ESTABLISHING AND IMPLEMENTING AN IPM PROGRAM FOR THE REDBANDED STINK BUG: AN EMERGING SOYBEAN PEST IN THE SOUTHERN REGION

A Dissertation

by

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ABSTRACT

Redbanded stink bug (RBSB), (*Piezodorus guildinii* Westwood) has recently emerged as an economic pest of soybean, *Glycine max* (L.) Merrill in the southern US. Having only recently emerged as a pest in the US, little information exists on RBSB in this country. Information on RBSB life history is needed to provide the basis for development of an effective management plan for this Neotropical pentatomid. This dissertation research was undertaken to gather information which will help achieve the long term goal of developing an integrated pest management (IPM) program for RBSB.

Soybean field surveys conducted over three years across the Upper Gulf Coast of Texas showed that RBSB has become the most abundant stink bug species attacking soybean in this region, accounting for 65% of the entire population of the stink bug pest complex. Field cage experiments showed that highest yield losses from RBSB occurred when soybeans at R5-R6 stages were infested. Our data also showed that a relatively high RBSB density (8 RBSB adults/0.3 m) during R4-R5 stage soybean triggered development of delayed maturity indicated by green leaf retention. In addition, field experiments conducted to determine if reduced pod load or alteration of sink-source ratio is involved in delayed maturity showed no relationship between reduced pod load and occurrence of soybean delayed maturity. However, RBSB density was found to have a significant positive correlation to the occurrence of soybean delayed maturity. These findings suggest that RBSB-induced soybean delayed maturity may not be solely due to reduced pod load or altered sink-source ratio, but additional mechanisms also may be involved. Finally, results from an insecticide field trial and laboratory bioassays showed that RBSBs are more susceptible to neonicotinic and pyrethroid insecticides than to the widely used organophosphate, acephate. This dissertation research has provided valuable information in regard to RBSB and soybean, which will help develop, and establish an IPM program for this emergent pest of US soybean. Development of an IPM program will reduce dependence on chemical insecticides for RBSB management. Reductions in insecticide use will eventually benefit the environment and human health.

DEDICATION

To my grandparents, Anna and Bai, it all started only because of you. I would not have even dreamt of this if you weren’t there.

To my mother, Chaya. Thank you for your love, sacrifice and patience.

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CHAPTER I

INTRODUCTION

Soybean (*Glycine max (L.)*) Merrill, is an important crop globally due to its multipurpose uses. Soybeans are processed for its oil and protein and are used to produce soy milk, soy flour and used as ingredients of many processed food products. It emerged as a domesticated crop around the 11th century BC in China (Hymowitz 1970). During the first three decades of the twentieth century soybean production was mainly confined to the Far East. China, Indonesia, Japan, and Korea were the major soybean producing countries in the 1930s (Burtis 1950). In the late 1940's and early 1950's, the US surpassed soybean production in China and eventually surpassed the entire soybean production of the Far East. From 1960 to 1973 soybean production in the US doubled with the greatest rate of increase occurring in southern states (ASA 1975). With the establishment of soybean as a major food source and due to its multiple uses, its continuous and rapid expansion was expected, particularly in tropical and subtropical latitudes (ASA 1972). Reduction in production cost and consistent improvements in average yields have steadily improved the competitive position of soybeans among arable crops. Soybean is the world's leading provider of protein and oil. It accounts for 35% of worldwide harvested areas dedicated to oil crops and for 44% of global oil crop production (FAO 2009). Currently, only five countries- US, Brazil, Argentina, China, and India contribute to over 90% of world soybean production (FAO 2011). US and Brazil contribute around 41 and 26 % of global soybean production, respectively (FAO

2011). In US agriculture, soybean has great importance. Farmers in more than 30 states grow soybean, making it the US' second largest crop in cash sales and the number 1 value export crop (http://soystats.com). In 2012, soybeans were planted on 77.2 million acres in the US, producing 82 million metric tons of soybeans (http://soystats.com/). The total value of the US soybean crop in 2012 was more than $43 billion. In the same year, soybeans accounted for 57% of world oilseed production of which 35% was produced in the US. In 2012, the US exported 38.4 million metric tons of soybeans, which accounted for 37% of the world's soybean trade, making the US the second largest soybean exporter in the world.

There are two types of soybean cultivars produced in the US *viz.* indeterminate and determinate. Indeterminate type cultivars are grown in northern states in which terminal buds continuously produce vegetative growth during most of the growing season. In these cultivars, inflorescences are on axillary racemes giving even distribution of pods on all branches. Determinate type cultivars are grown in southern states. In these cultivars, vegetative growth of terminal buds stop when they begins to flower. Determinate cultivars have both axillary and terminal racemes and are identified by a dense cluster of pods at terminals (Teare and Hodges 1994).

Soybean flowering and maturity during the growing season is controlled primarily by day length (Teare and Hodges 1994). As northern latitudes have longer day lengths, the period between seed emergence to flowering is longer. Soybean plant breeding efforts have developed cultivars suitable for different day lengths. These cultivars fall into 12 maturity groups ranging from 00 to X. The 00 cultivars require longer days to bloom and develop seed, therefore they are grown in southern Canada and northern US while Group X cultivars mature in tropical latitudes (Teare and Hodges 1994).

Soybean response to insect pest injury depends upon the crop developmental stage during which injury occurs (Teare and Hodges 1994). Therefore, considering soybean developmental stages is essential in describing the potential impact of insect pests. A letter designates soybean growth stages: V for vegetative and R for reproductive, followed by a number (Fehr and Caviness 1977). Nodes on the main stem are counted to designate the vegetative growth stages (V stages). A V1 stage is when the plant develops a first node with a trifoliate leaf; V2 is when a second node is present and so on for V3, V4, V5, etc. Reproductive stages of soybeans (R stages) are based on flowering, pod development, seed development, and plant maturation (Fehr and Caviness 1977). R1 is the beginning bloom; R2 is the full bloom stage; R3 includes plants at the beginning of pod development; R4 comprises plants at the full pod stage with no seeds present; R5 expands from the beginning of the seed stage, when pods are filling with seeds, to the full seed stage where pods are filled with the final number of seeds yet not fully developed; R6 includes plants in which pods are filled with full-sized seeds; the maturity stage R7 (beginning maturity), is characterized by the presence of at least one pod on the main stem reaching its mature color (tan or brown), and the R8 stage (full maturity) includes plant in which 95% of their pods have reached their mature color (Fehr and Caviness 1977).

During different developmental stages such as from germination to maturity, soybeans are attacked by a diverse community of arthropods seeking nourishment. During reproductive stages, soybean is primarily attacked by a complex of pod-attacking stink bugs (Way 1994). Stink bugs are the primary pests of soybeans in the southern US (Drees and Rice 1990, Baur et al. 2000). The southern green stink bug (SGSB), *Nezara viridula (L.)*, the green stink bug (GSB) *Chinavia hilaris (Say)*, and the brown stink bug (BSB) *Euschistus servus* (Say), were the most damaging members of the stink bug complex (McPherson et al. 1993) across the southern US up until 2000. Since, 2000, the redbanded stink bug (RBSB), *Piezodorus guildinii* (Westwood) has increased its numbers and currently has become a major soybean stink bug pest in Louisiana (Temple et al. 2009) and Texas (Vyavhare and Way 2013).

Stink bugs cause damage by feeding on young, tender growth and developing seeds (McPherson et al. 1994). They inject salivary secretions into seeds to form a slurry, which they ingest. Damaged seeds exhibit decreased germination, reduced emergence and low survival (Todd and Turnipseed 1974). The RBSB causes more damage per insect than other stink bug species on soybean (Correa-Ferreira and de Azevedo 2002), as the deleterious action of salivary enzymes is greater for this stink bug compared to others (Depieri and Panizzi 2011). Despite the fact that RBSB causes more damage than other stink bug species, action thresholds for this pest have been defined based on other stink bugs species (i.e., SGSB, GSB, and BSB). Further, even though it is known that vulnerability of soybeans to stink bug damage varies across soybean lifespan

(Musser et al. 2011), current action thresholds for stink bugs are constant throughout soybean reproductive development.

RBSB is also associated with the delayed maturity syndrome in soybean. Soybeans grown in Texas and Louisiana commonly exhibit this disorder. In this disorder, pods mature and get ready to harvest normally, but stems fail to mature (Schwenk and Nickell 1980). The presence of green stems makes operating harvesters difficult and cause seed loss by pod shattering. Although RBSB is known to cause delayed maturity in soybean, it is not clear which stink bug density triggers it nor it is known if it occurs due to changes in plant hormonal balance, due to alterations in the sink/source dynamics within the plant and/or due to microbial pathogens.

One of the major concerns in RBSB management is reduced susceptibility to labeled insecticides (Davis et al. 2011). Thus, the occurrence of the RBSB in Louisiana and Texas soybeans has significantly increased the number of insecticide applications in these states, therefore, increasing the potential for this insect to develop insecticide resistance (Davis et al. 2011, Vyavhare and Way 2013). Currently, multiple insecticide applications for stink bugs are common in Louisiana and Texas where soybean is an important crop accounting for 457,000 and 51,000hectares, respectively. Until recently, RBSB management was dependent upon a single insecticide i.e. acephate. Its repeated applications targeting mainly RBSB has resulted in reduced susceptibility to this organophosphate (http://www.tsusinvasives.org/database/Red_Banded_Stink_Bug.html). However, little or no information exists on RBSB susceptibility to insecticides.

RBSB has emerged as the most serious soybean pest throughout Louisiana and Texas, but no information is available to provide the basis for its effective management. Therefore, the overall goal of this dissertation was to provide basic information aimed to aid in the development and implementation of economical, effective, and sustainable management strategies against RBSB in soybean. My specific objectives were:

1. To determine the relative abundance of major stink bug species (SGSB, GSB, BSB, and RBSB) across different soybean growth stages on the Upper Gulf Coast of Texas
2. To determine the growth stage specific response of soybean to varying densities of RBSB
3. To determine RBSB threshold that triggers delayed maturity and if delayed maturity is due to reduced pod load
4. To generate baseline data on insecticide susceptibility of RBSB field population and evaluate efficacy of commonly used insecticides against RBSB

Stink bug relative abundance

In addressing the first objective, it was hypothesized that relative abundance of SGSB, BSB, GSB, and RBSB varies across soybean reproductive stages. To study this hypothesis, commercial soybean fields across the Upper Gulf Coast of Texas were sampled weekly during reproductive crop growth stages (R2-R7) over the period of three years (2010-2013) using a sweep net (Chapter II) and numbers of individuals of each species were recorded.

Growth stage specific response of soybean to RBSB

In order to determine the growth stage specific response of soybean to RBSB infestation, field grown soybeans were infested with varying densities of field collected RBSB adults using field cages. The plant response to RBSB feeding was measured in terms of numbers of flat pods, seed yield, and test weight (weight of 100 seeds) (chapter III).

RBSB and soybean delayed maturity

In order to determine what RBSB threshold triggers delayed maturity and if soybean delayed maturity is due to altered sink-source ratio (reduced pod load), two experiments were conducted on field grown soybeans. One with different levels of RBSB infestation and another with different levels of mechanical pod removal. Plant response to RBSB feeding and mechanical pod removal during R4 stage was recorded in terms of yield, leaf chlorophyll content, rate of photosynthesis, and green leaf retention at maturity (chapter IV).

Insecticide susceptibility

Chemical insecticides are currently the major line of defense against stink bug pests. In order to generate baseline data on insecticide susceptibility of RBSB field population, glass vial bioassays were conducted using technical grade insecticides. RBSB adults collected from commercial soybean fields were used in glass vial bioassays to determine LC_{50} values for pyrethroids (bifenthrin and cyfluthrin), neonicotinoids (thiamethoxam and imidacloprid), and an organophosphate (acephate). The efficacy of commonly used organophosphate, pyrethroid, and mixtures of pyrethroid and neonicotinoid insecticides against RBSB was also evaluated using a field trial (chapter V).

The information gathered from this dissertation will help develop improved management strategies against RBSB. These improved strategies aim to reduce insecticide applications and diminish insecticide-related risks to human health and the environment. Results from this research will be fundamental in the establishment of a RBSB specific action threshold for monitoring RBSB in soybean fields. A revised action threshold will help soybean producers by allowing them to fine-tune decision making on the proper use of management tactics. Furthermore, reduced insecticide applications will allow conservation of natural enemies and avoid further destabilization of the soybean agro-ecosystem due to insecticides. All the soybean-producing states in the southern region are in dire need of effective management strategies for RBSB. Results of this research will have direct and immediate impact throughout the southern region where sustainable and profitable soybean production is threatened by RBSB.

CHAPTER II

ABUNDANCE OF REDBANDED STINK BUG (HEMIPTERA: PENTATOMIDAE) IN SOYBEAN ON THE UPPER GULF COAST OF TEXAS

Synopsis

Stink bugs are the primary arthropod soybean pests in the southern United States. They mainly feed on young, tender growth and developing seeds with their piercing-sucking mouthparts. Historically, important stink bug species damaging soybeans in the southern United States included the southern green stink bug (SGSB) *Nezara viridula* (L.), the green stink bug (GSB) *Chinavia hilaris* (Say), and the brown stink bug (BSB) *Euschistus servus* (Say) (Hemiptera: Pentatomidae). The redbanded stink bug (RBSB), *Piezodorus guildinii (*Westwood), has recently become an economic pest of soybean in the southern region of the Unites States, especially in Louisiana and Texas. Little is known about the relative abundance of stink bug species in the soybean agro-ecosystems of Texas. To fill this gap, commercial soybean fields in the Upper Gulf Coast of Texas were sampled weekly during the growing season using a sweep net from R2 (full flowering) to R7 (beginning maturity) from 2011 to 2013. Adults and nymphs (3rd, 4th and 5th instars) of RBSB, SGSB, GSB, and BSB were counted in each sample (25 sweeps). The relative proportion of RBSB was significantly higher than any other stink bug species from R5 toR7. Over 65% of the total stink bugs collected during this period were RBSB and about 19% were SGSB. The highest RBSB densities and the highest ratio of RBSB nymphs to adults were recorded at R7. Results from this study show that

RBSB has become the most abundant species in soybean across the Upper Gulf Coast of Texas.

Introduction

Stink bugs are polyphagous pests that feed on a wide range of cultivated crops including cotton (*Gossypium hirsutum* L.), soybean (*Glycine max* L. Merr.), and corn (*Zea mays* L.) (Panizzi 1997). They also subsist on a variety of wild and non-agronomic hosts (Panizzi 1997). Stink bugs have recently become primary pests of soybean in the southern United States (Drees and Rice 1990, Baur et al. 2000). The upsurge in stink bug populations in the southern United States is believed to be due to the advent of *Bt* crops combined with the boll weevil eradication program that reduced the number of insecticide sprays in cotton, which in the past, provided indirect control of stink bug populations in soybean (Greene and Herzog 1999). Also, a shift in soybean production from May-planted maturity group (MG) V and VI in conventional soybean production systems to April-planted MG III and IV in early season soybean production, may have contributed to stink bug population growth in recent years (Heatherly 2005). The increased pressure of stink bugs on early planted soybeans may be due to the early availability of pods (Baur et al. 2000). After colonizing early-planted soybeans, stink bugs successively move to later planted soybeans as the developing pods become available.

In the southern United States, three key stink bug species viz. the southern green stink bug (SGSB), *Nezara viridula L.*; the green stink bug (GSB), *Chinavia hilaris* Say;

and the brown stink bug (BSB), *Euschistus servus* Say, have historically been considered as of substantial economic importance (McPherson et al. 1993). In the past, SGSB, the most cosmopolitan of the pentatomids attacking soybean, has represented the highest proportion of all stink bug species in soybean fields from Texas in the west through southern Arkansas to Virginia in the east (Turnipseed and Kogan 1976). However, during the past decade, a new Neotropical pentatomid, the redbanded stink bug (RBSB), *Piezodorus guildinii* Westwood, has become more common than any other stink bug species in Louisiana (Temple et al. 2011) threatening soybean production in other southern states.

RBSB was first reported on the island of St. Vincent (Stoner 1922) and has been a serious pest of soybean in the Neotropics since the 1960s (Panizzi et al. 2000). In the late 1970s, RBSB began replacing SGSB on Brazilian soybeans (Turnipseed and Kogan 1976, Kogan and Turnipseed 1987). The expansion of soybean cultivation in South America during the 1960s and 1970s could be the principal reason for the increase in RBSB populations (Panizzi and Slansky 1985a). Consequently, most of the information available about RBSB impact on soybean comes from Brazil (Panizzi et al. 1980, Panizzi and Slansky 1985c, a, b). In the United States, RBSB was first reported in the 1960s (Genung et al. 1964), but it was never considered an economic pest of soybeans until the late 1990s. It was frequently observed in low numbers in Florida and Georgia in the1980s (Panizzi and Slansky 1985c). Since its first report in the United States, RBSB has expanded its distribution from Florida (Menezes 1981) to South Carolina (Jones and

Sullivan 1982), Georgia (McPherson et al. 1993), Arkansas (Smith et al. 2009), Louisiana (Temple et al. 2009), and Missouri (Tindall and Fothergill 2011).

