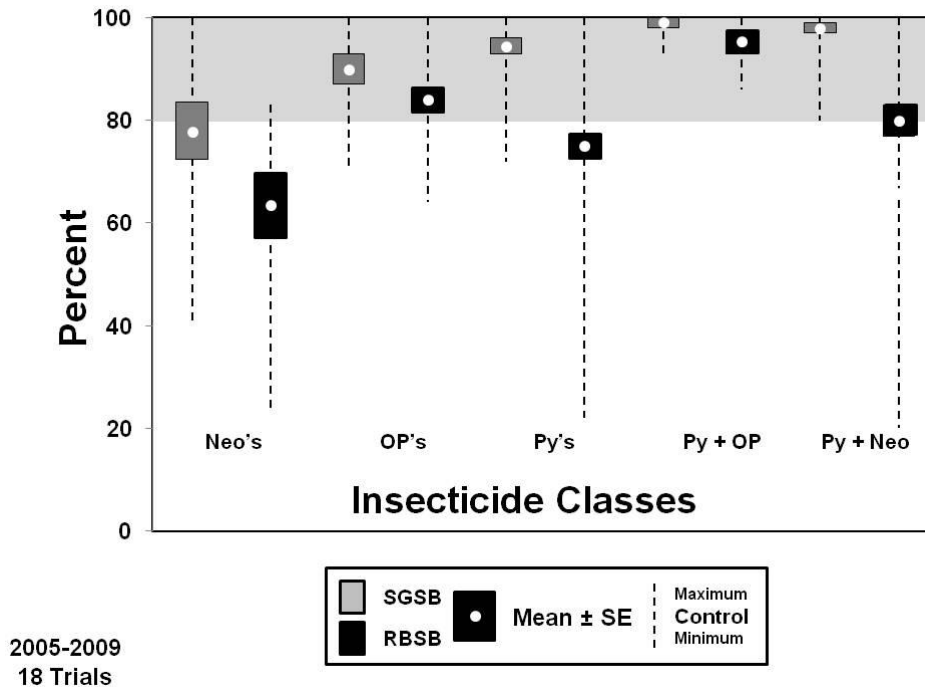


Figure 5.2. Control of southern green (SGSB) and redbanded stink bug (RBSB) with neonicotinoids (Neo's), organophosphates (OP's), Pyrethroids (PY's), and insecticide combinations in Louisiana soybean averaged across years and insecticide classes, 2005-2009.

The relative control levels for southern green and redbanded stink bugs with neonicotinoids ranged from 40-100% and 24-83%, respectively. Southern green stink bug control levels with imidacloprid ($70.8 \pm 8.8\%$) and thiamethoxam ($80.4 \pm 6.9\%$) were relatively higher compared to that for redbanded stink bug, but only thiamethoxam provided acceptable control of southern green stink bug in these trials (Figure 5.3).

