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Ecology and Biology of the Redbanded Stink Bug, Piezodorus guildinii (Westwood) in Louisiana

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ECOLOGY AND BIOLOGY OF THE REDBANDED STINK BUG, *PIEZODORUS GUILDINII* (WESTWOOD) IN LOUISIANA

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ABSTRACT

The redbanded stink bug, *Piezodorus guildinii* (Westwood) is an invasive stink bug species that was first documented as a soybean pest in Louisiana during the year 2000. This species continues to expand northward in the MidSouth but information is lacking on its biology and ecology in the U.S. In order to determine *P. guildinii*'s northern range, studies were designed to investigate the cold tolerance ability of this species. The mean supercooling points of adult *P. guildinii* ranged from highest -8.3 ± 0.2°C in March to the lowest of -11.0 ± 0.2°C in January. Evaluation of lethal exposure time (LT_{50}) and (LT_{90}) at subzero temperatures of 0°C, -2°C, and -5°C respectively showed that this insect had high mortality due to chill injury at these temperatures. Winter survival under field conditions was significantly different in two years of the study as mortality increased with progression of winter months. Next, in order to determine spring bridging hosts, field studies were conducted to evaluate the preference of *P. guildinii* to six leguminous cover crops. Our study showed that crimson clover, *Trifolium incarnatum* (L.) and white clover, *Trifolium repens* (L.) are the preferred spring hosts as well as being the main reproductive host plants of *P. guildinii.* These hosts are therefore important linking hosts leading to *P. guildinii* infestation into soybean production field in Louisiana. In order to predict recolonization of soybean fields after spray applications, studies were conducted on *P. guildinii* movement. The dispersion of adult and nymph stink bugs was monitored using protein marking via the mark-captured method. Protein marking was a viable option which provided direct proof of insect movement. The adult *P. guildinii* dispersed up to 137 m along and 15.3 m across the soybean rows. Evidence of dispersion of nymphs up to 122 m along and 11.7 m across the soybean rows was also documented.

CHAPTER 1: LITERATURE REVIEW

1.1. Introduction

Soybean, *Glycine max* (L.) Merrill is a major leguminous crop grown all over the world (Singh and Hymowitz 1999). It is an important plant source of oil and protein grown worldwide (Clemente and Cahoon 2009). Soybean has been a key agronomic crop cultivated throughout the U.S. since the 1940s (Heatherly and Hodges 1998). The U.S. is an overall leader of soybean production in the world and accounts for about 33% of the soybean produced globally (NASS 2017, SoyStats 2017). In 2016, soybean was planted in 33.7 million hectares in the U.S. (NASS 2017). The average yield was 3.1 metric tons per hectare making gross revenue of nearly $50 billion (NASS 2017). In 2014, soybean was planted on 497,763 hectares in Louisiana and the average yield was 2.9 metric tons per hectare (NASS 2017). Statewide, soybean production in Louisiana during 2016 was valued over $500 million (NASS 2017). Therefore, the soybean contribution to the overall economy of the state is highly important.

Numerous insect pests threaten both yield and quality of soybean (Kogan and Turnipseed 1987). The economically important insect pests that threaten soybean production throughout southern regions of the U.S. are comprised of guilds of defoliators, stem feeders, and pod feeders (Turnipseed and Kogan 1976, Way 1994). The important defoliators that primarily forage on soybean leaves include soybean looper *Chrysodexis includes* (Walker), velvetbean caterpillar *Anticarsia gemmatalis* (Hübner), green clover worm *Hypena scabra* (F.), Mexican bean beetle *Epilachna varivestis* (Mulsant), spotted cucumber beetles *Diabrotica undecimpunctata howardi* (Barber), and bean leaf beetle *Cerotoma trifurcata* (Forster) (Turnipseed 1974, Way 1994). The stem feeding guild consists of threecornered alfalfa hopper *Spissistilus festinus* (Say), soybean stem borer *Dectes texanus* (LeConte), and lesser corn stalk borer *Elasmopalpus lignosellus*