Identification and characterization of the species involved in the stink bug complex is important to determine effective economic thresholds in soybean. This is because different species within the stink bug complex have different damage potentials. For example, RBSB in soybean causes more damage per insect than any other stink bug species (Correa-Ferreira and de Azevedo 2002) while SGSB and GSB cause similar damage, and BSB cause comparatively less damage (Miner 1966, McPherson et al. 1979b). Nevertheless, the economic threshold level is the same for all these species in many of the soybean-producing states in United States including Texas, where RBSB populations have recently reached damaging levels (Vyavhare and Way 2013). No extensive field surveys have been conducted to understand the current composition of stink bug species in Texas soybean. In Texas, an economic threshold of 8 stink bugs/25 sweeps (38.1 cm diameter sweep net) is used for the stink bug pest complex throughout all reproductive stages of soybean (https://insects.tamu.edu/extension/bulletins/b-1501.html#Soybean). Although it is common to find multiple stink bug species in the field, little is known about how to incorporate species composition into considerations aimed to determine economic thresholds to justify use of chemical control against this pest complex. Also, it is important to understand the relative proportion of stink bug nymphs versus adults across different crop growth stages because the amount of injury per individual varies from nymphs to adults and the vulnerability of soybean to stink bug damage vary with plant growth stages. For example, both quality and yield loss are most affected when soybeans are exposed to stink bug feeding during R5-R6 (Fehr and Caviness 1977) while damage at R7 is much less than at earlier stages (McPherson et al. 1979b).

Currently, stink bug control in soybean is solely dependent upon chemical applications. Susceptibility to insecticides has been reported to vary among stink bug species and life stages (McPherson et al. 1979a). Therefore, knowledge of stink bug species involved, their relative abundance, and relative proportion of stink bug developmental stages across crop growth stages is needed. For example, pyrethroids are more effective against SGSB and GSB than for BSB (Willrich et al. 2003). Also, LD50s of methyl parathion for fifth instar nymphs of SGSB, GSB, and BSB are higher than for their corresponding adults (McPherson et al. 1979a).

The occurrence of RBSB populations in Texas has been responsible for a significant increase in the amount of insecticides applied to soybean. This increase in chemical control threatens beneficial organisms in the soybean agro-ecosystem and could result in the development of insecticide resistance. This study was conducted to determine the relative abundance of stink bug species and their developmental stages across R2-R7 soybeans in the Upper Gulf Coast of Texas.

Materials and methods

Stink bug collection

Densities of stink bug species were monitored from 2011 to 2013 in commercial soybean fields across the Upper Gulf Coast of Texas. Each year five soybean fields were chosen for the study. In 2011, study fields were located in Jefferson, Matagorda, Colorado, and Liberty Counties. In 2012, all fields were in Jefferson County. While in 2013 soybean fields in Jefferson, Liberty, and Wharton counties were sampled. Fields were kept insecticide-free and sampled at weekly intervals from R2 (full flowering) to R7 (beginning maturity) soybean growth stages (Fehr and Caviness 1977). Sampling began in mid-June and continued weekly through early October with 5 sets of 25 sweeps (38.1 cm diameter sweep net) taken at random locations in each soybean field on each sample date. Insect sampling was done by swinging the sweep net with as much force as possible through the top of the canopy so that the top of the net passed through the uppermost leaves (Rudd and Jensen 1977). Each sample consisted of sting bugs collected in 25 consecutive sweeps taken in a row while walking forward. After collection, stink bugs were separated from foliage and placed in plastic zip-lock bags along with a label (label showed location, crop growth stage, and sampling date) and brought to the laboratory. Plastic bags containing insects were stored at 3^0 C for further processing. Laboratory processing included identification of stink bug species and counting of nymphs (3rd, 4th, and 5th instars only) and adults of each stink bug species found per sample. First and 2nd nymphal instars were not included in counts because their impact on soybean damage is negligible (Simmons and Yeargan 1988).

Data analysis

Analysis of variance was used to determine variation in stink bug numbers (ANOVA) (SAS-Institute 2010). Stink bug species (i.e., RBSB, SGSB, GSB and BSB), stink bug developmental stage (i.e., adults and nymphs), soybean growth stage (i.e., R2-

R7), and their interactions were considered as fixed effects while year and location were considered as random effects. LS-means for number of stink bugs per 25 sweeps were computed and multiple comparisons were made using the Bonferroni correction (SAS-Institute 2010). The ratio of RBSB nymphs to adults, and percentage of samples that reached economic threshold during respective crop growth stages were calculated using MS Excel spreadsheets.

Results

There was a significant effect of crop growth stage, stink bug species, stink bug developmental stage, and their interactions, on stink bug mean abundance (Table 1). The relative abundance of stink bug species was significantly different depending upon soybean growth stage (Fig. 1). The mean abundance of RBSB, SGSB, GSB and BSB did not differ from R2-R4. However, after R4 there was a significant increase in mean abundance of RBSB for each soybean growth stage. Mean abundance of RBSB at R7 (13.4 RBSBs/25 sweeps) was significantly higher than at any other soybean growth stage. During R5-R7, mean abundances of RBSB were significantly higher than that of all other stink bug species. Mean abundances of SGSB increased significantly from R5 to R6 and R6 to R7. Mean abundance of SGSB was significantly higher than that of GSB and BSB during R6 and R7. There was no significant difference in mean abundance between GSB and BSB at any soybean growth stage. Also, the mean abundance of GSB and BSB did not vary significantly from R2-R7.

Table 1. Analysis of variance indicating the significance of crop growth stage, stink bug species, stink bug development stage, and interaction among them on mean number of stink bugs/25 sweeps. 2011-2013.

Source	DF	F Value	Pr > F
Crop growth stage	5	306.55	<.0001
Stink bug species	3	614.53	<.0001
Stink bug developmental stage	1	4.15	0.0418
Crop growth stage X stink bug species	15	127.24	<.0001
Crop growth stage X stink bug developmental stage	5	15.69	<.0001
Stink bug species X stink bug developmental stage	3	9.14	<.0001
Crop growth stage X stink bug species X stink bug developmental stage	15	5.53	<.0001

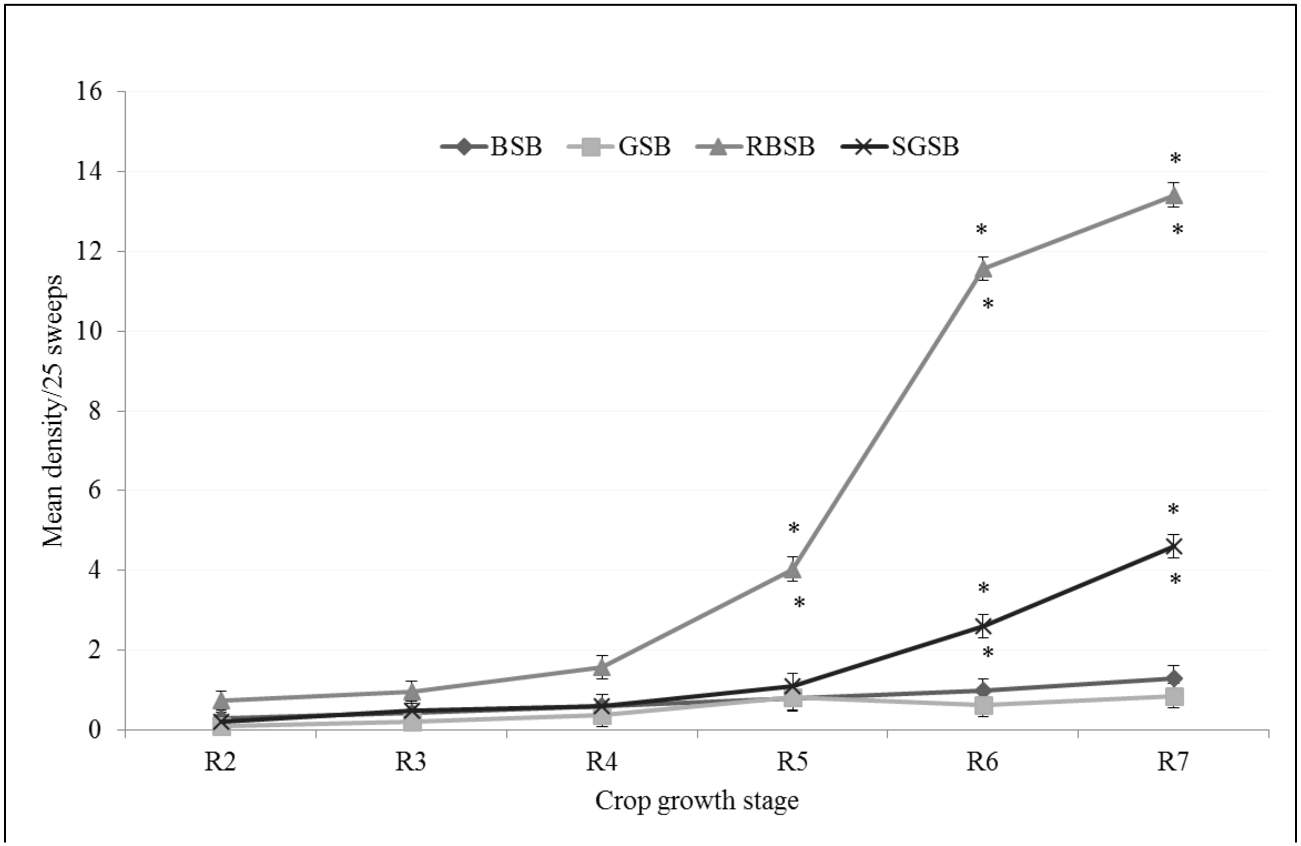

Figure 1. Relative abundance of stink bug species across R2-R7 soybean growth stages on the Upper Gulf Coast of Texas. * above line =significant difference among growth stages. * below line =significant difference among species at a particular crop growth stage (alpha = 0.05). RBSB= redbanded stink bug, SGSB= southern green stink bug, BSB= brown stink bug, GSB= green stink bug. 2011-2013.

Number of RBSB adults and nymphs was not significantly different during R2-R4 (Fig. 2). However, from R4 onwards, both RBSB adults and nymphs showed a significant increase in mean abundances. Highest abundance of RBSB adults (6.5/25 sweeps) was recorded at R6 while highest mean abundance of RBSB nymphs (7.8/25 sweeps) was recorded at R7. Mean adult and nymph abundance of SGSB did not vary significantly from R2 to R5. However, after R5, both adults and nymphs increased in number peaking at R7. At R7, mean abundance of SGSB nymphs was significantly higher than that of adults. BSB and GSB nymph and adult mean abundances remained constant from R2 to R7. No significant differences in mean abundance were observed between adults and nymphs for BSB and GSB at any of the soybean growth stages.

The ratio of RBSB nymphs to adults was the least at R2 (Table 2). However, as the crop progressed from R2 to older growth stages, numbers of RBSB nymphs in proportion to adults increased. During R2-R5, the ratio of RBSB nymphs to adults remained less than one. However, at R7, the ratio of RBSB nymphs to adults was greater than one (1.41).

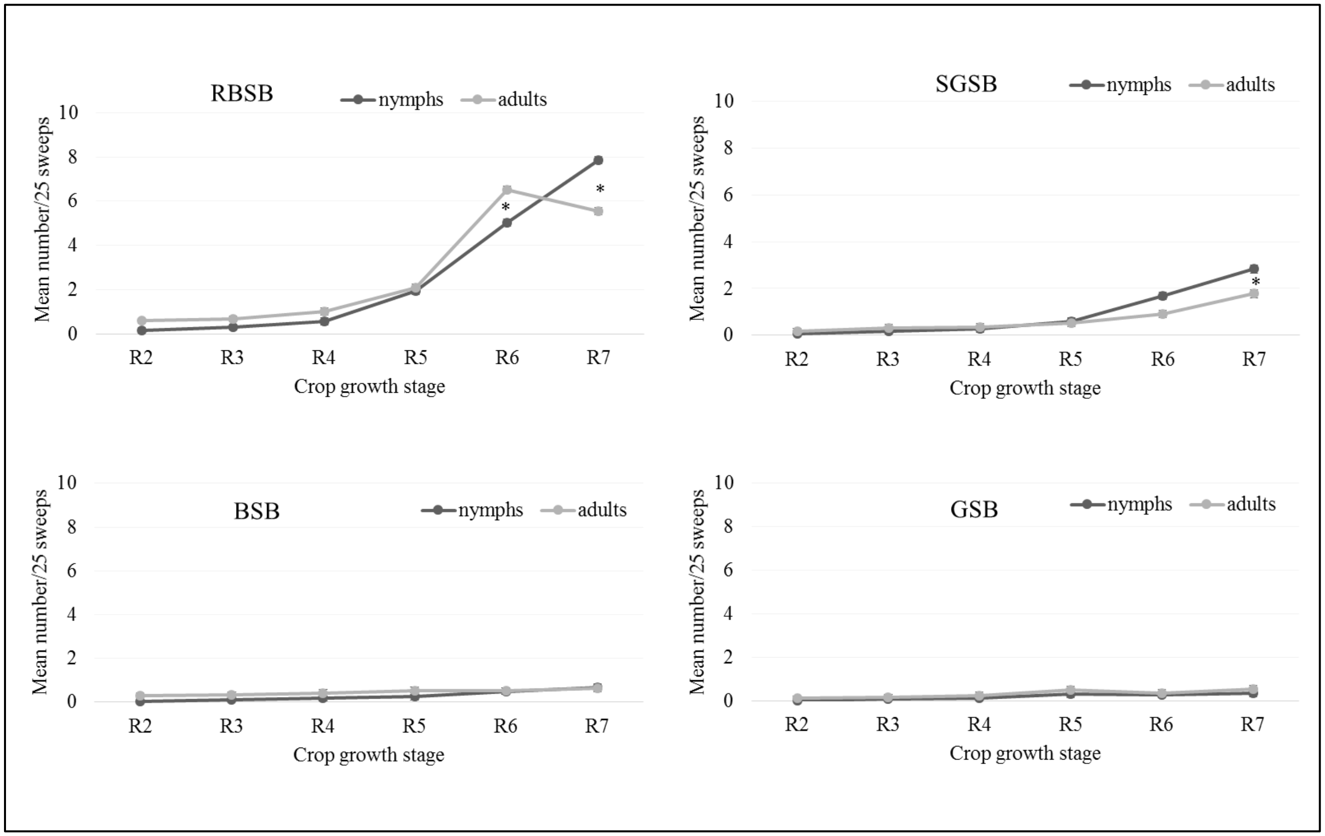

Figure 2. Nymph and adult mean abundances of four stink bug species across R2-R7 soybean growth stages in the Upper Gulf Coast of Texas. * indicates significant differences between nymph and adult mean density (alpha = 0.05). RBSB= redbanded stink bug, SGSB= southern green stink bug, BSB= brown stink bug, GSB= green stink bug. 2011-2013.

Table 2. Ratio of redbanded stink bug (RBSB) nymphs to adults across soybean growth stages. 2011-2013

Crop stage	Ratio of nymphs to adults
R2	0.26
R3	0.45
R4	0.55
R5	0.93
R6	0.77
R7	1.41

Compared to other insects, stink bugs were relatively abundant on R2-R7 soybeans. For three years, 86% of our field samples (each sample = 25 sweeps) contained at least one stink bug. Out of all the stink bugs collected over our three year field survey, 65% were RBSB, followed by SGSB (19%), BSBS (9%), and GSB (6%) (Fig. 3).

Although mean abundance of stink bugs was found to vary significantly across soybean growth stages, very few samples reached the economic threshold (i.e., 8 stink bugs/25 sweeps; including RBSB, SGSB, BSB, and GSB) during R2 – R4 (Table 3). However, during later growth stages (R5-R7) the majority of samples were found to have stink bug densities at or above the economic threshold. The highest number of samples with stink bug populations at or above the economic threshold occurred during R7. In 2011, 2012, and 2013, 75, 100 and 85% of our samples collected at R7 contained stink bug numbers at or above the economic threshold, respectively. At R6, 45, 100, and 50% of our samples contained stink bug numbers at or above the economic threshold during the same years. Overall, RBSB density reached the economic threshold in 21% of samples, while SGSB reached the economic threshold in only 3% of our samples. BSB and GSB never reached threshold levels.