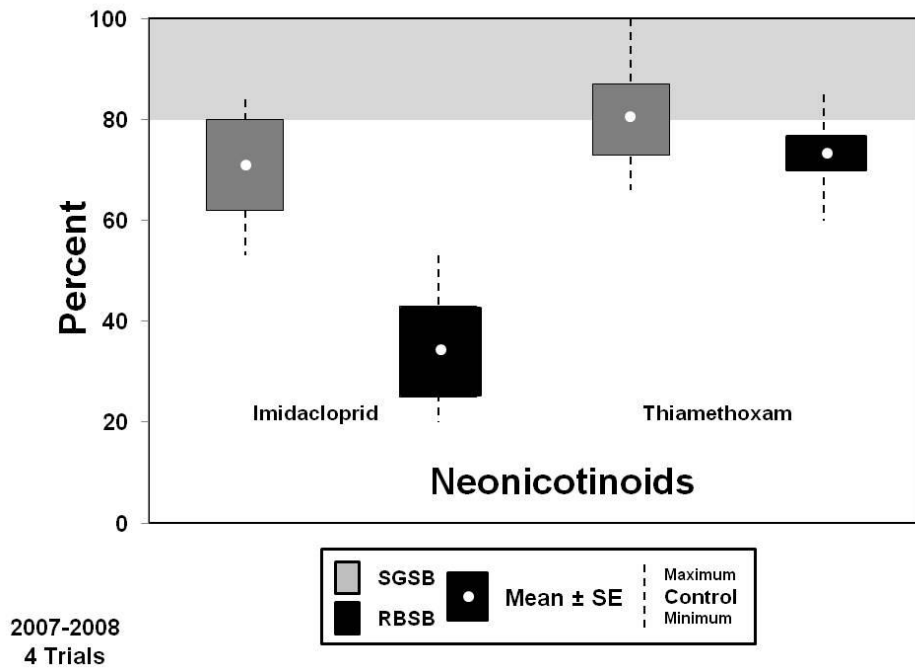


Figure 5.3. Control of southern green (SGSB) and redbanded stink bug (RBSB) with neonicotinoids in Louisiana soybean averaged across years, 2005-2009.

Neither of the neonicotinoids, imidacloprid ($34.3 \pm 9.3\%$) or thiamethoxam ($72.8 \pm 3.5\%$), provided acceptable levels of control for the redbanded stink bug in these trials. Mean control of southern green and redbanded stink bugs with organophosphate insecticides was $89.8 \pm 2.7\%$ and $84.8 \pm 1.7\%$, respectively (Figure 5.2). The relative control levels for southern green and redbanded stink bugs with organophosphates ranged from 55-100% and 64-100%, respectively. Acephate provided acceptable levels of

control against the southern green stink bug ($96.9 \pm 1.0\%$) and redbanded stink bug ($86.0 \pm 2.4\%$) in these trials (Figure 5.4), respectively. Methyl parathion provided acceptable levels of control for both the southern green stink bug ($90.9 \pm 6.3\%$) and redbanded stink bug ($82.2 \pm 3.8\%$) in these trials, respectively.

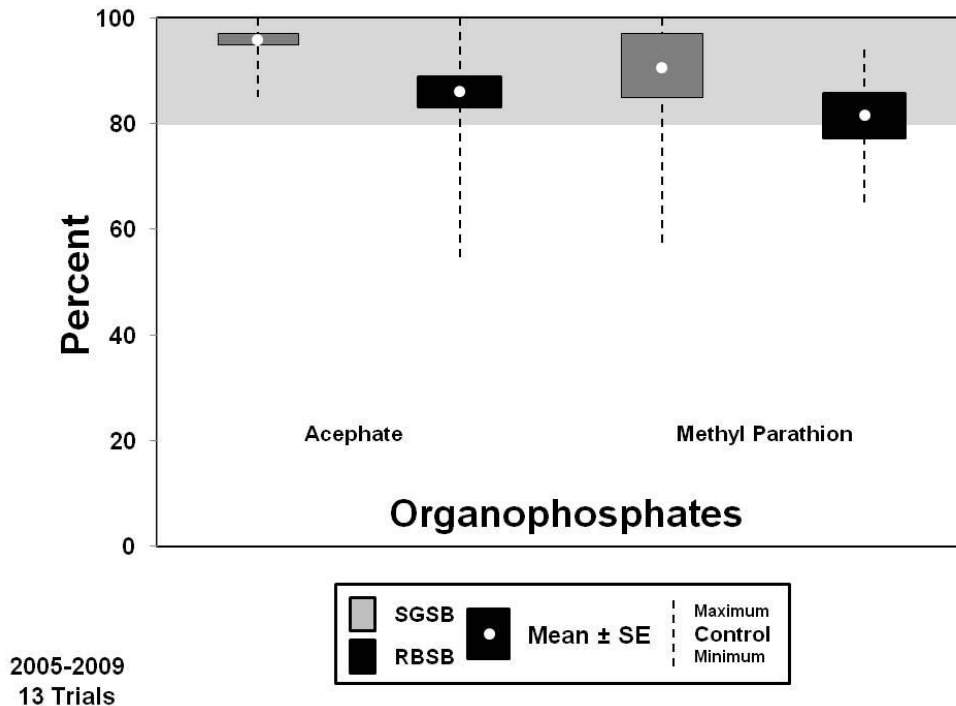


Figure 5.4. Control of southern green (SGSB) and redbanded stink bug (RBSB) with organophosphates in Louisiana soybean averaged across years, 2005-2009.