(Zeller) (Boethel and Highely 1994, Boyd et al. 1997). The insect groups that attack pods are pod-feeding caterpillars like fall armyworm *Spodoptera frugiperd*a (Smith), and corn earworm *Helicoverpa zea* (Boddie) and a complex of stink bug species (Turnipseed and Kogan 1976, Funderburk et al. 1999). A cluster of phytophagous stink bug species (Hemiptera: Pentatomidae) found in the southern U.S. is a key yield limiting soybean pest group in the region (Turnipseed and Kogan 1976, Boethel and Higley1994, Way 1994). A survey of 2014 soybean insect losses in the southern U.S. showed that stink bugs infested 81.9% of planted soybean acreages and accounted for 24.6% of total loss due to insect pests (Musser et al. 2016). The key species of stink bugs infesting soybean across southern U. S. are the southern green stink bug *Nezara viridula* (L.), the green stink bug *Chinavia hilaris* (say), and the brown stink bug *Euschistus servus* (say) (Drees and Rice 1990, Funderburk et al. 1999). A survey from southeast Texas by Drees and Rice (1990) revealed southern green stink bug as the predominant species, with the brown stink bug, green stink bug, redshouldered stink bug, *Thyanta custator* (F.) and *Euschistus quadrator* (Rolston). The major stink bug species found in Mississippi are the green stink bug, the southern green stink bug, and the brown stink bug (Gore et al. 2006). The brown and southern green are the principle stink bugs found in Arkansas (Smith et al. 2009). The earlier surveys showed that the major stink bugs in Louisiana are the green stink bug, the southern green stink bug, the dusky stink bug, *Euschistus tristigmus* (Say) and the brown stink bug, (McPherson et al. 1979). Later studies reported that the most important species found in Louisiana are the green, the southern green, and the brown stink bugs, with the southern green being the most prevalent species in the complex (Boyd et al. 1997, Boethel and Higley1994, Peters et al. 2004). Nevertheless, in the latest decade a novel stink bug species, the redbanded stink bug, *Piezodorus*

guildinii (Westwood), has become a serious soybean pest in Louisiana and neighboring states (Temple et al. 2007). It was the primary species in Louisiana soybean during 2006-2010 (Temple et al. 2013a).

1.2. Geographic distribution of redbanded stink bug

Piezodorus guildinii (Westwood) is distributed throughout the tropic and semi tropic regions of the New World stretching across the southern U.S., Central America, and northern regions of South America (Panizzi and Slansky 1985b, McPherson and McPherson 2000a). The first documentation of this species can be tracked back to the1920s from St. Vincent Island located in the Caribbean (Stoner 1922). *Piezodorus guildinii* is the most widespread soybean pest in South America where it has persisted as a major economic pest since the 1960s (Panizzi and Slansky 1985b, Kogan and Turnipseed 1987, Panizzi et al. 2000). A massive rise in soybean acreages in South America during the 1960's and 1970's is believed to be the reason behind the upsurge of *P. guildinii* in the region (Turnipseed and Kogan 1976, Panizzi and Slansky 1985b, Kogan and Turnipseed 1987). *P. guildinii* is a main soybean pest in Brazil (Kogan and Turnipseed 1987), Uruguay (Molina and Trumper 2012), and Argentina (Zerbino et al. 2014). In South America, *P. guildinii* thrives on extensive varieties of wild host plants and domesticated crop plants, especially legumes. Its infestation can cause economic loss to soybean, pea, lentil, alfalfa, and many other leguminous crops (Panizzi and Slansky 1985a, Panizzi 1997).

In U. S., *P. guildinii* was first reported during the 1960's (McPherson and McPherson 2000a). Prior to 2000, *P. guildinii* had never been an economic concern to U.S. agriculture but had been reported only in Georgia, South Carolina, and Florida (McPherson et al. 1993, McPherson and McPherson 2000a). In Louisianan, *P. guildinii* was recognized as a pest of

soybean during the year 2000 (Baldwin 2004). Since then it has turned out to be a serious yield limiting pest species in Louisiana. At present, it is established as a dominant species in Louisiana soybean comprising of 54% of the stink bug composition (Temple et al. 2013a). Furthermore, over the past few years *P. guildinii* has also turned out as a key species in the coastal regions of Texas (Vyavhare et al. 2014). *P. guildinii* has been reported from southern Arkansas in 2005 (Smith et al. 2009) and from Mississippi during 2007 (Catchot 2009). By 2009, this pest had reached Tennessee and southeastern Missouri (Tindall and Fothergill 2011). Based on the reports of *P. guildinii* incidence, its present distribution range is east as far as South Carolina, west as far as New Mexico, and north as far as Missouri (Temple 2011). Thus, *P. guildinii* is an emerging threat to soybean enterprises in the southern as well as mid-south areas of the U.S.