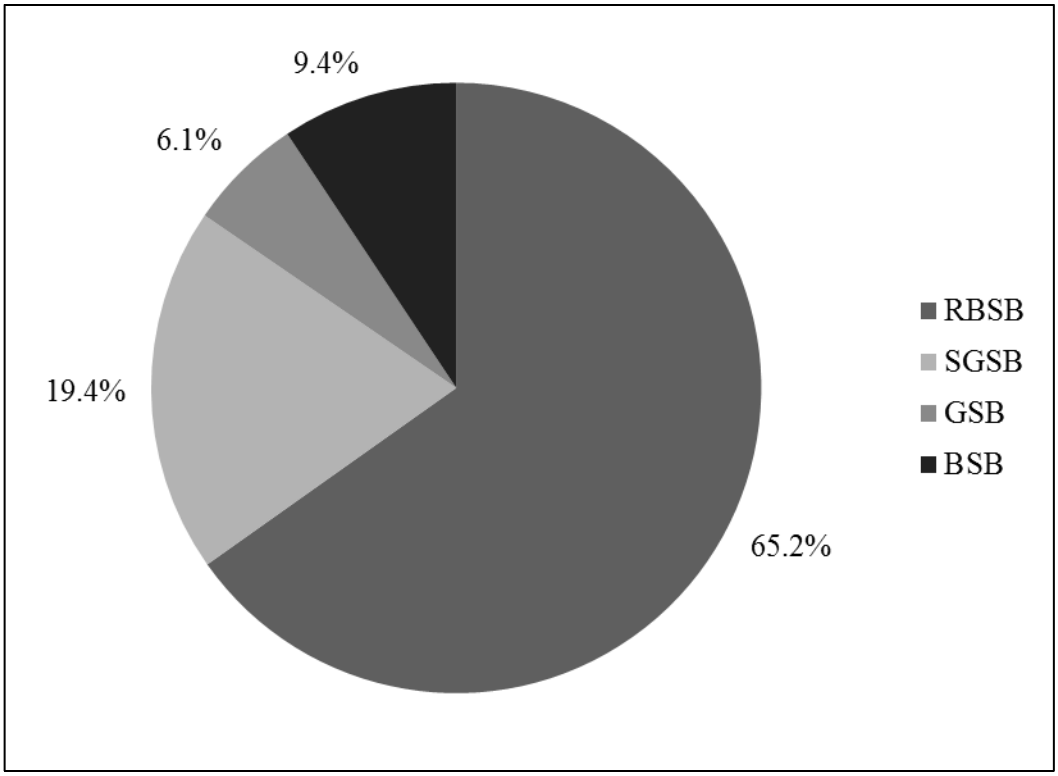

Figure 3. Stink bug species composition in soybean fields across the Upper Gulf Coast of Texas during 2011-2013. RBSB= redbanded stink bug, SGSB= southern green stink bug, BSB= brown stink bug, GSB= green stink bug.

Table 3. Percentage of samples in which stink bug counts reached economic threshold (ET). Percentages include nymphs and adults of RBSB, SGSB, BSB and GSB.

	Percentage of samples at or above ET (8 stink bugs/25 sweeps)		
Crop stage	2011	2012	2013
R2	0	0	0
R3	0	2.50	1.82
R4	0	5.00	0
R5	40	60	10
R6	45	100	50
R7	75	100	85

RBSB= redbanded stink bug, SGSB= southern green stink bug, BSB= brown stink bug, GSB= green stink bug

Discussion

The RBSB has become the most dominant stink bug species in Texas and Louisiana soybeans. The shift in the composition and relative abundance of the stink bug complex in these states calls for a revised economic threshold for the stink bug complex (McPherson et al. 1994). The currently used economic threshold for the soybean stink bug complex has limitations since it is based on outdated data excluding RBSB. The currently used economic threshold was determined when the stink bug complex was mainly composed by SGSB, GSB, and BSB (McPherson et al. 1994). Our study shows that RBSB alone represented more than 65% of the stink bugs found in our samples from 2011 to 2013, while SGSB, BSB and GSB altogether accounted for less than 35% (Fig. 3). Therefore, taking into account the upsurge in densities of RBSB and its higher damage potential compared to other common stink bug species (Correa-Ferreira and de Azevedo 2002), we believe a revised economic threshold for the soybean stink bug complex is needed.

The RBSB is known to be less susceptible to products available for stink bug control on soybeans (Davis et al. 2011), as a result, insecticide applications have significantly increased in regions where RBSB has become a soybean pest. For example, because the RBSB has become more prevalent in Louisiana soybeans, the average number of insecticide applications has increased from one or two per season during the late 1990s to three to five per season in 2013, with the bulk of those targeting RBSB (Temple et al. 2011). Similarly, in Texas, predominance of RBSB has been responsible for a significant increase in the amount of insecticides applied in soybean. Under these circumstances, insecticide resistance is possible. Increased insecticide use may also have negative impacts on natural enemies and increase soybean production costs.

The co-occurrence of multiple developmental stages and species of phytophagous stink bugs with different damage potentials and insecticide susceptibilities makes difficult to determine accurate economic thresholds and selection of the proper insecticide. Information about stink bug species composition and abundance relative to soybean growth stages, such as provided in this study, call for the need to design revised economic thresholds. Also, because susceptibility to insecticides varies with stink bug developmental stages, knowing the relative proportion of less mobile immatures and more mobile adult stink bugs across soybean growth stages may increase the efficiency in timing of insecticide applications.

It is not clear why RBSB geographic range has expanded since the first report of this insect in the United States in the 1960s (Panizzi and Slansky 1985c). It is also unclear what has caused the rise in RBSB populations resulting in this insect becoming the most serious pest of soybean in Louisiana and Texas in recent years. We observed during early reproductive stages of soybean (R2 to R4), populations of RBSB, SGSB, BSB, and GSB were not significantly different (Fig. 1). However, during later reproductive stages (R5 to R7), number of RBSBs significantly increased compared to other insect species. This was in part due to the relatively greater increase in RBSB nymphs vs adults compared to other stink bug species as the crop progresses towards maturity (Fig. 2). This suggests RBSB possesses a higher reproductive rate of increase than the other stink bug species found in Texas soybean. Also, the greater insecticide susceptibility of SGSB, BSB and GSB may make it difficult for them to compete with RBSB, which might displace them from the crop. More research needs to be conducted to fully understand the geographic expansion and increased abundance of RBSB and its interactions with other stink bug species in soybean.

CHAPTER III

DETERMINATION OF GROWTH STAGE-SPECIFIC RESPONSE OF SOYBEANS TO VARYING DENSITIES OF REDBANDED STINK BUG, *Piezodorus guildinii* WESTWOOD, (HEMIPTERA: PENTATOMIDAE)

Synopsis

The redbanded stink bug (RBSB), *Piezodorus guildinii* Westwood, (Hemiptera: Pentatomidae) is an emerging pest of soybeans in the southern states of the US. It has become the most abundant stink bug species in Texas soybeans. Field cage studies were conducted to determine the damage potential of RBSB during R2 to R6 growth stages of soybeans. Soybeans at respective growth stages were infested with varying densities (0, 1, 2 and 4 RBSB adults/cage) of field collected RBSB adults. At each growth stage four adjacent plants were randomly selected and cylindrical wire mesh cages were installed to confine RBSBs on the plants. RBSB infestation was maintained for 10 days after which cages were removed and plants were repeatedly sprayed with acephate. Plant response was measured in terms of number of flat pods, seed yield, 100 seed weight, and number of seeds per pod. RBSB infestation during R5-R6 growth stages significantly decreased soybean yield. Decrease in soybean yield in response to RBSB infestation was mainly due to reduced seed weight and increased numbers of flat pods.

In addition, a field experiment was conducted to determine if flat pods are localized only to the regions of RBSB feeding. RBSB adults were confined to certain portions of the plants (bottom, top, and both) using specially designed cages isolating

these portions of the plants. Results from this experiment showed significantly higher numbers of flat pods on plant portions infested with RBSBs than those kept free of RBSB infestation indicating that flat pods are result of direct RBSB damage and are localized only to the area of RBSB feeding.

Introduction

Stink bugs are the primary arthropod pests of soybeans in the southern US (Drees and Rice 1990, McPherson et al. 1994). Historically, important stink bug species damaging soybeans in this region include the southern green stink bug (SGSB) *Nezara viridula* (L.), the green stink bug (GSB) *Chinavia hilaris* (Say), and the brown stink bug (BSB) *Euschistus servus* (Say) (Hemiptera: Pentatomidae) (Miner 1966, McPherson et al. 1993). The redbanded stink bug (RBSB), *Piezodorus guildinii (*Westwood), has recently emerged as an economic pest of soybean in the southern US.

RBSB was first reported on the island of St. Vincent (Stoner 1922). It's been known to cause serious damage to soybeans in the Neotropics since the 1960s (Panizzi et al. 2000). In the late 1970s, RBSB began replacing SGSB on soybeans in Brazil (Turnipseed and Kogan 1976, Kogan and Turnipseed 1987). Consequently, most of the information available about its impact on soybean comes from Brazil (Panizzi et al. 1980, Panizzi and Slansky 1985c, a, b). The expansion of soybean cultivation in South America during the 1960s and 1970s could be the principal reason for the increase in RBSB populations in this region (Panizzi and Slansky 1985a).

In the US, RBSB was first reported in the 1960s in Florida (Genung et al. 1964), but it was never reported to cause economic damage to US soybeans until the late 1990s. Since its first report, RBSB has expanded its distribution from Florida (Menezes 1981) to South Carolina (Jones and Sullivan 1982), Georgia (McPherson et al. 1993), Arkansas (Smith et al. 2009), Louisiana (Temple et al. 2009), Missouri (Tindall and Fothergill 2011) and Texas (Vyavhare and Way 2013). In the late 1990s, RBSB was recognized as an economic pest of soybean in Louisiana. Currently, RBSB has become the most abundant stink bug species in soybean in Louisiana and Texas (see Chapter 1) and poses a substantial threat to soybean production in the US.

Stink bugs mainly feed on young, tender growth and developing seeds with their piercing-sucking mouth parts (McPherson et al. 1994). They inject salivary secretions into seeds to form a slurry, which they ingest. Damaged seeds exhibit decreased germination, reduced emergence and low survival (Todd and Turnipseed 1974). Damage by stink bugs is caused not only by direct mechanical damage but also by the transmission of disease agents. For example, stink bugs are vectors of yeast spot disease in soybeans (Daugherty 1967) and they have also been reported to be associated with soybean delayed maturity syndrome (Daugherty et al. 1964, Duncan 1968, Panizzi et al. 1979).

The damage potential of stink bugs in soybean varies with the species of stink bug. For example, the rate of damage per insect in soybeans is equivalent for the southern green stink bug and green stink bug, while the brown stink bug is slightly less damaging (McPherson et al. 1979b). The RBSB causes more damage per insect than southern green, green and brown stink bug on soybean (Correa-Ferreira and de Azevedo 2002) because of the greater deleterious action of its salivary enzymes (Depieri and Panizzi 2011). Similarly, the extent of feeding damage by RBSB could vary with the phenological (crop growth) stages of soybean. Little is known about the impact of RBSB injury on soybean yield during specific crop growth stages.

The highest densities of stink bug populations generally occur from mid to late pod fill (R5-R7) (McPherson et al. 1993, Baur et al. 2000, Smith et al. 2009, Vyavhare and Way 2013). Stink bug feeding during full pod to early seed development stages (R4 to R5) can cause large numbers of flat pods i.e. pods without seeds. Although occurrence of flat pods in soybean fields infested with stink bugs is very common, no study has been done to understand the relationship between stink bug feeding and development of flat pods. Flat pods can be observed throughout the plant, but stink bug feeding signs are present only on certain pods (Fig. 4). This observation suggests that localized RBSB feeding could trigger development of flat pods throughout the plant possibly through translocation of deleterious insect enzymes injected while feeding.

Figure 4. Flat pods on soybean plant (red circle shows stink bug feeding site)

The objectives of this study were: 1) to determine the yield response of soybean to RBSB infestation during different growth stages: from full bloom to full seed (R2 to R6) and 2) to determine if flat pods are localized only to the regions of RBSB feeding or if RBSB triggers flat pod development throughout the plant. This study is the first to investigate damage potential of RBSB and its relationship with the occurrence of flat pods in TX soybeans. Understanding the growth stage specific response of soybean to RBSB is necessary to determine the most vulnerable soybean growth stages to this pest. Also, this information is critical to develop action thresholds specific to crop growth stages rather than having a constant action threshold throughout the soybean reproductive development.

Materials and methods

Field cage studies were conducted at the Texas A&M AgriLife Research and Extension Center, Beaumont.

RBSB source

Field collected RBSB adults were used in the study. The day before each infestation, RBSB adults were collected using a standard 15 inch diameter sweep net from commercial soybean fields in Jefferson County, TX. Upon collection, RBSBs were held in the laboratory and provided with fresh soybean pods for 24 hours before infestation. This allowed exclusion of RBSBs which could have been injured when collected from the field and selection of healthy, robust adults for infestation.

Growth stage specific response of soybean to RBSB

Soybeans, AG 6732 (Asgrow, St. Louis, MO) were planted in the field on May 20, 2012 and May 30, 2013. Fields were irrigated regularly, so soil moisture was not a limiting factor that could potentially mask treatment effects by RBSBs. Agronomic practices used were those recommended for soybean production in Louisiana by the Louisiana Agricultural Experiment Station and Louisiana Cooperative Extension Service (Levy 2012). Soybeans were planted at ~6-7 seeds/row-foot with one ft spacing between rows. However, in order to maintain a uniform plant density throughout the treatments, plants were thinned after emergence to keep 4 plants/row-foot at randomly selected spots in the field. In order to protect treatment plants from any kind of insect damage other than confined RBSBs, plants were sprayed with methyl parathion at 0.75 lb AI/ac whenever insect activity was observed using a hand sprayer. Methyl parathion has a relatively short half-life on foliage. Two weeks before the experimental infestation with RBSBs, plants were kept free of any insecticide application to diminish residual effects of pesticides on RBSBs. Because RBSB mainly feed on reproductive structures, soybeans in the reproductive stages R2 (full flowering) to R6 (full seed) were infested with RBSBs. R1 was not used because this stage is characterized by the appearance of a single flower at one of the top internodes. The presence of only one flower at this stage may reduce the opportunities to visualize differences in RBSBs among our different treatments. To determine response of R2-R6 soybeans to RBSB damage, we used a range of RBSB densities (0, 1, 2 and 4 RBSB adults/cage) at soybean growth stages R2, R3, R4, R5, and R6. When soybeans approached R2 (full flowering), cylindrical, wire mesh cages (1ft X 5ft) were placed over plants at randomly selected spots in the field. Prior to caging, selected plants were visually checked and made insect free. RBSB were kept in cages for 10 days. Plants were inspected daily and dead RBSBs were replaced to keep herbivore pressure constant. There were four and six replications for each treatment in 2012 and 2013 studies, respectively. The different soybean growth stages were considered as treatments and the different RBSB densities were considered as sub-treatments. After 10 days of infestation, cages and insects were removed and plants were sprayed with acephate (Orthene 75% SP, Arysta NC) at 1 lb. AI/ac to eliminate further insect activity that could mask treatment effects. The control was caged and maintained without any infestation during any of the plant growth stages and was also treated with insecticides. At maturity, plants were threshed and yield parameters, such as number of flat pods, 100 seed weight, total seed yield, and number of seeds per pod, were recorded. Data were analyzed using PROC GLM (SAS-Institute 2003). Contrasts among specific treatments were determined using Bonferroni test.

RBSB and flat pods

Soybean variety AG 6730 was planted in the field under irrigated conditions on May 12, 2011. Weeds were controlled by hand and Round-up spray at 1% concentration by volume. Plants were sprayed with lambda-cyhalothrin at 0.03 lb ai/ac and methyl parathion at 20 gm/gal of water alternatively in order to protect plants from any kind of insect damage. About 10 days before the infestation of bugs, plants were kept free of any insecticide application to avoid residual effects of pesticides. When soybeans approached R4-R5, plants of uniform height were selected and field collected RBSB were confined to certain portions of the plants (bottom, top, and both) using specially designed cages isolating these portions of the plants. The top two internodes of the plants were considered as the top portion and the rest of the plant as the bottom portion. Overall, there were 4 treatments: infestation of only the top portion, infestation of only the bottom portion, infestation of both portions, and a control without infestation. Two field collected RBSB adults were put into each cage. Infestations were maintained for 3 days after which cages were removed and plants were repeatedly sprayed with acephate to avoid further insect damage. At maturity, pods were harvested separately from each plant portion and the number of flat pods was counted. Data were analyzed using PROC GLM (SAS-Institute 1999). Differences in numbers of flat pods on top and bottom portions of plants under each treatment were determined using Tukey test.