Pyrethroids provided $94.4 \pm 1.3\%$ and $75.1 \pm 1.9\%$ mean control of southern green and redbanded stink bug, respectively (Figure 5.2). The relative control of southern green stink bug and redbanded stink bugs with pyrethroids ranged from 22-100% and 72-100%, respectively. All pyrethroids provided >90% of southern green stink bug, but <80% control of redbanded stink bug (Figure 5.5). Gamma-cyhalothrin and cyfluthrin provided the highest levels of southern green stink bug control, 99.2 ± 0.8

and $97.3 \pm 0.9\%$, respectively. Cyfluthrin and zeta-cypermethrin provided the highest levels of control of redbanded stink bug, 79.9 ± 3.2 and $79.7 \pm 4.0\%$, respectively.

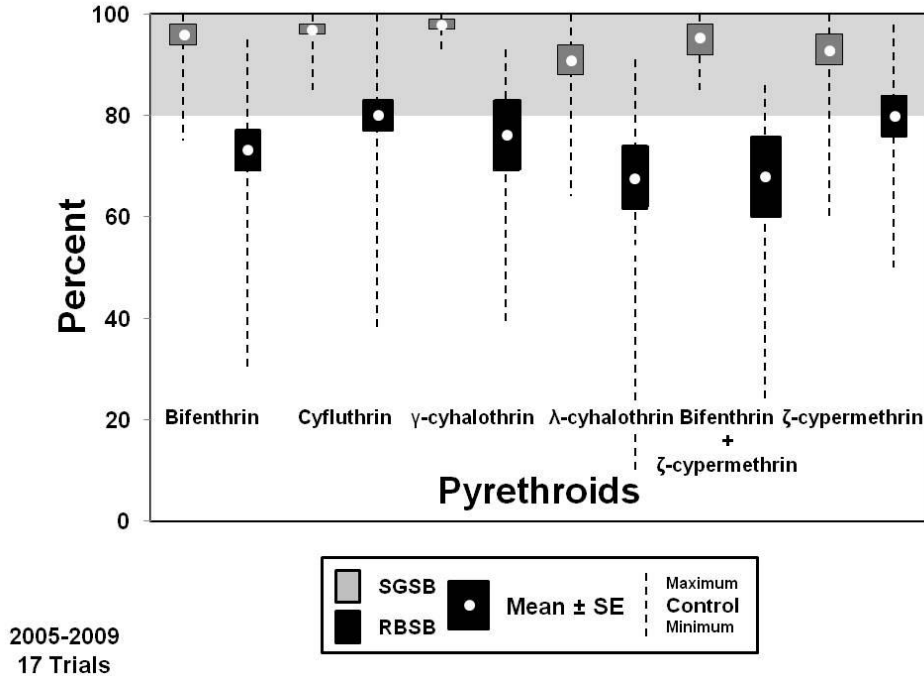


Figure 5.5. Control of southern green (SGSB) and redbanded stink bug (RBSB) with pyrethroids in Louisiana soybean averaged across years, 2005-2009.

The insecticide combinations of a pyrethroid + an organophosphate (cyfluthrin + acephate, bifenthrin + acephate, or gamma-cyhalothrin + methyl parathion) provided the highest efficacies for southern green stink bug ($99.5 \pm 0.5\%$) and redbanded stink bug ($94.7 \pm 1.7\%$) in these trials (Figure 5.2). The pyrethroid + neonicotinoid combinations (bifenthrin + imidacloprid, cyfluthrin + imidacloprid, and lambda-cyhalothrin + thiamethoxam) provided acceptable control of southern green stink bug ($98.1 \pm 0.8\%$) and redbanded stink bug ($80.1 \pm 2.9\%$).

Laboratory Bioassays. For southern green stink bugs exposed to organophosphates, LC_{50} 's ranged from 0.41 to 2.36 $\mu\text{g}/\text{vial}$ (Table 5.2). The LC_{50} 's for

southern green stink bugs exposed to pyrethroids ranged from 0.02 to 0.72 µg/vial. The pyrethroids, bifenthrin, cyfluthrin, and lambda-cyhalothrin, were more toxic to southern green stink bugs than the two organophosphates.