1.3. Identification, life history, and biology of redbanded stink bug

Piezodorus guildinii (Westwood) is commonly known as the "redbanded stink bug" in North America and the "neotropical green stink bug" or "small green stink bug" in South America (Panizzi and Slansky 1985b, Zerbino et al. 2013). The adult *P. guildinii* is 10-12 mm in length. The body is relatively slender compared to other common stink bug species (Akin et al. 2011b). The color may vary from shiny green to somewhat yellowish with red, yellow or a brown stripe on the scutellum (Kamminga et al. 2009). It is more likely to be misidentified as the redshouldered stink bug (*Thyanta custrator*) (Akin et al. 2011b). The distinctive morphological characteristic of adult *P. guildinii* to other similar looking stink bug species is the abdominal spine present on the ventral surface in between where the posterior legs are joined into the body (Greene et al. 2006). *Piezodorus guildinii* eggs are dark red to black in color with

spines and white bands around the outer edge. The eggs are laid in double rows consisting of roughly around 30 eggs per cluster (Kamminga et al. 2009). The average hatching time *P. guildinii* egg in laboratory conditions is 8 days (Panizzi and Smith 1977). The hemimetabolous life cycle comprises five nymphal stages. The nymphs from the 1st to 5th instar in order are 1.30 mm, 2.25 mm, 2.58 mm, 4.60 mm, and 7.87 mm in length (Grazia et al. 1980). Early instars are dark brown with dark spots while later instars are greenish brown to yellowish-brown (Kamminga et al. 2009).The 1st instars of *P. guildinii* are non-feeding and stay nearby the oviposition spots (Panizzi and Slansky 1985b). The 2nd and 3rd instars are gregarious and remain congregated. However, 4th instars and the 5th instars often disperse rapidly (Panizzi et al. 1980).

Most of the available literature on biology of *P. guildinii* is from South America where it has been an economic pest for a long time. *Piezodorus guildinii* survival, longevity, body weight and reproductive capability vary greatly depending on host plant (Panizzi 1997, Panizzi 2000). *Piezodorus guildinii* reproduce rather poorly and has a shorter adult life span on soybean in comparison to various wild hosts (Panizzi 2000). Additionally, the number of egg clusters and total number of eggs per cluster laid by female *P. guildinii* may vary greatly depending on the host plant (Panizzi 2000). An oviposition study showed that the mean number of *P. guildinii* eggs on soybean was 17.5 from 542 egg masses with egg numbers ranging from 4-39 per mass (Link and Concatto 1979). *Piezodorus guildinii* egg depositions were found on pods as well as stem, upper and lower leaf surfaces, with pods (80%) as the preferred oviposition site (Link and Concatto 1979). Comparable results were found in another field study where the mean number of eggs was 15.1, and 60% of the eggs were found deposited on soybean pods (Panizzi and Smith 1977). A study from the U.S. showed that the mean number of eggs deposited on a

soybean plant per cluster was 16.6 (Temple et al. 2016). It was also reported that egg masses deposited by *P. guildinii* on the soybean plant is influenced by maturity group (MG) with 79.4% of egg mass deposited on leaves for soybean MG IV, whereas 72.7% of egg masses were deposited on pods for soybean MG V (Temple et al. 2016). *Piezodorus guildinii* eggs reared in the lab settings at 80% RH showed that at 24°C, it took about 7.5 days to hatch (Panizzi and Smith 1977). Average development time was 4.3, 5.9, 6.1, and 9.7 days respectively from first instar to fifth instar (Panizzi and Smith 1977). The average developing period from oviposition to adult took 39 days at 25°C. The sex ratio of the field population was 1.4 females to one male (Panizzi and Smith 1977). It is reported that the sex ratio of *P. guildinii* in soybean was even during all phonological stages excluding the R5 stage, where the ratio was 1.4:1 in favor of female corresponding with increased oviposition (Temple et al. 2016). In a laboratory study, nymphal development was shortest with lowest mortality at 25°C with 14:10 h L:D while it was longest with highest mortality at 20°C with 10.14 h L:D (Zerbino et al. 2013).