Results

Development of flat pods was significantly impacted by RBSB density and timing of infestation during R2 to R6 stages (Table 4). Further, the interaction between RBSB density and infestation timing was significant. Relatively higher numbers of flat pods were produced in response to RBSB infestations at R5 and R6 stages than during R2 to R4 (Fig. 5). Percent of flat pods was highest when R5 soybeans were infested with 1 and 4 adult RBSB/0.3m and when R6 soybeans were infested 4 adult RBSB/0.3 m.

Table 4. Analysis of variance indicating the significance of infestation timing, RBSB density, and interaction between infestation timing and RBSB density on development of flat pods

Source	DF	F Value	P value
Infestation timing (soybean growth stage)	4	8.19	<0.0001
RBSB density	3	3.53	0.0164
Infestation timing X RBSB density	12	2.01	0.0261

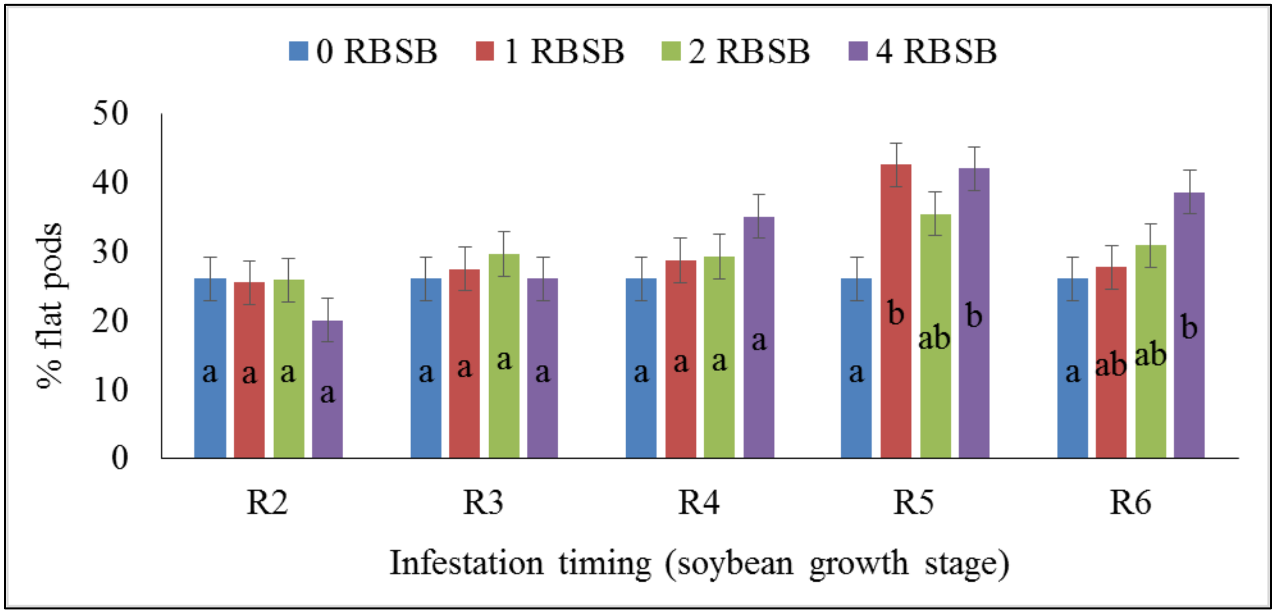

Figure 5. Development of flat pods in response to RBSB infestation in R2-R6 soybeans (RBSB densities: 0, 1, 2, and 4 adults/0.3 m). Bars with same letters are not significantly different (Bonferroni, alpha = 0.05)

Yield response to RBSB density and timing of RBSB infestation during soybean reproductive development was significant (Table 5). Soybean yield did not vary significantly due to RBSB infestation during R2 and R3 stages. However, RBSB infestation during soybean stages R4 to R6 showed significant yield reduction (Fig. 6). The least amount of yield was produced when soybeans at R5 and R6 stages were infested with RBSB densities of 1, 2, and 4 adults/0.3 m. Yield did not vary significantly across RBSB densities 1-4 adults/0.3 m at R5 and R6.

There was no significant impact of RBSB infestation on numbers of seeds per pod (*F* = 0.68, *df* = 3, *P* = 0.5652). As a result, numbers of seeds per pod remained constant across all treatments (Fig. 7).

Seed weight was significantly impacted by RBSB infestation across soybean growth stages (Table 6). At R6, there was a significant reduction in mean seed weight in response to RBSB infestation at 2 adults/0.3 m (Fig. 8). RBSB infestation during R2-R5 stages, however, had no effect on seed weight. Also, seed weight did not vary significantly among RBSB densities.

Table 5. Analysis of variance indicating the significance of infestation timing, RBSB density, and interaction between infestation timing and RBSB density on soybean yield

Source	DF	F Value	P value
Infestation timing (soybean growth stage)	4	19.18	<0.0001
RBSB density	3	42.92	<0.0001
Infestation timing X RBSB density	12	2.56	0.004

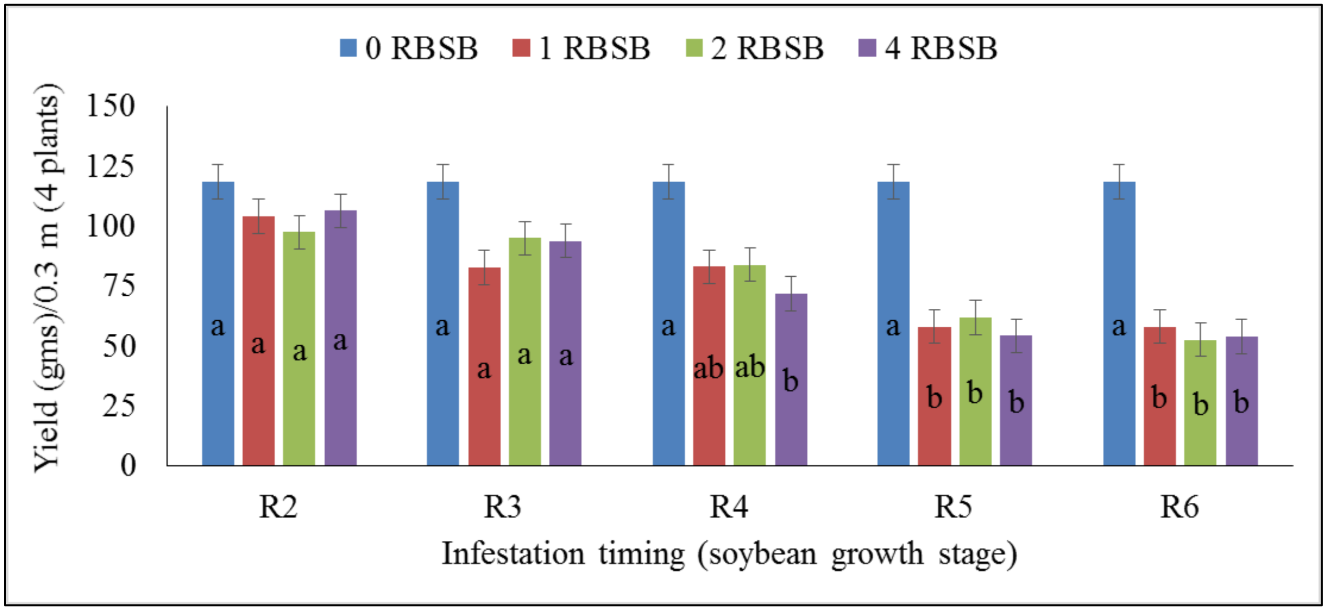

Figure 6. Growth stage specific response of soybean to varying densities of RBSB (0, 1, 2, and 4 adults/0.3 m). Bars showing same letters within each soybean growth stage are not significantly different (Bonferroni, alpha = 0.05)

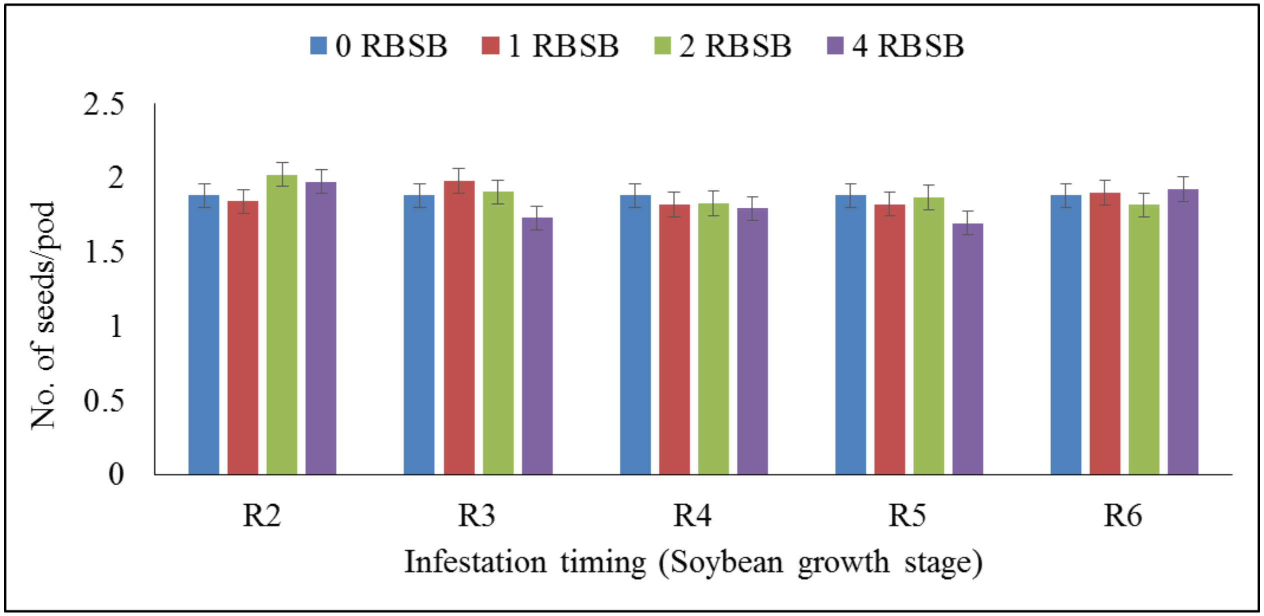

Figure 7. Effect of RBSB infestation during R2-R6 stage soybeans on numbers of seeds per pod.

Table 6. Analysis of variance indicating the significance of infestation timing, RBSB density, and interaction between infestation timing and RBSB density on seed weight/100 seeds

Source	DF	F Value	P value
Infestation timing (soybean growth stage)	4	16.96	<0.0001
RBSB density	3	0.56	0.6429
Infestation timing X RBSB density	12	2.7	0.0025

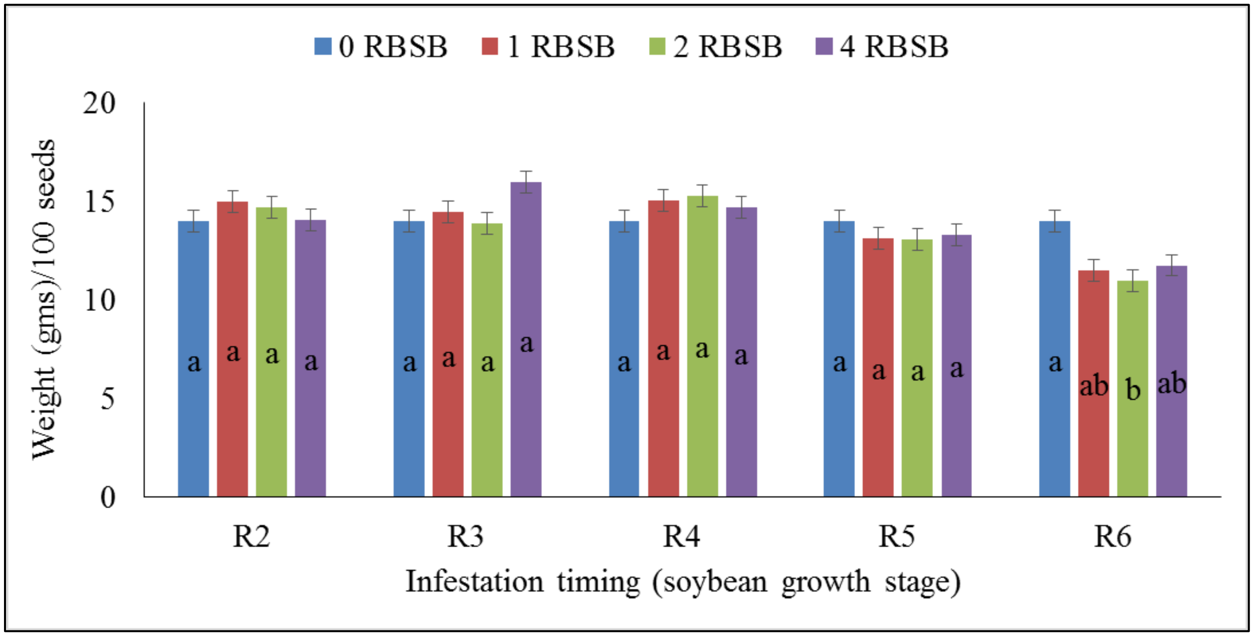

Figure 8. Mean seed weight in response to RBSB infestation at varying densities (0, 1, 2, and 4 adults/0.3 m) at R2-R6 stage soybeans. Bars showing same letters within each soybean growth stage are not significantly different (Bonferroni, alpha = 0.05)

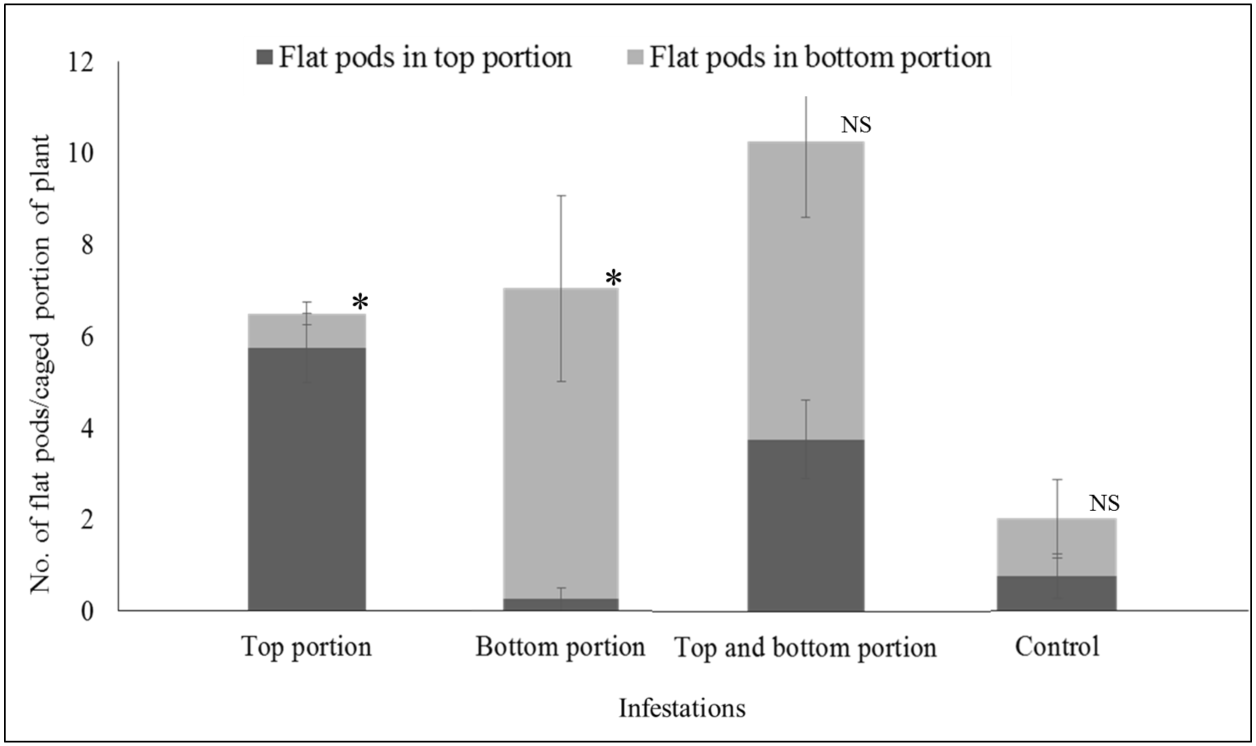

Figure 9. Number of flat pods in response to RBSB infestation on particular plant parts.
* indicates significant difference between numbers of flat pods between top and bottom portions in each treatment, NS = not significant (alpha = 0.05)

Numbers of flat pods on different plant sections (i.e., top and bottom) varied significantly in response to RBSB infestation. When the top portion of soybean plants was infested, significantly higher numbers of flat pods developed on the top portion as compared to the bottom portion (*F* = 35.2, *df* = 1, *P* = 0.0019). Similarly, when only the bottom portion of the plant was infested, significantly higher numbers of flat pods were present on the bottom than on the top portion (*F* = 17.7, *df* = 1, *P* = 0.0085). When both top and bottom portions were infested, similar numbers of flat pods were produced in both portions (*F* = 2.65, *df* = 1, *P* = 0.1644). Few flat pods were produced in control plants with no significant difference in numbers of flat pods between top and bottom portions of the plant (*F* = 0.54, *df* = 1, *P* = 0.494) (Fig. 9).