Redbanded stink bug responses were not significantly different between the organophosphates, acephate and methamidophos, and LC₅₀'s ranged from 1.87 to 4.86 µg/vial (Table 5.3). The LC₅₀'s for redbanded stink bugs exposed to pyrethroids ranged from 0.08 to 3.85 µg/vial. The pyrethroids, bifenthrin, cyfluthrin, and lambda-cyhalothrin, were more toxic to redbanded stink bugs than the two organophosphates. All insecticides were significantly more toxic to the southern green stink bug when compared to redbanded stink bugs within each year, insecticide class, and location. The redbanded stink bug was 7.3-fold less sensitive to acephate and 2 to 3.8-fold less sensitive to methamidophos compared to the southern green stink bug. For pyrethroids, the redbanded stink bug was less sensitive to bifenthrin (3.9 to 4.6-fold), cyfluthrin (4-fold), cypermethrin (4 to 8-fold), and lambda-cyhalothrin (7.5-fold) compared to southern green stink bug.

Discussion

This study provides baseline field and laboratory data on the current susceptibility of Louisiana southern green and redbanded stink bugs to pyrethroids and organophosphates. Results of field trials confirm reports from agricultural consultants and producers that the redbanded stink bug is less susceptible than the southern green stink bug to several insecticides. In field studies during 2003-2006, Baur et al. (2010), reported redbanded stink bug control levels of 81 and 69% for acephate and methyl parathion, respectively.

Table 5.2. Response of southern green stink bug adults to organophosphate and pyrethroid insecticides at 4 h after exposure in the adult vial test.

Class^a	Insecticide	Year	Collection site	N^b	LC₅₀^c	95% CL^d	Slope	χ^2^e
OP	Acephate	2004	Acadia	210	0.41	0.23 - 0.66	1.00+-0.15	4.15
OP	Methamidophos	2005	Concordia	210	0.59	0.38 - 0.89	2.62+-0.57	1.22
OP	Methamidophos	2008	Franklin	180	0.89	0.50 - 1.45	1.12+-0.20	3.82
OP	Methamidophos	2009	Franklin	180	2.05	1.58 - 2.59	2.55+-0.33	2.53
OP	Methamidophos	2010	East Baton Rouge	260	2.36	1.85 - 2.94	2.15+-0.23	2.08
PY	Bifenthrin	2009	Franklin	230	0.11	0.08 - 0.14	1.66+-0.21	5.28
PY	Bifenthrin	2010	East Baton Rouge	240	0.13	0.10 - 0.17	1.68+-0.22	4.92
PY	Cyfluthrin	2008	Franklin	200	0.02	0.01 - 0.03	2.31+-0.39	1.25
PY	Cypermethrin	2004	Acadia	263	0.16	0.12 - 0.22	1.39+-0.17	4.73
PY	Cypermethrin	2009	Franklin	120	0.72	0.47 - 1.05	1.82+-0.31	2.47
PY	Cypermethrin	2010	East Baton Rouge	150	0.33	0.10 - 0.87	1.29+-0.21	3.40
PY	Lambda-cyhalothrin	2008	Franklin	160	0.02	0.01 - 0.02	1.88+-0.37	3.84

^aInsecticide class: Organophosphate (OP) and Pyrethroid (PY)

^bTotal number of insects tested including non-treated controls.

^cConcentrations reported in μg insecticide per vial: LC₅₀ values are significantly different if 95% confidence limits did not overlap.

^dConfidence limits

^eChi-square (no significant values)

Table 5.3. Response of redbanded stink bug adults to organophosphate and pyrethroid insecticides at 4 h after exposure in the adult vial test.

Class^a	Insecticide	Year	Collection site	N^b	LC₅₀^c	95% CL^d	Slope	χ^2^e
OP	Acephate	2004	Acadia	300	3.01	2.05 - 4.27	1.65+-0.24	4.98
OP	Methamidophos	2005	Concordia	250	1.87	1.30 - 2.63	2.56+-0.30	3.12
OP	Methamidophos	2008	Franklin	150	3.41	1.79 - 8.08	2.21+-0.34	4.90
OP	Methamidophos	2009	Franklin	180	4.66	3.83 - 5.53	3.67+-0.54	0.83
OP	Methamidophos	2009	Concordia	150	3.42	2.71 - 4.70	2.09+-0.36	2.13
OP	Methamidophos	2010	East Baton Rouge	190	4.86	3.98 - 5.69	3.49+-0.53	1.43
PY	Bifenthrin	2009	Caldwell	160	0.21	0.15 - 0.30	1.76+-0.26	1.81
PY	Bifenthrin	2009	Franklin	120	0.48	0.34 - 0.69	2.43+-0.48	2.45
PY	Bifenthrin	2009	Concordia	120	0.45	0.33 - 0.62	2.26+-0.39	2.07
PY	Bifenthrin	2010	East Baton Rouge	165	0.51	0.31 - 0.83	2.41+-0.35	3.32
PY	Cyfluthrin	2008	Franklin	200	0.08	0.06 - 0.10	1.77+-0.29	1.02
PY	Cypermethrin	2004	Acadia	200	0.55	0.30 - 0.99	1.32+-0.23	3.51
PY	Cypermethrin	2009	Franklin	150	2.90	1.71 - 4.76	2.08+-0.32	4.76
PY	Cypermethrin	2009	Concordia	150	3.85	2.92 - 4.85	2.71+-0.42	3.43
PY	Cypermethrin	2010	East Baton Rouge	150	2.66	1.66 - 4.39	1.30+-0.22	1.61
PY	Lambda-cyhalothrin	2008	Franklin	210	0.15	0.11 - 0.25	2.43+-0.55	2.19