1.4. Redbanded stink bug in Louisiana agroecosystem

Piezodorus guildinii (Westwood) in Louisiana was first reported from the south-central region of Louisiana in 2000 (Baldwin 2004). *P. guildinii* infestation in south Louisiana reached action threshold levels as early as 2002 (Baldwin et al. 2009). This mobile pest reached the northeastern part of Louisiana by 2004 (Baur and Baldwin 2006, Tindall and Fothergill 2011). Different studies have suggested that this species is well established throughout Louisiana as an annual soybean pest (Temple 2011). It is now the most prevailing and destructive stink bug species present in soybean grown all over the state (Temple et al. 2013a). The current economic threshold for *P. guildinii* in Louisiana is 16 insects per 100 sweeps (Davis 2016). Besides

soybean, *P. guildinii* can infest various cultivated crops such as lentil, kidney bean, peanut, sunflower, clover, and alfalfa (Baur and Baldwin 2006). *Piezodorus guildinii* infest all major soybean-growing areas in Louisiana with the number in southern Louisiana reaching 3-4 times higher than these economic threshold levels (Temple et al. 2011). Although *P. guildinii* is established as a major soybean insect pest, other crops in Louisiana such as corn, cotton, grain sorghum, and rice are not infested. Very low numbers of *P. guildinii* incidence in these crops are reported when soybean is absent in the adjacent areas (Temple 2011). This observation contrasts from other common generalist herbivorious stink bug species like the southern green, green, and brown stink bugs whose populations within these crops often exceed economic threshold limits (Temple 2011). It has been reported that *P. guildinii* are found in leguminous plants host like medic, clover, and vetch in the spring (Baur and Baldwin 2006). Following the senescence of spring hosts, *P. guildinii* populations concentrate in soybean fields in early summer. *Piezodorus guildinii* population in soybean is then capable of quickly reaching to economically damaging levels (Baur and Baldwin 2006, Paxton et al. 2007). Typically, two to three separate *P. guildinii* population peaks are observed in Louisiana soybean. The first peak is evident in June, the second in July and the third occurs later in August or September (Baur and Baldwin 2006. In addition, the increasing trend of growing early soybean varieties compared to late maturity groups in conventional soybean production systems may also have contributed to the recent rise in stink bug populations (Baur et al. 2000, Temple 2011).

1.5. Redbanded stink bug damage to soybean

Piezodorus guildinii can damage soybean by consuming plant saps from foliage, stems, and flowers but both nymph and adult equally prefer to feed on pods and seeds (Panizzi 1997).

P. guildinii infestation at any soybean stages from R4 – R7 reduces yield and the most serious yield loss occurs during infestation at the R5 stage (Parker 2012). Similar to other phytophagous pentatomids, *P. guildinii* inject salivary secretion into soybean seeds through piercing-sucking mouthparts, and consumes the food slurry created by salivary enzymes (McPherson and McPherson 2000b). Feeding punctures on seeds and pods are visible as small black or brown mark points. These punctures can facilitate access points for fungi into the seedpods (Daugherty 1967, Russin et al. 1988).

Higher incidence of the seed borne pathogen, *Fusarium* spp., has been reported in response to stink bug damage to seed pods (Russin et al. 1988). Stink bug injury on the pods and seeds reduce both seed quality and quantity. In addition, feeding results in decreased pod fill and seed weight, reduced oil content, increased seed protein levels and reduced germination (Jensen and Newsom 1972, Tood and Turnipseed 1974, Kogan and Turnipseed 1987, Bansal et al. 2013). Studies have shown that higher numbers of *P. guildinii* in soybean are associated with a delay in crop maturity in which plant retains green leaves, green stems, or green pods even when it has already reached maturity (Panizzi et al. 1978, Costa and Link 2008, Vyavhare et al. 2015). This leads to delayed harvest and decreased seed quality. It has been reported that *P. guildinii* feeding results in greater damage to the soybean seed endosperm, which signifies that the injurious action of salivary enzymes for *P. guildinii* is more severe compare to other species (Depieri and Panizzi 2011). *Piezodorus guildinii* infestation in soybean may result in more loss per capita compared to other species (Corrêa-Ferreira and De Azevedo 2002). Research on soybean damage conducted on Argentina has also shown *P. guildinii* to be the most detrimental stink bug

species compared to other common stink bugs species infesting soybean (Vicentini and Jimenez 1977).