Discussion

The first objective of this study was to determine soybean yield response to RBSB infestation across R2 to R6 stages. Results from this study showed significant yield reduction across soybean R4 to R6 growth stages in response to RBSB infestation (Fig. 6). The highest yield losses occurred at R5 and R6. Most of the yield reduction during R5 and R6 was the result of the relative increase in the number of flat pods and the reduction in mean seed weight (weight of 100 seeds) as compared to early reproductive stages (R2 to R4). Our results support previous studies done on other stink bug species (e.g. SGSB and GSB) reporting that yield losses are most affected when soybeans are exposed to stink bug feeding during R5to R6 stages. Damage from RBSB particularly during R6 can be severe. Our data suggest that RBSB infestation cause substantial yield loss during the same stage (i.e., R6) at which RBSB populations reach their peak in soybean fields (Vyavhare and Way 2013) which can lead to severe damage.

Results from this study stress the need to revise action thresholds for stink bugs on soybean. Improved thresholds should consider not only the RBSB density but also the variable susceptibility of soybean at different growth stages. Current action thresholds for RBSB are constant throughout the soybean reproductive stages. The incorporation of soybean growth stage into action threshold calculations will increase their accuracy and consequently reduce and optimize the use of insecticides in the agro-ecosystem.

Results from this study show that flat pods are the result of direct feeding by RBSB and that the damage is localized to the region of feeding. Interestingly, the presence of flat pods in the control treatment suggests that there might be other factors involved in the development of flat pods in addition to stink bug feeding. Because flat pods are restricted to the area of feeding by RBSB, the existence of any long-range translocation substance transmitted by RBSB saliva seems unlikely.

Future research needs to be done to examine the extent of damage at different growth stages of not only by RBSB but also by SGSB, GSB, and BSB. This information will allow the development of a combined action threshold for all the species within the stink bug complex. Consideration of both the variable damage potential of different stink bug species and the variable vulnerability of soybean growth stages to stink bug damage needs to be taken under consideration in the development of revised and improved action thresholds for the stink bug complex in soybean.

The present study determined damage potential of RBSB only in terms of yield parameters. However, determination of yield reduction is not enough to estimate overall damage from RBSB, as seed quality can also affect the market value of a crop. Stink bug feeding during late reproductive stages (R6-R7) is known to affect seed quality adversely. For example, SGSB infestation during R7 soybeans have shown to significantly reduce crop value (Musser et al. 2011). We showed significant yield reduction due to RBSB infestation during R5-R6, but seed quality in response to RBSB infestation was not assessed. Future studies need to address seed quality to better estimate RBSB damage and to incorporate this information into action threshold calculations.

To summarize, this is the first study to determine damage potential of RBSB in TX soybean and its association with the occurrence of flat pods. Results from this study have shown that impact of RBSB infestation in soybean depends upon the timing of infestation during crop phonological stages. In addition, this study has shown that direct RBSB feeding on developing pods results in the production of flat pods.

CHAPTER IV

REDBANDED STINK BUG (HEMIPTERA: PENTATOMIDAE) INFESTATION AND OCCURRENCE OF DELAYED MATURITY IN SOYBEAN

Synopsis

Studies done in Brazilian soybean in 1970s indicates that redbanded stink bug (RBSB), *Piezodorus guildinii* (Westwood) is principally responsible for delayed maturity disorder in soybean *Glycine max* (L.) Merr. probably due to its ability to cause more damage per individual than other phytophagous stink bug species found in soybean. This species of stink bug has recently emerged as a serious pest of soybean in the southern US, particularly in the states of Louisiana and Texas. As RBSB has only recently gained the status of serious pest in US soybean, little is known about its association with the occurrence of soybean delayed maturity syndrome in the US. Also, the mechanism behind stink bug induced soybean delayed maturity remains unknown. Though no definitive evidence is present, one of the major hypothesis about stink bug induced delayed maturity is that stink bug feeding during pod and seed development stages result in reduced pod/seed load causing alteration of source-sink ratio in soybeans and eventually in delayed maturity. In order to determine what RBSB threshold triggers delayed maturity in soybean, experiments were conducted with different levels of RBSB infestation (0, 2, 4, and 8 adults/0.3 m) during R4 to R5 stages. In addition, to determine if soybean delayed maturity is exclusively due to reduced pod load by RBSB, experiments with different levels of mechanical pod removal (0%, 25%, 50%, and 75%

pod removal) were conducted on field grown soybeans. RBSB density up to 4 adults/0.3 m did not trigger occurrence of delayed maturity indicated by green leaf retention. However, RBSB density of 8 adults/0.3 m showed a significant increase in the number of green leaves retained on plants at maturity. Results from the mechanical pod removal experiment showed no effect of pod removal on green leaf retention. Significant positive correlation was observed between RBSB density and occurrence of soybean delayed maturity while no significant correlation was observed between mechanical pod removal and number of green leaves retained on plant at maturity. Indicating the involvement of additional mechanism/s than just reduced pod load or alteration of sink-source ratio behind delayed maturity disorder of soybean.

Introduction

Delayed maturity is a common disorder in soybean, *Glycine max* (L.) Merr. Throughout the US (Holshouser 2009). It consists of stems failing to mature even though pods mature and are ready to harvest (Schwenk and Nickell 1980). The presence of green stems at harvesting makes the use of combines difficult and may cause seed loss by pod shattering. Many causes of soybean delayed maturity have been reported such as bean pod mottle virus, environmental stress, and insect feeding, mainly by stink bugs (Duncan 1968, Todd and Turnipseed 1974, Panizzi et al. 1979, Schwenk and Nickell 1980, Holshouser 2009).

An association between stink bug feeding and occurrence of delayed maturity in soybeans has been found in several studies (Daugherty et al. 1964, Duncan 1968, Todd and Turnipseed 1974). Specifically, redbanded stink bug (RBSB), *Piezodorus guildinii* (Westwood) has been found to be a major cause of soybean delayed maturity, because it causes more damage per insect than any other stink bug species (Sosa-Gomez 1995, Correa-Ferreira and de Azevedo 2002). Consequently, increased occurrence of both delayed maturity and RBSB populations have been observed in Louisiana (Davis et al. 2011) and Texas (Vyavhare and Way 2013) in recent years. Studies done in Brazilian soybean in 1970s have related RBSB densities to occurrence of delayed maturity (Costa and Link 1977, Panizzi et al. 1979). However, little or no latest information is available regarding RBSB infestation and occurrence of delayed maturity in the US where RBSB has recently emerged as a serious pest of soybean. It is not known what RBSB threshold during pod development stage causes delayed maturity nor the mechanism/s involved in the induction of delayed maturity as a result of RBSB feeding.

Reduction in pod load is the common denominator between all factors thought to cause soybean delayed maturity (i.e., stink bugs, diseases and environmental stress). The current base of knowledge suggests that reduced pod load altering the ratio of photosynthetic source organs (e.g., leaves) to non-photosynthetic sink organs (e.g., pods) could be behind soybean delayed maturity. In plants photosynthetic matter production is regulated by photosynthetic source-sink balance (Kasai 2008)… In general, photosynthate move from the leaf to the regions of energy utilization such as the floral buds, developing seeds and pods (the sinks) (Egli et al. 1976). Stink bug feeding during R4-R5 stage soybeans is known to cause significant yield reduction through producing flat pods (empty pods) and reduced seed weight (Yeargan 1977). Fewer seed bearing pods and seeds as a result of RBSB feeding during R4-R5 stages would result in greater amount of photosynthate being directed into vegetative rather than reproductive plant parts resulting into delayed maturity.

Alteration of source-sink balance through mechanical pod removal have been shown to cause high levels of leaf chlorophyll retention and reduced rate of photosynthesis in soybean (Wittenbach 1982). Source-sink balance particularly at the grain filling stages (R5-R6) is a crucial factor in the regulation of leaf senescence (Miao et al. 2009). Nitrogen in grain is derived from nutrients that are taken up from roots and remobilized from vegetative organs of the plant (e.g. leaves, stems) to reproductive organs (e.g. flowers, pods) (Pan et al. 1986). Reduced nitrogen uptake during grain filling stages cause nutrients to remobilize from leaves and the stem to developing pods, subsequently leading to leaf senescence (Htwe et al. 2011). However, removal of sink decreases the nutrient remobilization from leaves and stem causing them to stay green longer (Htwe et al. 2011).

This study was conducted with two objectives. The first objective was to determine RBSB threshold causing delayed maturity in soybean. The second objective was to determine if reduced pod load (alteration of sink-source ratio) is the main cause of soybean delayed maturity or if some other mechanism/s could be involved. In order to determine the RBSB threshold for soybean delayed maturity to 51ccur, field cage experiments were conducted with different levels of RBSB infestation during R4-R5 stages in field grown soybeans. While in order to determine if reduced pod load was associated with soybean delayed maturity, experiments with different levels of mechanical pod removal were conducted. This study is the first to correlate sink reduction (mechanically and by RBSB feeding) with the green leaf retention at maturity in soybean.

Materials and methods

Field experiments were conducted at Texas A&M AgriLife Research and Extension Center in Beaumont, Texas, US over two years. The experiments were laid out as a completely randomized design. Glyphosate tolerant soybean variety (AG 6732) were planted in the field on June 10, 2012 and May 30, 2013, under irrigated conditions. Agronomic practices recommended by the Louisiana Agricultural Experiment Station and Louisiana Cooperative Extension Service (Levy 2012) for soybean production were used. The size of the study field was 27 m X 27 m and the spacing between rows was 0.3 m. Plant density of 4 plants/ 0.3 m was maintained by thinning out extra plants at early vegetative stage in the central eight rows of the study field. Four rows of which were used for RBSB infestation and the other four rows for mechanical pod removal experiments.

RBSB infestation experiment

Within the rows dedicated for the RBSB infestation experiment, spots with 4 plants/0.3 m were randomly selected and the adjacent plants from both sides were removed mechanically to make space around selected plants to install cages. When soybeans approached R4 (full pod), cylindrical, wire mesh cages (1.5 ft X 5 ft) were placed over plants with the top of cages enclosed with fine wire mesh. Cages were used such that they would not cause shading effect and alter plant canopy temperature (Hourly plant canopy temperature recorded for 24 hr under caged and uncaged conditions showed no significant effect of cages on canopy temperature). Prior to enclosure, plants were visually checked and made insect free. Plants were infested with four densities of RBSB: 0, 2, 4, and 8 adults/0.3 m (0, 0.5, 1, and 2 adults/plant). In 2012 our experiment consisted of four replications while in 2013 there were six. Hence, in 2012, 16 cages were examined. While in 2013, 24 such cages were examined in the field. Treatments (i.e., RBSB densities) were assigned randomly to each cage. RBSB adults were collected from soybean fields located in Jefferson County, TX a day prior to placing them inside field cages. Upon collection, RBSB adults were kept in the laboratory on fresh soybean pods taken from the same field where they were collected. On the day of infestation, only healthy and robust adults were confined inside field cages in the ratio of 1:1 male and female. Stink bugs were kept inside cages for 10 days. Plants were inspected daily and dead RBSB were replaced to keep herbivore pressure constant. After 10 days of infestation, cages were removed and plants were sprayed alternatively with λ-cyhalothrin (Karate EC, Zeneca, Wilmington, DE) @ 0.03 lb AI/ac, methyl parathion @ 0.75 lb AI/ac, and acephate (Orthene 75% SP, Arysta NC) @ 1 lb. AI/ac to insure minimum further insect activity that would mask the treatment effect.

Mechanical pod removal experiment

Within the rows dedicated for the mechanical pod removal experiment, spots with 4 plants/0.3 m were randomly selected and the adjacent plants from both sides were removed mechanically to make space around selected plants as in the RBSB infestation experiment. There were no cages used in this experiment. There were four levels of pod removal treatments: removal of 25%, 50%, 75% pods, and a control with no pod removal. Pod removal treatments were assigned when plants had approached R4 stage (full pod). In 2012, study was consisted of four replications while in 2013 there were six. For the first treatment i.e. 25% pod removal, one pod set from every four pod sets on each plant was removed by hand; for the 50% pod removal treatment, every alternate pod set from the stem was removed; for the 75% pod removal three of the every four pod sets were removed. After mechanical pod removal, plants were irrigated regularly to allow growth. Plants were sprayed with λ-cyhalothrin (Karate EC, Zeneca, Wilmington, DE) @ 0.03 lb AI/ac, methyl parathion @ 0.75 lb AI/ac, and acephate (Orthene 75% SP, Arysta NC) @ 1 lb. AI/ac using a hand sprayer in order to protect plants from any kind of insect damage which may mask treatment effects.

Parameters recorded

In both the experiments, rate of photosynthesis and leaf chlorophyll content were recorded at soybean stage R6 (full seed), number of green leaves/0.3 m were recorded at maturity as an indicator of delayed maturity syndrome, and yield parameters such as 100 seed weight and yield were recorded upon harvesting. Number of flat pods per plant was recorded for the RBSB infestation experiment to determine the extent of sink (pod) removal at each level of RBSB infestation.

Photosynthetic measurements were taken following the procedure used by Macedo et al. 2003. Within each replication, we randomly selected two individual leaflets from any two plants out of the four that comprised each treatment, with the restriction that only fully expanded leaflets from the uppermost three nodes were used (to ensure leaves used were of comparable age). A portable photosynthesis system (Model Licor-6400, Li-Cor, Lincoln, NE) with CO2 injector and light source (in order to maintain stable CO2 and light concentrations for all measurements) was used to measure gas exchange parameters. Rate of photosynthesis was measured on 6 cm^2 leaf sections, the maximum leaf area measured by LI-6400. Photosynthetic measurements were taken at 1600 µmol photons m^{-2} s^{-1} light intensity, 400 intercellular CO_2 concentration, and 45-55% of chamber humidity.

Leaf chlorophyll content was determined in the same leaflets used for photosynthetic measurements using a chlorophyll meter, (Model, Spad-502, Minolta, Japan). Within each replication, four chlorophyll readings were taken (two per leaflet) which were then averaged to be used as a single replication.

Data analysis

Data were subjected to analysis of variance (ANOVA) for the response variables of yield, number of green leaves, leaf chlorophyll content, and rate of photosynthesis using PROC GLM procedure (SAS Institute 2010). Data from RBSB infestation and mechanical pod removal were analyzed separately. Pairwise treatment differences were determined using Tukey test. In addition to the overall ANOVA, correlation and regression analysis were used to examine relationship among response variables (numbers of RBSBs, flat pods, green leaves at maturity, and % of mechanical pod removal).

Results

RBSB infestation had a significant effect on soybean yield (*F* = 6.89, *df* = 3, *P* = 0.0014) (Fig. 10A). The highest yield (86.17g) was recorded in our control treatment while it was the lowest (41.27g) when RBSB density was 8 adults/0.3 m. The decrease in yield correlated an increased number of flat pods in response to RBSB feeding. Numbers of flat pods varied significantly when RBSB density was 8 adults/0.3 m (*F* = 16.55, *df* = 3, *P* <0.0001) (Fig. 11). Numbers of green leaves retained on plants at maturity where the highest when RBSB density was 8 adults/0.3 m (*F* = 35.62, *df* = 3, *P* <0.0001) (Fig. 10B).

Even though there was significant yield reduction in response to RBSB infestation at 8 adults/0.3 m, no significant effect on the rate of photosynthesis was observed (*F* = 0.94, *df* = 3, *P* = 42.81) (Fig. 12). Leaf chlorophyll content was significantly affected by RBSB infestation (*F* = 6.88, *df* = 3, *P* = 0.0014) (Fig. 12). Significant increase in leaf chlorophyll content was observed under all RBSB infestation levels as compared to control plants.