^aInsecticide class: Organophosphate (OP) and Pyrethroid (PY)

^bTotal number of insects tested including non-treated controls.

^cConcentrations reported in μg insecticide per vial: LC₅₀ values are significantly different if 95% confidence limits did not overlap.

^dConfidence limits

^eChi-square (no significant values)

In the current study, control for acephate (86%) was similar, but slightly higher for methyl parathion (82%). This may be attributed to the use of field rates in the present study approximately 25% higher than the rates used by Baur et al. (2010). Furthermore, Baur et al. (2010) found that control of redbanded stink bugs with pyrethroids (bifenthrin, cyfluthrin, gamma-cyhalothrin, lambda-cyhalothrin, and zeta-cypermethrin) ranged from 52-80%. Similar results were seen in the present study with all pyrethroids providing $\leq 80\%$ control of redbanded stink bug. These results have been further substantiated by field trials during 2009 across Arkansas, Mississippi, and Texas. Pyrethroid performance in these trials was highly variable and inconsistent (41-100%) against redbanded stink bug (Akin and Howard 2010, Smith et al. 2010a, Smith et al. 2010b, Way et al. 2010). In these same trials, satisfactory control of redbanded stink bug was achieved with the acephate (87-94%), pyrethroid + acephate (90-100%), and lambda-cyhalothrin + thiamethoxam (90-97%) treatments.

Differences between species in field susceptibility to pyrethroids and organophosphates were confirmed with laboratory bioassays in the present study. Baur et al. (2010) reported the first baseline toxicity data on Louisiana redbanded stink bug populations during 2004 and 2005. In those studies, LC_{50} 's for a pyrethroid (cypermethrin) ranged between 0.44-0.88 $\mu\text{g}/\text{vial}$. The LC_{50} for an organophosphate (methamidophos) was 1.64 $\mu\text{g}/\text{vial}$. Similar results were reported in the current study the results were generally similar with for additional redbanded stink bug collections from different locations in Louisiana during 2004 and 2005. LC_{50} values (cypermethrin: 0.55 $\mu\text{g}/\text{vial}$ and methamidophos: 1.87 $\mu\text{g}/\text{vial}$) for redbanded stink bug populations (Acadia and Concordia) in the current study were comparable to those reported by Baur et al.

(2010). However, field collections of redbanded stink bug from 2009-2010 (Franklin, Concordia, and East Baton Rouge) had significantly higher LC₅₀ values for both cypermethrin and methamidophos compared to all values reported for previous years in this and Baur et al. (2010) studies.

Pyrethroid and organophosphates have been used to control redbanded stink bug in Louisiana since it reached pest status. Changes in susceptibility to both classes of chemistry are surprising, but not unexpected since high application rates for insecticides in both classes are used to obtain satisfactory control of this pest. High infestation levels (two –five X action threshold) often require sequential treatments with a co-application of pyrethroid and organophosphate (acephate) to reduce the pest populations below the action threshold level. The only labeled insecticides recommended for control of the redbanded stink bug in Louisiana are acephate, bifenthrin, bifenthrin + zeta-cypermethrin, and lambda-cyhalothrin + thiamethoxam. An additional six products are recommended to control southern green stink bug in Louisiana, which include beta-cyfluthrin, cyfluthrin, gamma-cyhalothrin, lambda-cyhalothrin, methyl parathion, and zeta-cypermethrin (Pollet et al. 2010). The frequency of applications, high use rates, and limited insecticide MOA's could quickly reduce the useful life of the currently available chemical control options.