1.6. Redbanded stink bug management challenges

Chemical control is an extensively used approach to manage *P. guildinii* in Louisiana (Temple et al. 2013b). It has been reported that *P. guildinii* is less sensitive to commonly used insecticides in comparison to other stink bugs species (Temple et al. 2013b). Farmers, therefore, must spray more frequently to manage *P. guildinii* infestation (Davis et al. 2011). Since the invasion of *P. guildinii*, the average number of insecticides applications in Louisiana soybean has increased from1-2 per season to 3-5 per season to account for redbanded stink bug infestation (Temple et al. 2013b). Due to its high damage potential, the economic threshold for *P. guildinii* has been revised twice since its introduction, from nine to six stink bug per 25 sweeps and then to four stink bug per 25 sweep most recently (Davis 2014). Likewise, the rise in *P. guildinii* abundance in soybean has resulted in increased insecticide applications in Texas (Vyavhare et al. 2014). The heavy reliance on insecticides to control *P. guildinii* could make this pest more insecticide resistant as well as kill many important beneficial insects and other arthropods.

Besides chemical controls, there are other alternatives such as cultural and biological control methods to manage stink bug in an agroecosystem (Knight and Gurr 2007). Cultural control approaches being used to manage stink bug populations includes the use of an early season production system (McPherson et al. 2001) and the trap crop approach (Newsom and Herzog 1977, Hokkanen 1991). It has been shown that the stink bug populations on early maturity group IV soybean was lower compared to a conventional late maturing group (Gore et

al. 2006). In addition, utilizing early soybean varieties as a trap crop to manage stink bugs in late maturing varieties has also been proposed (McPherson et al. 2001, Gore et al. 2006). Egg parasitoids were also found as a viable biological control means for *P. guildinii* with about a 57% egg parasitism rate (Correa-Ferreira and Moscardi 1995).

It has been reported that with the expansion of the soybean crop, *P. guildinii* displaced the southern green stink bug to become the dominant pest in some soybean producing regions across Brazil also (Turnipseed and Kogan 1976, Panizzi and Slansky 1985b, Kogan and Turnipseed 1987). The unique characteristics like small size, high mobility, overlapping generations, adaptation to cooler climates, low incidence of natural enemies, and a higher tolerance to common insecticides might be the contributing factors in this shift of species composition (Kogan and Turnipseed 1987). Similar phenomena of *P. guildinii* replacing southern green stink bug to establish itself as number one pest of soybean might be in progress across Louisiana and other southern states (Temple 2011, Parker 2012). This species possess a real threat and can cause substantial damage to soybean enterprise throughout the U.S. unless management strategies based on multiple tactics are developed (Akin et al. 2011a).

Control strategies based on the biological and ecological information are important to develop a species-specific management plan. In contrary to the previously well-known species in the complex, there is limited information available on basic ecology and biology of this pest. Virtually no information is available on overwintering biology and cold tolerance ability of this species in the U.S. This species has shown a large scale geographical expansion in the last decade (Temple et al. 2011) and continues to expand into relatively cooler climatic regions of Mid-South (Tindall and Fothergill 2011). Information on cold tolerance of this species is

therefore valuable in predicting its northern distributional limits. The other little known area in the ecology of *P. guildinii* in the US is its alternative hosts. Little is known about potential spring hosts of this species where its population can build up early in the spring before infesting soybean. In addition, movement of this highly mobile pest species among different host plants, its colonization of soybean, and dispersal within soybean fields is largely unknown. This has left farmers with fewer options for effectively controlling this pest species. In consideration to previous research and prevailing needs, the following research objectives are proposed:

1.7. Study objectives

1. To determine cold tolerance and supercooling capacity of the redbanded stink bug, *Piezodorus guildinii* (Westwood).
2. To evaluate preference of the redbanded stink bug, *Piezodorus guildinii* (Westwood) to selected spring host plants.
3. To monitor the dispersion of the redbanded stink bug, *Piezodorus guildinii* (Westwood) in soybean using a protein based mark-capture method.