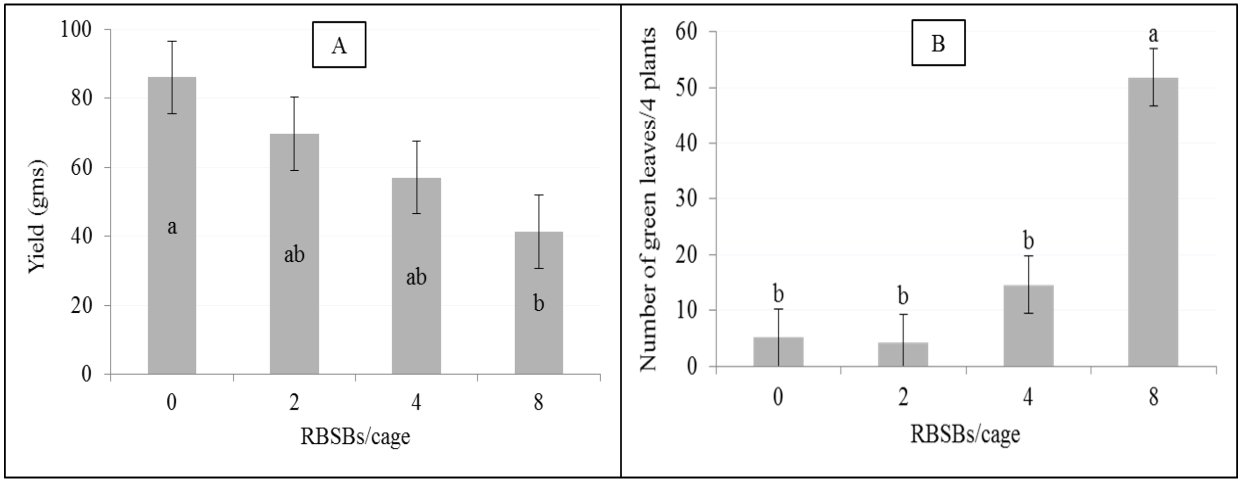

Figure 10. Effect of RBSB on yield (A) and numbers of green leaves at maturity (B).

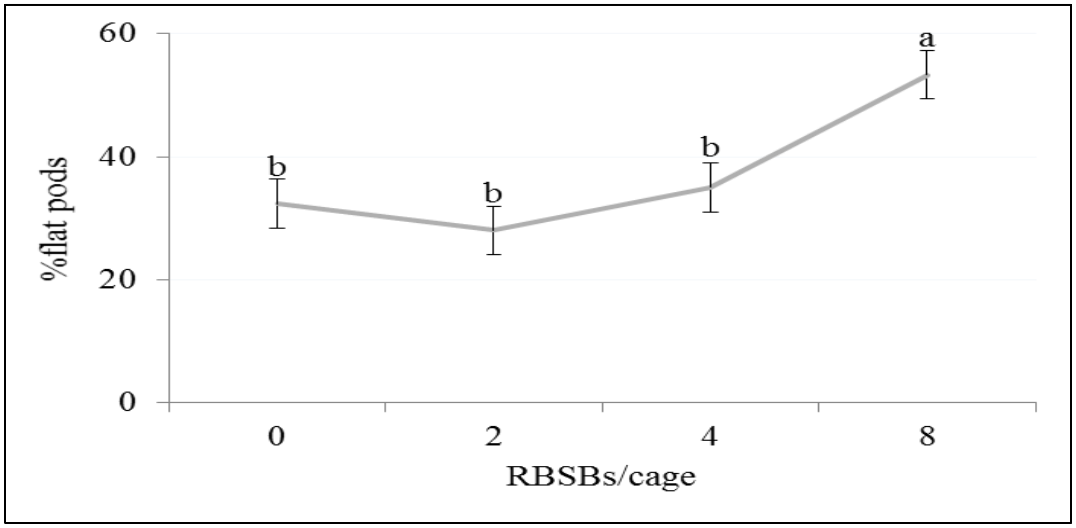

Figure 11. RBSB infestation and development of flat pods.

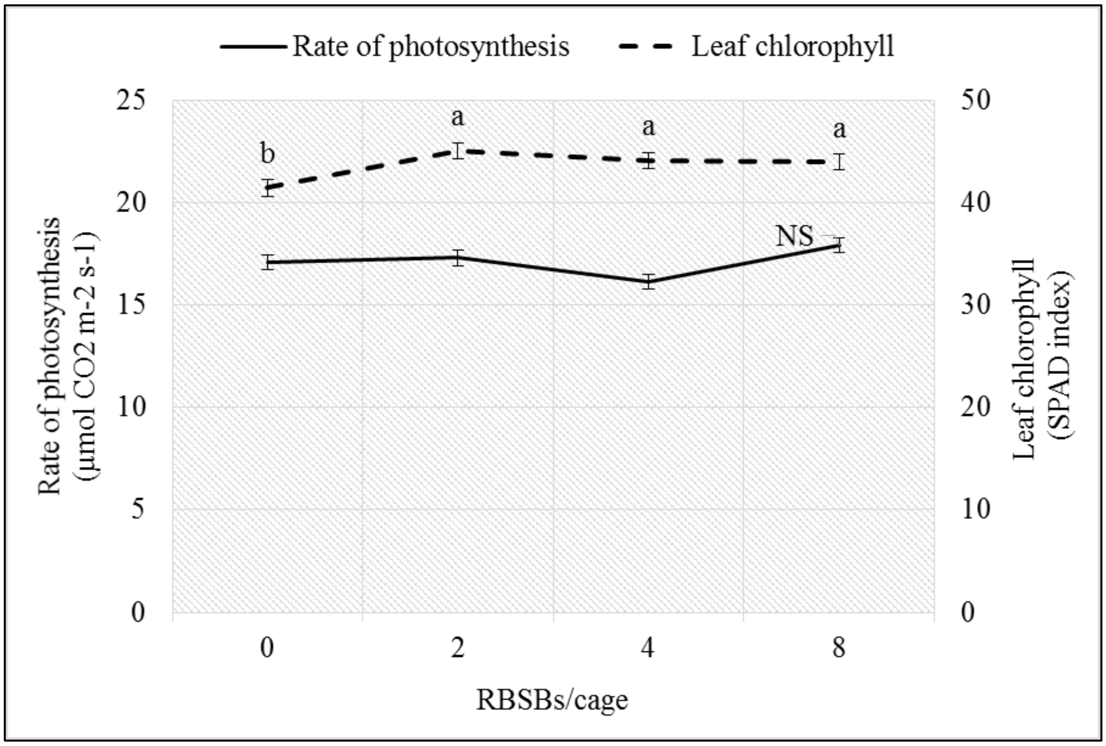

Figure 12. Effect of RBSB on rate of photosynthesis and leaf chlorophyll content. Means showing same lowercase letter are not significantly different. NS=not significant

A mechanical pod removal of 75% had a significant effect on yield (*F* = 3.65, *df* = 3, *P* = 0.0354) (Fig. 13A). However, no significant effect of mechanical pod removal was observed on green leaf retention at maturity (*F* = 2.12, *df* = 3, *P* = 0.138) (Fig. 13B).

The effects of pod removal on leaf chlorophyll content and rate of photosynthesis are shown in Fig. 14. The amount of leaf chlorophyll remained constant across treatments indicating no effect of pod removal on leaf chlorophyll content (*F* = 0.17, *df* = 3, *P* = 0.9174). However, significant reduction in rate of photosynthesis was observed following mechanical pod removal (*F* = 4.83, *df* = 3, *P* = 0.0042).

There was significant positive correlation between RBSB density and green leaf retention in soybean (r = 0.72, *P* < 0.0001) (Table 7). Also, significant positive correlation was observed between RBSB density and % flat pods; and % flat pods and green leaf retention. On the other hand, no significant correlation between mechanical pod removal and green leaf retention was observed.

Figures 15 and 16 further illustrate the difference between mechanical pod removal and RBSB infestation treatments. There was no relationship between mechanical pod removal and green leaf retention in soybean. While RBSB induced pod removal (flat pods) was correlated with green leaf retention at maturity. The relationship between RBSB density and green leaf retention was the strongest (Fig. 16).

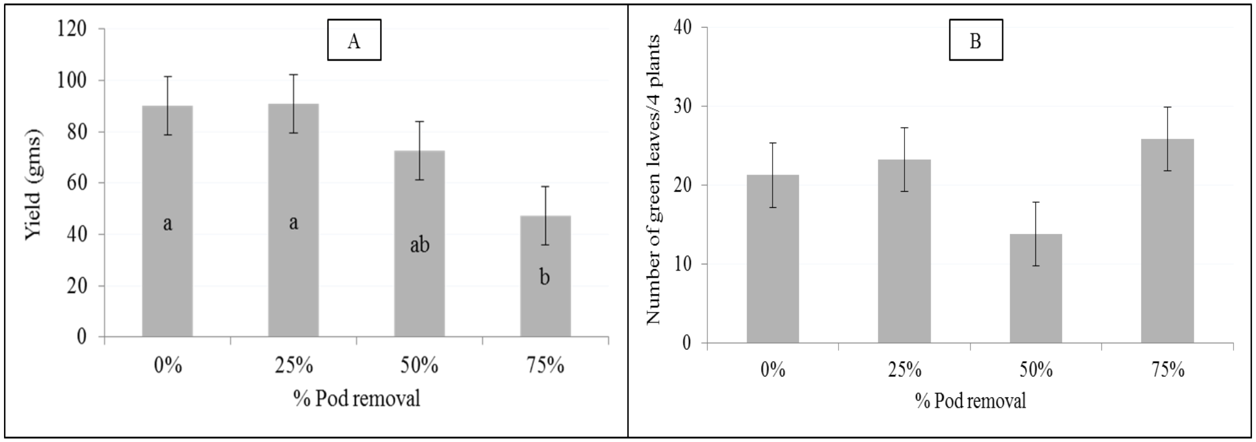

Figure 13. Effect of pod removal on yield (A) and green leaf retention at maturity (B) in soybean

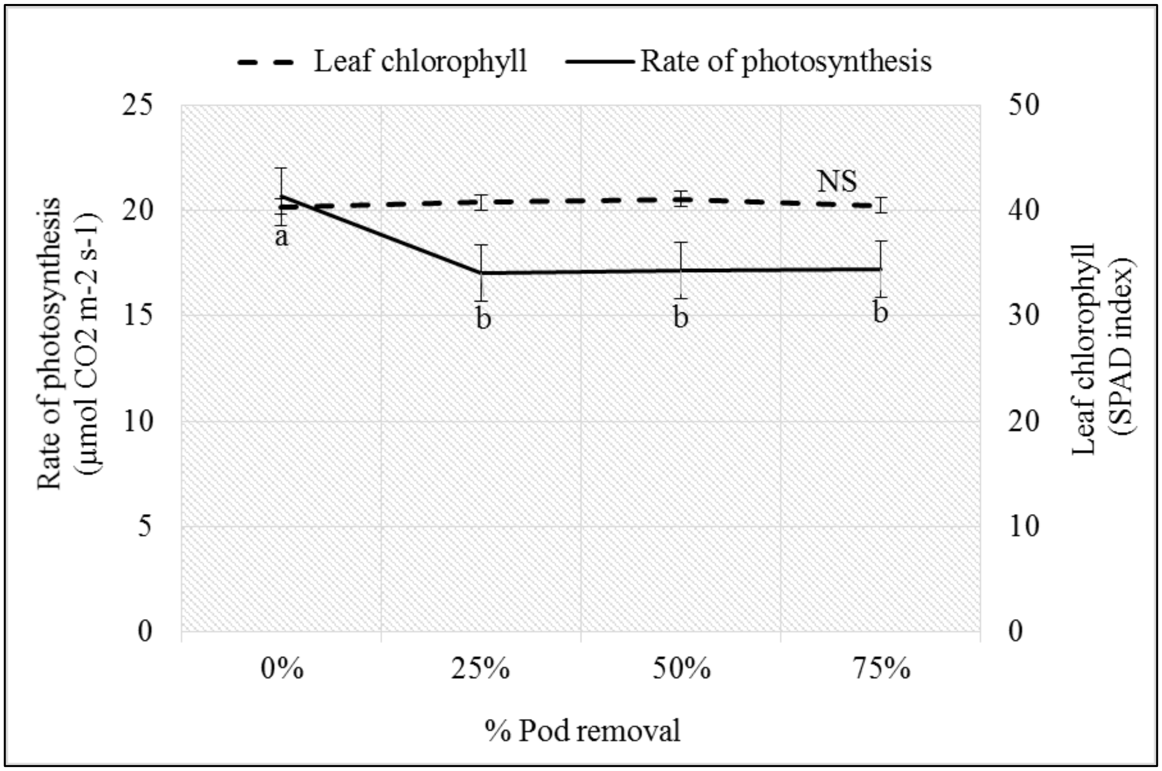

Figure 14. The effect of pod removal on leaf chlorophyll content and rate of photosynthesis in soybean. NS=not significant

Table 7. Correlation coefficients (R) for mechanical pod removal, RBSB induced flat pods, RBSB density, and green leaf retention at maturity and RBSB density

	Mechanical pod removal	RBSB induced flat pods	RBSB density
Green leaf retention	0.26^{ns} (0.0988)	0.38* (0.0159)	0.72* (<0.0001)
RBSB density		0.49* (0.0014)	

*Significant at the 0.05 probability level
ns= not significant; (*P value*)

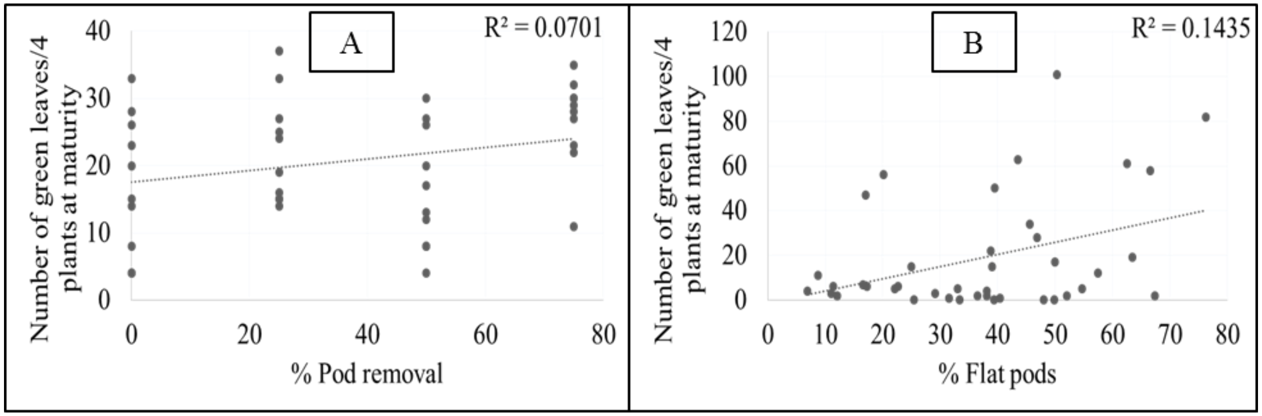

Figure 15. Relationship between mechanical pod removal and green leaf retention (A); and RBSB induced pod removal (flat pods) and green leaf retention (B). Coefficients of determination (R^2) are noted.

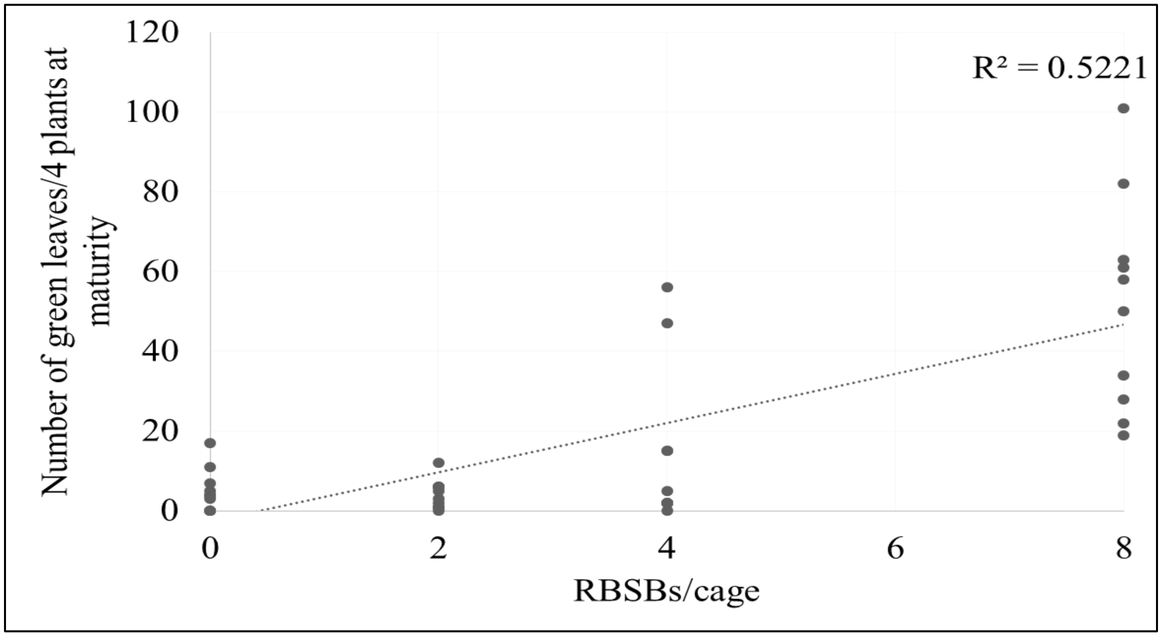

Figure 16. Relationship between RBSB density and green leaf retention at maturity in soybean. Coefficient of determination (R^2) is noted.