The southern green stink bug was highly sensitive to all organophosphates and pyrethroids tested in the AVT. Southern green stink bug susceptibility to insecticides in the present study was similar to that previously reported by Willrich et al. (2003) for acephate, bifenthrin, cyfluthrin, cypermethrin, and lambda-cyhalothrin. Results of the

present study confirm that the southern green stink bug is still highly susceptible to the currently recommended insecticides.

Differences in susceptibility between southern green stink bug and redbanded stink bug caused a revision in the action threshold and chemical control recommendations for Louisiana soybean pest management. The action threshold for redbanded stink bug in Louisiana soybean is six insects/25 sweeps, while the action threshold for southern green, green, and brown stink bugs remains at nine insects/25 sweeps (Pollet et al. 2010). This summary of field and laboratory results should enable scientists to monitor for changes in insecticide susceptibility of the southern green stink bug and redbanded stink bug, not only in Louisiana, but across the southern United States.

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CHAPTER 6

SUMMARY AND CONCLUSIONS

A complex of stink bug species including green stink bug, *Acrosternum hilare* (Say), southern green stink bug, *Nezara viridula* L., and the brown stink complex, *Euschistus spp.*, are annual pests in Louisiana soybean fields. In 2002, infestations of another phytophagous pentatomid, the redbanded stink bug, *Piezodorus guildinii* (Westwood), were detected at levels exceeding the current action threshold (nine insects per 25 sweeps) in Louisiana soybean. Since that discovery, this species has become a perennial stink bug pest in Louisiana soybean production systems and has become distributed across the Mid-Southern United States.

The native range of the redbanded stink bug ranges includes regions in Argentina to the Southern United States where it is considered an annual pest. Considerable work has been done on this species in Brazil where it is particularly damaging to soybean. It was first reported in United States soybean during the 1960's with historical reports in several states including South Carolina, Florida, Georgia, and New Mexico. However, none of these references considered it as an economical pest.

As a relatively new pest, very little information is available on the biology and ecology of the redbanded stink bug in the Louisiana soybean agro-ecosystem. However, preliminary work has shown that the redbanded stink bug appears to be less susceptible than other stink bugs to selected insecticides. In addition, the redbanded stink bug also has shown a propensity to develop high infestations (> five-fold action thresholds) within Louisiana soybean fields in short periods of time. In order to develop sustainable IPM strategies against this pest, several studies were proposed. The objectives of these

studies were to determine species composition and seasonal abundance of the Louisiana soybean pentatomids, characterize oviposition habits of redbanded stink bug in soybean, determine redbanded stink bug effects on soybean yield and seed quality, and to determine the susceptibility of redbanded stink bugs to selected insecticides in laboratory bioassays and field trials.

Soybean representing four maturity groups (MG) III, IV, V, and VI were sampled weekly from R1 (first flower) through R8 (physiological maturity) growth stages during 2008 to 2010 at five locations across Louisiana to determine Pentatomidae composition. These locations included the Jeanerette (southwest), Baton Rouge (southeast), Alexandria (central), Winnsboro (northeast), and Bossier City (northwest). A total of 13,146 stink bugs were captured and subsequently identified to species. The predominant species (>90%) captured included the redbanded stink bug (54.2%); southern green stink bug (27.1), *Nezara viridula* L.; brown stink bug (6.6%), *Euschistus servus* (Say); and the green stink bug (5.5%), *Acrosternum hilare* (Say). Other minor pentatomid pests captured included *Euschistus quadrator* Rolston (1.6%), *E. tristigmus* (Say) (1.6%), *Thyanta accera* McAtee (0.9%), *Oebalus pugnax* F. (0.2%), *Edessa bifida* (Say) (0.1%), *Euschistus consperus* Uhler (0.1%), and *Euschistus ictericus* L. (0.1%). The spined soldier bug, *Podisus maculiventris* (Say); comprised 1.6% of the stink bug population.