Discussion

Results from RBSB infestation experiments demonstrated that RBSB density of 8 adults/0.3 m (2 adults/plant) during R4-R5 stages triggers delayed maturity in soybean variety AG 6732. Studies done in Brazilian soybean have also found a correlation between RBSB feeding and occurrence of soybean delayed maturity. For example, (Panizzi et al. 1979) reported foliage retention at RBSB densities 0.3-1.3 adults/0.3 m with infestation maintained for 25 d during R3-R8 stage soybeans. In contrast, the current study found that higher RBSB density (8 adults/0.3 m) was required to cause delay maturity in soybean. This could be because of different climatic conditions under both the regions where experiments were conducted which may affect plant growth and/or variable response of different soybean varieties as occurrence of delayed maturity is known to vary with the soybean varieties (Holshouser 2009). Several studies have related feeding by other stink bug species to delayed maturity in soybean. For example, (Boethel et al. 2000) showed that *N. viridula* infestation and occurrence of soybean delayed maturity. *E. servus* was also shown to cause delayed maturity in soybean (Daugherty et al. 1964). However, little have been done on elucidating the mechanism behind stink bug induced delayed maturity in soybean. Reduced pod load and altered source-sink ratio due to stink bug feeding was thought to be a potential mechanism causing delayed maturity, but before the current study, no study had compared the response of same soybean variety to sink removal by stink bug feeding and mechanical pod removal through the point of view of delayed maturity.

Results from mechanical pod removal experiments showed no relationship between reduced pod load and occurrence of delayed maturity. Also, the correlation between RBSB induced pod removal (flat pods) and green leaf retention was weak (Table 7). RBSB density, however, had strong positive correlation with the occurrence of soybean delayed maturity indicated by green leaf retention. These results indicate that occurrence of soybean delayed maturity in response to RBSB feeding may not be attributed exclusively to reduction in pod load. If soybean delayed maturity was solely due to reduced pod load, plants with up to 75% of mechanical pod removal would have retained green leaves as well. However, green leaves were retained only in response to high RBSB density (8 adults/0.3 m) but not in response to mechanical pod removal which indicates the possible involvement of additional mechanism(s) than just pod reduction or alteration of sink-source ratio behind RBSB induced soybean delayed maturity.

The literature suggests that reduced pod load or altered sink-source ratio can have significant effects on the rate of photosynthesis or on the accumulation of photosynthate and ultimately plant maturity in soybean. For example, soybean is known to have an ability to adjust its rate of photosynthesis depending upon the demand from sinks or depending upon source-sink ratio (Kasai 2008). There is evidence showing reduction in the rate of photosynthesis in response to removal of developing pods which exert high demand for photosynthate (Thomas and Stoddart 1980). Similarly, there is evidence demonstrating a decrease in the rate of photosynthesis in soybeans in response to mechanical pod removal due to reduced demand for assimilate in depodded plants, even though leaf chlorophyll content was not decreased (Nooden 1984). Our results from the mechanical pod removal experiment were in agreement with those from Nooden (1984) study as we also observed reduction in rate of photosynthesis in response to pod removal and no effect on leaf chlorophyll content (Fig. 14). In contrast, in the RBSB infestation experiment, even though there was significant decrease in the amount of sink (increase in numbers of flat pods and decrease in yield) at the RBSB density of 8 adults/0.3 m, there was no reduction in the rate of photosynthesis and leaf chlorophyll content was significantly increased (Fig. 12). Thus, soybean plants responded differently to sink removal by mechanical pod removal than by RBSB feeding. In the case of sink removal by RBSB, instead of showing signs of senescence such as reduced rate of photosynthesis, plants continued photosynthesis, showed an increase in the amount of leaf chlorophyll (Fig. 12), and retained significantly higher numbers of green leaves at maturity (Fig. 10B).

Some caution is advice before using the results of this study to exclude the possibility of the involvement of sink-source imbalance in the occurrence of soybean delayed maturity. I did not measure the partitioning of assimilate into vegetative and reproductive plant parts in response to sink removal by both mechanical removal and RBSB feeding. This partitioning will shed more light on the understanding of the role of sink removal on photosynthate accumulation in source organs and on the tendency of plants to stay green. Therefore, further studies are needed to estimate the partioning of assimilate between different plant organs in response to sink removal by both mechanical pod removal and RBSB feeding. Also, although it is possible that the iron mesh cages installed to confine RBSBs on plants could have produced a cage effect on these treatments, I find that possibility unlikely. Temperature was compared within and outside cages and no variation was detected. Furthermore, as RBSB is known to have more deleterious salivary enzymes than other stink bug species (Depieri and Panizzi 2011), exploring if RBSB feeding cause hormonal imbalances in plants could shed light into the mechanisms by which this insect produce delayed maturity. Hormones such as cytokinin, gibberellin, and ethylene are known to regulate senescence in plants (Nooden et al. 1997). Thus, the effect of RBSB in changing the expression of specific phytohormones is one of the areas in which future research could be conducted.

In conclusion, this study indicates that high RBSB density (8 adults/0.3 m) during R4-R5 stage soybeans can delay soybean maturity. Results of this study suggest that the RBSB induced delayed maturity in soybean may not be solely due to reduced pod load or alteration of source-sink ratio, but that additional mechanisms may be involved.

CHAPTER V

BASELINE INSECTICIDE SUSCEPTIBILITY OF REDBANDED STINK BUG (HEMIPTERA: PENTATOMIDAE) FIELD POPULATIONS IN TEXAS SOYBEAN

Synopsis

Redbanded stink bug (RBSB), *Piezodorus guildinii* (Westwood) is a relatively new pest of soybean, Glycine max (L.) Merr. in the southern US. Invasion by this neotropic pentatomid has been responsible for a substantial increase in the amount of insecticides applied in soybean potentially triggering the development of insecticide resistance in RBSB. This study was conducted to generate baseline data on insecticide susceptibility levels in current RBSB field populations. RBSB adults collected from commercial soybean fields were used in glass vial bioassay to determine LC50 values for pyrethroids (bifenthrin and cyfluthrin), neonicotinoids (thiamethoxam and imidacloprid), and an organophosphate (acephate) using technical grade materials. In addition, a small plot field trial was conducted to determine the efficacy of some commonly used pyrethroid, neonicotenoid and organophosphate formulations against RBSB. Glass-vial bioassays generated LC50 values of 0.76 µg/vial for bifenthrin, 0.18 µg/vial for cyfluthrin, 2.32 µg/vial for thiamethoxam, and 1.07 µg/vial, for imidacloprid after 4h of exposure. When RBSBs were exposed to acephate for 4h in vial bioassays, no more than 20% of mortality was recorded preventing the calculation of LC50 while after 24 hours of exposure a LC50 of 2.84 µg/vial was generated. Results from the field trial found significant reduction in numbers of surviving RBSBs one day after treatment (DAT) in all insecticide treatments except acephate when compared to untreated plots. While at 12 DAT, all insecticide treatments including acephate showed significant reduction in numbers of RBSBs. Results from both laboratory bioassay and field trial showed that RBSBs are more susceptible to neonicotinic and pyrethroid insecticides than to the organophosphate acephate, which took longer time to show RBSB mortality.

Keywords: Redbanded stink bug, insecticides, susceptibility, LC50.

Introduction

Historically, the soybean stink bug pest complex in the southern US has consisted of mainly three species, viz., southern green stink bug (SGSB) *Nezara viridula* (L.), the green stink bug (GSB) *Chinavia hilaris* (Say), and the brown stink bug (BSB) *Euschistus servus* (Say) (McPherson et al. 1993). However, during the past decade, the redbanded stink bug (RBSB), *Piezodorus guildinii* (Westwood), has emerged as a major soybean pest in this region. RBSB, has been known as a serious pest of soybeans in the neotropics since the 1960s (Panizzi et al. 2000). In the US, it was first reported in Florida in the 1960s (Genung et al. 1964), however, it was never considered as an important pest of US soybeans until 2000 when this species was first reported in south Louisiana (Temple et al. 2011). By 2002, the RBSB in southern Louisiana was found to exceed the action threshold commonly used for the stink bug complex (i.e., southern green stink bug, green stinkbug and brown stink bug together). Soon after, RBSB spread rapidly, infesting entire soybean growing areas in Louisiana by 2006 (Davis et al. 2011).

Currently, the RBSB has become a significant portion of the overall stink bug complex in Louisiana soybeans (Temple et al. 2011). Similarly, soybean field surveys conducted during 2011-2013 across the Upper Gulf Coast of Texas have shown RBSB as the most abundant stink bug species in this area (see chapter 2).

Stink bug pests mainly feed on young, tender growth and developing seeds (McPherson et al. 1994). The RBSB causes more damage per insect than other stink bug species on soybean (Correa-Ferreira and de Azevedo 2002). The relatively higher damage caused by RBSB when compared with other stink bug pests is thought to be due to the more deleterious action of RBSB salivary enzymes when compared to other species (Depieri and Panizzi 2011). In addition to direct damage, RBSB is mainly responsible for delayed maturity syndrome in soybeans (Sosa-Gomez 1995)

Control of RBSB relies almost exclusively on insecticides. One of the most serious concerns regarding RBSB management is its low susceptibility to labeled insecticides. For example, RBSB populations in Louisiana have been observed to be less susceptible to currently available products compared to other commonly known stink bug species which has resulted in a significant increase in insecticide applications on soybeans (Davis et al. 2011). Esterases are responsible for insect resistance to organophosphates and pyrethroids (Li et al. 2007). Baur et al. (2010) reported higher esterase activity in RBSB populations in Louisiana than in Brazil, even though the later have a longer history of organophosphate use (>30 years). Similarly, although the US has a shorter history than Brazil in controlling RBSB populations using organophosphates, RBSB populations in the US are more tolerant to organophosphates than in Brazil (Baur et al. 2010). Reduced organophosphate susceptibility of RBSB populations in Louisiana might be caused by the relatively higher doses of insecticides used in US soybean when compared to Brazil (Baur et al. 2010). Until recently, acephate was the only product recommended for RBSB in the US. Dependence on this single product continuously for nearly a decade has raised concerns about development of resistance in RBSB. Recently, insecticides with different modes of action (e.g., neonicotinoids) have been recommended and registered for RBSB control (soybean Insect Control Guide, http://www.lsuagcenter.com/). Currently, no information exists about the susceptibility levels of RBSB field populations in Texas where multiple insecticide applications targeting RBSB have become more common. Surveys of insecticide susceptibility levels among insect populations are crucial for they help detecting any shift in insecticide performance and provide early warning to modify chemical control strategies so that resistance development in insect population is avoided or delayed. The objectives of this study were to establish baseline susceptibility data for RBSB field populations in Texas soybeans using vial bioassay with organophosphate (acephate), pyrethroids (bifenthrin and cyfluthrin), and neonicotinoids (imidacloprid and thiamethoxam). The Second objective was to determine the field efficacy of selected insecticide formulations currently recommended for RBSB management.

Materials and methods

Field trial for insecticide evaluation

Field trials were conducted at the Texas A&M AgriLife Research and Extension Center, Beaumont. Roundup ready AG 6732 soybean was planted on a 46 m^2 plot on July 11th, 2013 under irrigated conditions. A day after planting, pre-emergence herbicides, First Rate (Dow AgroSciences, Indianapolis, IN) @ 0.75 oz/A and Dual Magnum (Syngenta crop protection, Greensboro, NC) @ 2.5 pt/A were applied with a 2-person hand-held spray boom (13- No. 2 cone nozzles, 50 mesh screens, 15 gpa final spray volume). Plots were trimmed to 40 ft length after emergence. Spacing between rows was 30 inches and each plot consisted of four rows. Weeds were controlled by Roundup (Monsanto, St. Louis, MO) spray @ 1% concentration by volume. 5 ft alleys (3 rows) were left between plots as a buffer. Insecticide treatments were arranged in a randomized complete block design with six treatments: lambda-cyhalothrin (Karate Z, Syngenta Crop Protection, Greensboro, NC), acephate (Orthene 90S, AMVAC Chemical Corporation, Los Angeles, CA), beta cyfluthrin and imidacloprid (Leverage 360, Bayer CropScience, Research Triangle Park, NC), lambda-cyhalothrin and thiamethoxam (Endigo ZC, Syngenta Crop Protection, Greensboro, NC), bifenthrin (Brigade 2EC, FMC Corporation, Philadelphia, PA) and a control consisting of unsprayed soybeans. Each treatment had four replications. Treatments were applied at standard recommended rates (Table 8) at R6 (full seed) when stink bug populations were at their peak.

Table 8. Treatment descriptions and rates

Commercial Product	Active Compound	Rate (Kg AI /ha)
Orthene 90S	acephate	1.01
Leverage 360	beta cyfluthrin + imidacloprid	0.02 + 0.04
Endigo ZC	lambda cyhalothrin + thiamethoxam	0.028 + 0.037
Brigade 2EC	bifenthrin	0.09
Karate Z	lambda cyhalothrin	0.028

Insecticides were applied using a hand-held spray boom (2-nozzle boom (Conejet TSS cone nozzles, 50 mesh screens, 22 gpa final spray volume). Experimental plots were sampled for insects using a standard 38 cm diameter sweep net (12 sweeps/plot) 1 day after treatment (DAT) and 12 DAT. Samples were collected in zip lock plastic bags and taken to the laboratory for further processing. Laboratory processing consisted of quantifying RBSB adults and nymphs (3rd, 4th, and 5th instars only).

Data analysis

Analysis of variance (ANOVA) (PROC GLM, SAS Institute 2010) was used to determine if any of the insecticide treatments significantly affected RBSB mean densities. Multiple comparisons among treatment means were made using the Bonferroni mean separation test (PROC GLM, SAS Institute 2010).

Vial bioassay

Field collected RBSB adults were used for glass-vial bioassays. Insects were collected from soybean fields near Rosharon, TX during august 2013 using a standard 38 cm diameter sweep net. Adults were kept at room temperature for 24 hrs before bioassays and fed with washed pods of soybean, collected from the same field where RBSBs were collected. Vial bioassays were conducted using technical grade materials of three groups of insecticides with different modes of action: An organophosphate (acephate), neonicotinoids (thiamethoxam and imidacloprid) and pyrethroids (cyfluthrin and bifenthrin). Acephate (99.5% wt:wt) was obtained from Chem Service (West Chester, PA); Imidacloprid (98.80% wt:wt) and beta cyfluthrin (98.40% wt:wt) were obtained from Bayer Environmental Science (Durham, NC); Thiamethoxam (99.6%

wt:wt) was obtained from Sigma-Aldrich (Milwaukee, WI) and Bifentrhin (98.1% wt:wt) was obtained from FMC Corporation (Philadelphia, PA). Glass-vial bioassays followed Willrich et al. (2013) methodology. The range of concentrations of each insecticide that caused 20% to 80% mortality after 24 hrs of exposure in preliminary bioassays was used. Technical grade materials were dissolved in 99.5% acetone (Sigma-Aldrich, Milwaukee, WI) to make stock solutions, which were diluted to the bioassay concentrations on the day of the bioassay. Nine to ten concentrations of each compound were used. The concentration of acephate ranged from 0 to 6.0 µg/vial. The concentrations of pyrethroid and neonicotinoid insecticides ranged from 0 to 2.0 µg/vial. Control vials received only acetone. No modification in the vial bioassay was made for systemic insecticides imidacloprid and thiamethoxam because they also exhibit contact activity against sucking insects (Mullins 1993, Maienfisch et al. 2001). The interior surface of 20 ml glass scintillation vials was coated with 0.5 ml of appropriate insecticides diluted in acetone. Vials were rotated on a hot dog roller (with heating unit turned off) until all the acetone evaporated. Vials were placed in the dark until use in bioassays. There were ten replications of each treatment with one RBSB adult placed in each vial. Mortality was determined 4h and 24h after exposure. The criterion for mortality was inability of insects to assume an upright posture within 5 seconds after being dislodged from vials (Willrich et al. 2003).

Analysis of dose-response data

Mortality in untreated vials was never above 10% therefore, there was no need to determine corrected mortality (Abbott 1925). Mortality data was subjected to probit analysis using PoloPlus (LeOra Software, Petaluma, CA) to generate LC_{50} and 95% confidence intervals (CI). Chi-square values (χ^2) were used to estimate how well probit models fit the dose-mortality data.

Results

Results from field trials showed that all the insecticides used were effective at controlling RBSB (i.e., mean number of RBSBs per 12 sweeps for each treatment was significantly lower than the control) at 1 DAT except for acephate which showed no significant difference from untreated plots. No significant effect of sampling date was observed on RBSB population (Table 9). At 12 DAT, all insecticide treatments including acephate showed significantly less numbers of RBSBs/12 sweeps than untreated plots (Fig. 17).

Table 9. Analysis of variance indicating the significance of insecticide treatment, replication and sampling date on mean number of RBSBs/12 sweeps.

Source	DF	F value	*P* value
Treatment	5	14.35	<.0001
Replication	3	2.05	0.1263
Sampling date	1	1.91	0.1759

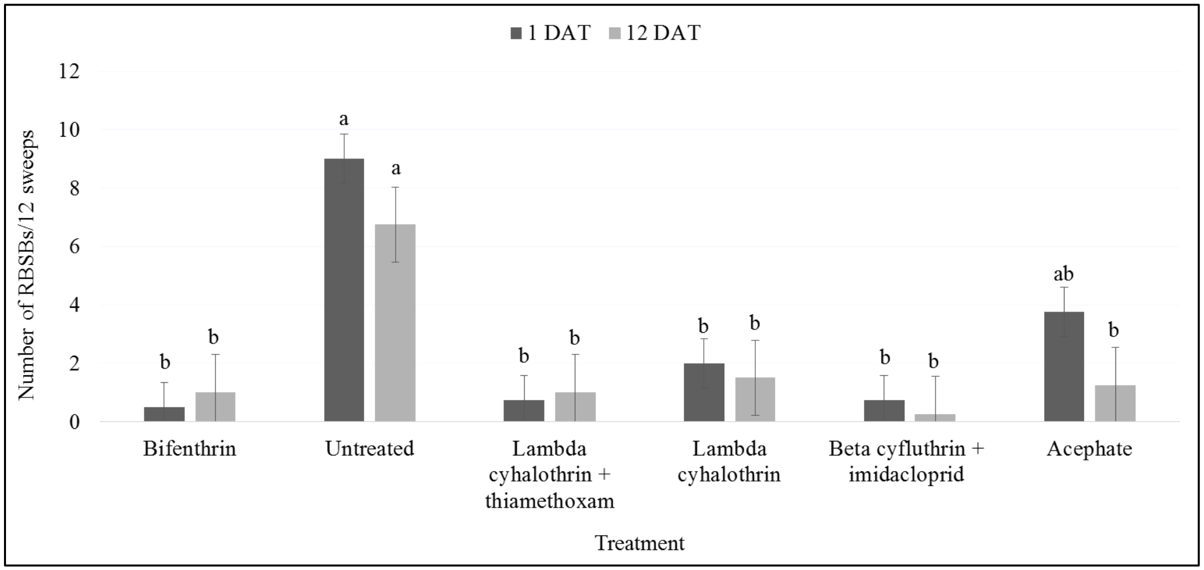

Figure 17. Number of RBSBs per 12 sweeps. Bars showing same letters are not significantly different (P <.0001, Bonferroni)

Estimates of LC_{50} and 95% confidence intervals were calculated for field collected RBSB adults exposed to insecticide treatments. The predicted values of the probit model did not differ significantly from the observed values in vial bioassays (Tables 10 and 11), indicating that the probit model was suitable for the dose-mortality analysis.

After 4h of exposure with acephate, no LC_{50} value was generated because no more than 20% mortality was recorded in acephate treated vials. This indicates that after 4h from vial bioassay, LC_{50} value with acephate should be more than 6 µg/vial in RBSB population we studied. In case of pyrethroid and neonicotinoid insecticides, the bioassay yielded enough numbers of knocked down individuals after 4h to be able to generate LC_{50} values (Table 10).

There was relatively higher mortality after 24h of exposure than after 4h of exposure in vial bioassay with acephate. As a result, after 24h vial bioassays with acephate, we were able to generate a LC_{50} value of 2.84 µg/vial (Table 11).

Table 10. Mortality recorded after 4h from vial bioassay on field populations of RBSB collected near Roshaton, TX in August 2013

Insecticide	N[a]	Slope ± SE	LC_{50}[b] (CI 95%)	χ^2 (df)
Acephate	110	-	-	-
Imidacloprid	110	1.51 ± 0.32	1.07 (0.56-2.90)	11.54
Thiamethoxam	110	0.91 ± 0.27	2.32 (1.02-24.90	4.23
Bifenthrin	100	2.01 ± 0.55	0.76 (0.50-1.06)	5.74
Cyfluthrin	110	1.10 ± 0.31	0.18 (0.08-0.32)	7.17

[a] Total number tested including controls

[b] Lethal concentration expressed in µg insecticide $vial^{-1}$ with 95% confidence intervals (CI)

Table 11. Mortality recorded after 24h from vial bioassays on field populations of RBSB collected near Roshaton, TX in August 2013

Insecticide	N[a]	Slope ± SE	LC_{50}[b] (CI 95%)	χ^2 (df)
Acephate	110	3.03 ± 0.73	2.84 (2.06-3.61)	2.25[NS]
Imidacloprid	110	1.07 ± 0.24	0.66 (0.37-1.34)	6.91
Thiamethoxam	110	1.15 ± 0.24	0.36 (0.20-0.64)	4.04
Bifenthrin	100	3.40 ± 0.69	0.76 (0.59-0.93)	6.04
Cyfluthrin	110	1.13 ± 0.30	0.46 (0.27-1.11)**	2.55 [NS]

[a] Total number tested including controls

[b] Lethal concentration expressed in µg insecticide $vial^{-1}$ with 95% confidence intervals (CI)

[NS] The predicted values of the probit model did not differ significantly from the observed values in vial bioassays ($P \leq 0.025$)

**Lesser numbers of RBSB individuals showed knock down symptoms at 24h than that of at 4h of exposure resulting in higher LC_{50} value with longer exposure

In case of the pyrethroid bifenthrin, after 24h of exposure in vial bioassay the overall mortality (dead + knocked down) remained the same as that recorded after 4h of exposure. However, there was an increase in the relative proportion of dead versus knocked down RBSBs. This resulted in the same bifenthrin LC_{50} value after 4h and 24h of exposure. The pyrethroid beta cyfluthrin, showed an unusual trend. The LC_{50} value after 24h of beta cyfluthrin exposure was greater than after 4h (Table 11). This is because, after 4h of exposure with beta cyfluthrin, several RBSBs were knocked down but recuperated after 24h of exposure. Some of the individuals that showed knocked down symptoms after 4h of exposure with beta cyfluthrin could have recovered from toxicity by the time when RBSB mortality was recorded at 24h. This resulted in overall lesser mortality at 24h after vial bioassay than at 4h generating higher LC_{50} value at 24h and lower LC_{50} value at 4h.

Discussion

Dose-mortality data from this study provides a benchmark for future evaluation of insecticide susceptibility of RBSB populations. Because currently no laboratory maintains standard susceptible colonies of RBSB, future studies to determine changes in insecticide susceptibility can compare back to historical values such as the ones generated in this study. This data can also be used for the immediate purpose of comparing current field data to that of previously determined LC_{50} values from other geographic locations. This kind of monitoring data may play an important role in adjusting chemical control strategies such that they would increase the durability of insecticides.

Baur et al. (2010) conducted glass-vial bioassay on RBSB populations collected in Iberville, Parish, LA in 2004 and recorded a LC_{50} value of 3.83 µg/vial after 4h of exposure to acephate. In our study, no LC_{50} value could be generated with acephate after 4 hr of exposure in vial bioassays. No more than 20% RBSB mortality was observed at any of the acephate concentrations used (0 to 6 µg/vial) (Table 10). This indicates that the LC_{50} for acephate after 4h of exposure in vial bioassay lies above 6 µg/vial in the RBSB population we studied. The relatively higher acephate LC_{50} in current Texas RBSB populations when compared with Louisiana populations tested in 2004 suggests higher tolerance to this insecticide. If one assumes that RBSBs from Louisiana and Texas belong to the same panmictic population, one could argue that RBSB populations seem to have developed resistance to acephate.

Following a day of treatment, acephate treated plots had a mean RBSB density which was not significantly different from untreated plots (Fig.17) which was still above the action threshold (~3 RBSBs/12 sweeps) (soybean Insect Control Guide, http://www.lsuagcenter.com/). While pyrethroids and mixtures of pyrethroids and neonicotinoids were able to bring down RBSB population below action threshold relatively fast, as indicated by the significant reduction in RBSB populations in treated plots at 1DAT. Similarly, vial bioassays showed slower activity of acephate against RBSB. After 4h in the acephate vial bioassay, RBSB mortality was too little to calculate LC_{50} (Table 3) while pyrethroids and neonicotinoids had a rapid knockdown effect on

RBSB. Our field results should be interpreted with caution. High RBSB mobility (Panizzi et al. 1980) combined with the relatively small size of our experimental plots may have affected our insecticide efficacy results.

In summary, this study provides baseline data on current resistance/susceptibility levels of RBSB field populations in Texas to insecticide chemistries with different modes of action. RBSB susceptibility to insecticides needs to be determined at other locations in Texas and Louisiana where RBSB has emerged as a serious soybean pest to determine the overall status of insecticide resistance in RBSB. Currently, RBSB management is solely dependent upon insecticide applications (5-7 applications per season). If current rates of insecticide use against RBSB continue, development of insecticide resistance in this pentatomid pest is likely.

CHAPTER VI

CONCLUSION

Historically, three stink bug species: southern green stink bug (SGSB), green stink bug (GSB), and brown sting bug (BSB), formed the stink bug complex in southern US soybean. However, in recent years, the redbanded sting bug (RBSB) has emerged as a pest, which poses a substantial threat to soybean production in this region. The geographic range of RBSB includes regions in Argentina and Brazil where it’s been known to cause economic damage to soybean since 1960s. Consequently, considerable work has been done on RBSB and its impact on soybean production in Brazil. However, in the US where RBSB has only recently emerged as a serious pest of soybean, little information exists regarding its biology, ecology, damage potential, and management tactics which is why this dissertation research was undertaken with the goal to gather information that will contribute towards achieving the long term goal of developing and implementing an integrated pest management program for RBSB.

The specific objectives of this study were to determine the relative abundance of major stink bug species (SGSB, GSB, BSB, and RBSB) across different soybean growth stages on the Upper Gulf Coast of Texas; to determine the growth stage specific response of soybean to RBSB; to determine the RBSB threshold that triggers delayed maturity and to find out if delayed maturity is due to reduced pod load; and finally, to generate baseline data on insecticide susceptibility of RBSB field population and evaluate efficacy of commonly used insecticides against RBSB.

In order to develop an integrated pest management program for any insect pest, it is of prime importance to know the abundance of the target species in the threaten commodity. Data on the relative abundance of target pests aid in assessments of the extent of risk and economic damage and improve the design of specific control actions. A three year survey study across commercial soybean production areas in the Upper Gulf Coast of Texas found that RBSB has become the most dominant stink bug species in this area. This is the first study reporting a shift in stink bug species composition relative to RBSB abundance in TX soybean. Increased abundance of RBSB over previously known major stink bug species (SGSB, GSB, and BSB) has become a major concern for soybean growers in TX, because RBSB causes more damage per insect than other stink bug species.

Soybean fields infested with RBSB not only shows substantial yield losses but also exhibit symptoms of delayed maturity. Although there are many potential causes known to be associated with soybean delayed maturity, RBSB is thought to be the major cause because of the deleterious action of its salivary enzymes. In order to understand what RBSB threshold triggers soybean delayed maturity and if this disorder is solely due to reduced pod load (causing alteration in sink-source ratio), field experiments were conducted with different levels of RBSB infestation and mechanical pod removal during R4-R5 stages of soybean. Results from these experiments showed that RBSB density of 8 adults/0.3 m is needed during R4 stage to trigger the development of delayed maturity in soybean variety AG 6732. Plants infested with RBSB densities below 8 adults/0.3 m did not show symptoms of delayed maturity (green leaf retention at maturity).

Mechanical pod removal experiments however, showed no effect of pod removal on occurrence of delayed maturity. There was a significant positive correlation between RBSB density and delayed maturity while no correlation existed between reduced pod load and occurrence of delayed maturity. These findings suggest that RBSB induced soybean delayed maturity may not be due just to the reduction in pod load, but additional factors may also be involved.

One of the major concern with RBSB management is its reduced susceptibility to labeled insecticides which has resulted into substantial increase in the amount of insecticides applied in soybean. A small plot field trial was conducted to determine the efficacy of selected insecticides against RBSB. Pyrethroids (bifenthrin and lambda cyhalothrin) and mixtures of pyrethroids + neonicotinoids (lambda cyhalothrin + thiamethoxam and beta cyfluthrin + imidacloprid) were found to have rapid action against RBSB. Soybean plots treated with these insecticides showed significant reduction in RBSB population one day after treatment. On the other hand, plots treated with the commonly used organophosphate, acephate, showed no significant difference in number of RBSBs when compared with control plots. Results from laboratory bioassays using insecticides showed a similar trend. Neonicotinoids and pyrethroids (imidacloprid, thiamethoxam, beta cyfluthrin, and bifenthrin) had rapid knockdown effect on RBSB while acephate took longer to cause RBSB mortality. After 4h of bioassays, LC_{50} values for neonicotinoids and pyrethroids ranged from 0.18 to 2.32 µg/vial. While in case of acephate, no enough mortality was recorded after 4h to be able to generate a LC_{50}. This suggests that the LC_{50} for acephate lies somewhere above 6 µg /vial. Comparing LC_{50}

values from this study to historical LC_{50} values, suggests that current RBSB populations have reduced susceptibility to acephate.

Future studies are needed to develop more effective management tactics against the new stink bug pest complex in soybean. General suggestions for future research include:

1. A revision of the economic threshold for the new stink bug complex in soybean. The current economic threshold used to justify chemical control against soybean stink bug complex is based upon historical data when RBSB was not an economic pest in US soybeans. The shift in stink bug species composition we have reported in Texas soybean and the RBSB ability to cause more injury per individual than other stink bug species, warrant the need to revise the action threshold for the stink bug complex where RBSB is present. A revised action threshold will help soybean producers by allowing them to fine-tune decision making on the proper use of management tactics.
2. Study the ecological interactions among RBSB and other stink bug species. It seems that upon increased numbers of RBSB, numbers of SGSB, BSB, and GSB have gone down. Studying the interactions among these species will provide information about the influence of invasive RBSB on other stink bug species fitness and whether it has displacing effects on their populations.
3. Elucidate mechanism/s involved in RBSB induced soybean delayed maturity. We found that high RBSB density triggers soybean delayed maturity and that this it is not just solely due to reduced pod load or to the alteration of sink-source ratio. Future studies may be conducted to elucidate the specific mechanism/s associated

with soybean delayed maturity. Testing if RBSB feeding alters the expression of key plant hormones would be an interesting possibility.

4. Study insecticide resistance mechanisms. This study found that RBSB field populations seem to have developed resistance to acephate. More studies may be conducted to monitor insecticide resistance development in RBSB by conducting laboratory bioassays on RBSB populations over larger geographic areas. Dose-mortality data could be compared between RBSB populations under different scenarios of selection pressure from insecticide applications. For example, RBSB populations in Lousiana soybeans have been under constant selection pressure from insecticide applications for longer time than in Texas and RBSB populations in Missouri soybeans have just begun to establish. Comparing RBSB tolerance to acephate among these locations will provide interesting data. Similarly, RBSB populations on different host plant species such as on durana clover, indigo, susbenia, etc.,which are hardly subjected to insecticide exposure, should be compared with RBSB populations in soybean in terms of insecticide susceptibility. Understanding the variability in resistance/susceptibility levels among different RBSB populations will improve area-wide management plan for RBSB.

Results from this dissertation research have provided information that will help in the implementation of economical, effective, and sustainable management strategies for RBSB in soybean, US’ number one crop in terms of value of crop export.

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