Redbanded stink bug comprised the largest percentage of the complex at all survey sites except northwest Louisiana (most northern survey site), representing 76%, 68%, 49%, 62%, and 33% of populations at Jeanerette (southwest), Baton Rouge (southeast), Alexandria (central), Winnsboro (northeast), and Bossier City (northwest), respectively. Stink bug infestations exceeding action thresholds were detected during R4,

R5, R6, and R7 growth stages with peak levels occurring during R6 (full seed) and R7 (beginning maturity) stages. Redbanded stink bugs accounted for the largest percentage of the stink bug complex in early maturing soybean varieties (MG III [86%] and IV [60%]) and declined in later maturing soybean (MG V [54%] and VI [50%]). This may be due to the absence of other suitable hosts for the redbanded stink bug, while the green, southern green, and brown stink bugs have a wider variety of crop and non-crop hosts available during the period MG III and IV soybean are most susceptible. These species may become more distributed across the general farmscape leaving redbanded stink bug populations concentrated in soybean. The early soybean production system that is widely utilized on much of the Louisiana acreage may allow the overwintering and first generation populations of redbanded stink bug to have a suitable host with little competition from other stink bug species that inhabit a wide range of alternate hosts.

Over 650 egg clusters (multiple eggs in a mass) from native populations of redbanded stink bug were collected during field surveys of ≈ 2400 plants during the two year period, 2008 to 2009. Across all years and samples, egg clusters were more common on soybean leaves (52%) and pods (45%) compared to that on main stems (3%). However, egg clusters were primarily recorded on leaves during the earlier soybean reproductive stages R1 (beginning bloom) to R4 (beginning pod). During the pod filling stages R5 (seed initiation) to R7 (beginning maturity) female oviposition was similar between leaves (52%) and pods (46%). Within the soybean plant, oviposition was distributed among the lower (43%), middle (36%) and upper (21%) plant canopies. The mean number of eggs within a single cluster was 17.6 ± 0.3 . These results suggest that redbanded stink bug adults have a preference for the lower plant canopy. As the nymphs

eclose from eggs, they do not appear to rapidly migrate into other plant strata. The current sampling strategy with a sweep net only measures infestations in the upper plant canopy. Sampling strategies for stink bugs may need to be modified in order to more accurately measure field infestations of this species. Furthermore, adults and nymphs are less likely to come into contact with insecticide residues in the lower portions of the plant canopy which may require a change in chemical control strategies.

During the study, over 2,000 redbanded stink bug adults were collected with an average sex ratio of 1.2 females to 1 male. During the R5 growth stage, females in this ratio increased to 1.4 females to 1 male. This change coincided with peak oviposition on plants. Peak oviposition during R5 ensures that redbanded nymphs will have an adequate food source as seed begins to form within pods.

The effects of artificial and natural redbanded stink bug infestations on soybean yield, seed quality, and delayed crop maturity were evaluated in field trials. In the artificial infestation study, redbanded stink bugs (0, 1, 2, 4 insects/0.6 row m) were caged for 7 d during the R4, R5, R6, or R7 growth stage. In this trial, redbanded stink bug infestations reduced soybean yield and quality while increasing frequency of plants exhibiting green stems at the R8 (physiological maturity) growth stage. Redbanded stink bug infestations during R4 (pod elongation) to R7 (beginning maturity) caused a reduction (2.7-16.1%) in yield. The R5 growth stage appeared to be the most sensitive stage for yield reductions. One and two redbanded stink bug/0.6 row m significantly reduced yields by 9.1% and 13%, respectively during the R5 stages. The current action thresholds in Louisiana soybean for native stink bugs are two stink bugs/0.6 row m or nine stink bugs/25 sweeps. Results from this study suggest the action threshold should be

lower for redbanded stink bug than that for native stink bugs and may be one to two insects per 0.6 row m depending on growth stage. The amount of stink bug-damaged seed in infested plots was also highly variable and ranged from 3.5% to 8.1%. All stink bug infestations (one, two, and four insects/ 0.6 row m) had significantly higher stink bug-damaged seed compared to the non-infested control at both R5 and R6 growth stages. During the R4 and R7 growth stages, only the highest infestation level (four insects/0.6 row m) significantly increased the number of stink bug-damaged seed compared to the non-infested control. The incidence of green stems at R8 in redbanded stink bug-infested plots was highest during the R4 and R5 growth stages. Native infestations of stink bugs (>90% redbanded stink bug) that exceeded the Louisiana action thresholds significantly reduced yield and 100 seed weights with an increase in the frequencies of stink bug-damaged seed, and purple-stained (fungal infection) seed compared to that in field plots treated with insecticides. Infestations exceeded an action threshold on Aug 21 (R5) and population remained above that for the duration of the trial in the non-treated control with a peak at 8X the action threshold (R6). In addition, overall crop maturity was affected with an increase in the frequencies of green pods (immature seed), and excessive green leaves at the R8 stage was associated with these infestations compared to plants in plots treated with insecticides.

A series of field experiments during 2005-2009 determined the efficacy of several classes of insecticides against the southern green stink bug and redbanded stink bug. The mean level of southern green stink bug and redbanded stink bug control was $94.4 \pm 1.3\%$ and $75.1 \pm 1.9\%$, respectively, with pyrethroids. Mean organophosphate efficacies against southern green stink bug and redbanded stink bug was $89.8 \pm 2.7\%$ for and $84.8 \pm$

1.7%, respectively. Neonicotinoids provided $78.0 \pm 5.6\%$ and $63.2 \pm 6.0\%$ control of southern green and redbanded stink bug, respectively. Selected pre-mixes or co-applications of products from multiple classes provided $98.6 \pm 0.6\%$ and $83.8 \pm 2.4\%$ control of southern green and redbanded stink bugs, respectively. The field trial results indicated consistently lower efficacy of the same treatment against the redbanded stink bug compared to the southern green stink bug.

In laboratory bioassays, both species were exposed to pyrethroids and organophosphates in adult vial bioassays. Dose responses (LC_{50} 's) ranged from 0.02 to 2.36 $\mu\text{g}/\text{vial}$ for southern green stink bug and from 0.21 to 4.86 $\mu\text{g}/\text{vial}$ for redbanded stink bugs. The redbanded stink bug was four to eight-fold less susceptible to pyrethroids (bifenthrin, cyfluthrin, lambda-cyhalothrin, and cypermethrin) compared to southern green stink bug. This species also was two to eight-fold less susceptible to organophosphates (methamidophos and acephate) compared to the southern green stink bug. The laboratory bioassays support the field trials and show the redbanded stink bug is less susceptible than the southern green stink bug to these selected insecticides. This differential susceptibility between stink bug species has required changes in action thresholds and insecticide recommendations for Louisiana soybean insect pest management. The current action threshold is six redbanded stink bugs/25 sweeps. Products currently recommended for control include the acephate (organophosphate) and bifenthrin (pyrethroid) or combinations of acephate + a pyrethroid and a neonicotinoid + a pyrethroid.

The results of these projects enhance the overall understanding of redbanded stink bug population dynamics and ecology in Louisiana soybean. Redbanded stink bug has

the potential to be a perennial pest of Louisiana soybean and during the course of this study was a dominant pest species. However, it has yet to be determined what impact adverse overwintering conditions will have on redbanded stink bug population development. During 2010, populations were much lower than that during the previous years of the study which may have been related to low winter temperatures and less favorable late-winter and spring habitat. The redbanded stink bug prefers to oviposit in the lower plant canopy which allows adults and nymphs to have lower exposure to direct applications of insecticides as well as residue on leaf surfaces. This species causes detrimental effects on soybean yield, seed quality, and crop maturity in a manner similar to that observed with other phytophagous stink bugs in soybean. Finally, the redbanded stink bug is less susceptible to numerous insecticides compared to the southern green stink bug. In summary, the redbanded stink bug has a greater damage potential in soybean than other native stink bugs which justifies a lower action threshold.

This project has addressed several critical issues with the redbanded stink bug in soybean, but opportunities exist for further research with this pest. General suggestions for future research include:

- 1) Laboratory and field studies should determine the cold tolerance and overwintering potential of this pest to provide an indication of the northern range of this pest within the United States.
- 2) Field surveys and host preference studies should be conducted to determine the suitability of alternate non-crop hosts in Louisiana. Little is currently known about how populations of the redbanded stink bug build in early spring prior to infestations occurring in soybean. A better understanding of native non-soybean

hosts may allow for effective management strategies to be implemented prior to high infestations occurring in soybean.

3) Further studies should be conducted on yield and quality losses associated with redbanded stink bug infestations to determine the cumulative effects of sub-action threshold levels for a sustained period of time.

4) Further insecticide screening trials should be done with currently available and novel insecticides to determine efficacy and rate structures for the optimum chemical control strategies to control this pest.

VITA

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