CHAPTER 2: COLD TOLERANCE AND SUPERCOOLING CAPACITY OF THE REDBANDED STINK BUG (HEMIPTERA: PENTATOMIDAE)

2.1. Introduction

The redbanded stink bug, *Piezodorus guildinii* (Westwood), is a stink bug species found in the tropic and in the semi-tropic regions of South and North America (Panizzi and Slansky 1985b, McPherson et al. 1993, McPherson and McPherson 2000a). *Piezodorus guildinii* was reported from the U.S. as early as 1960s. However, this species was not considered economically important (Panizzi and Slansky 1985b, McPherson et al. 1993, McPherson and McPherson 2000a). In 2000, *P. guildinii* was recognized as a major insect pest of soybean in Louisiana (Baldwin 2004). Subsequently, it has emerged as a serious stink bug pest species (Temple et al. 2013a). *Piezodorus guildinii* is more damaging to soybean compared to other stink bug species (Depieri and Panizzi 2011). In addition, it is less susceptible to commonly used insecticides compared to other species of stink bug (Temple et al. 2013b). *Piezodorus guildinii* is the dominant stink bug species in Louisiana soybean, comprising 54% of the insect species (Temple et al. 2013a). It is established as a major stink bug in southeastern Texas soybean as well (Vyavhare et al. 2014). Currently the *P. guildinii* range is distributed along the rice belt of Texas (Vyavhare et al. 2014), throughout Louisiana (Temple et al. 2013a), central Mississippi (Catchot 2009), southern Arkansas (Smith et al. 2009), and southeast Missouri (Tindall and Fothergill 2011). These reports clearly indicate there is a large-scale geographic expansion of *P. guildinii* populations in recent years.

Many biotic as well as abiotic factors influence the colonization, establishment, and expansion of an insect species in a given geographic range (Sakai et al. 2001, Parmesan 2006, Stotter and Terblanche 2009). Thermal tolerance, predominantly low temperatures in winter, is a critical determinant of the geographic range expansion of many insects (Bale 1991b). Though *P. guildinii* was the third most prevalent stink bug species in soybean in 2009 in southeastern Missouri, no *P. guildinii* were found in 2010 (Tindall and Fothergill 2011). These episodes of expansion and extirpation of *P. guildinii* populations in a particular area may be due to fluctuation in climatic conditions (Baur and Baldwin 2006) and low winter temperatures may be limiting its survivorship in northern states (Tindall and Fothergill 2011). Several studies have shown that winter mortality is the major factor that limits the distribution of the southern green stink bug, *Nezara viridula* (L.) (Kiritani 1966, Jones and Sullivan 1981), another Neotropical pest.

Cold severity and exposure time affect insect physiology and behaviors (Koštál et al. 2007). A prerequisite for an insect species to establish in a geographic area is that adequate numbers of individuals survive periods of winter (Bale 1996). Cold tolerance ability enables insect species to endure cold and consequently build populations rapidly after winter (Denlinger 1991). There are three central mechanisms of cold tolerance in insects based on their physiological response to extended exposure to cold temperatures (Salt 1961, Baust and Rojas 1985, Bale 1993, Zachariassen and Kristiansen 2000, Denlinger and Lee 2010). The first category is "freeze-tolerant insects" that can sustain ice formation outside the body cell. The second category is "freeze-intolerant insects" that evade the fatal temperatures by depressing the freezing point of watery solutions inside their own body (Sømme 1982, Baust and Rojas 1985,

Zachariassen 1985). Finally, the third category is "chill-intolerant insects" that die before freezing (Bale 1987, Bale 1993, Lee 2010).

Cold tolerance is the capability of an insect to endure short or extended exposure time to low temperatures (Salt 1961). Insect cold tolerance studies can help determine northern distribution limits, relative abundance, and voltinism of the insect species (Bale 1991a, Denlinger and Lee 2010). Evaluation of insect cold tolerance includes measuring of supercooling ability and examination of insect survival at low temperatures (Sømme 1982, Leather et al. 1992). Supercooling ability is a phenomenon in which water and watery solutions continue at an unfrozen state below the melting point (Bale 1987). Supercooling point determination is a simple procedure that gives the lowest fatal temperature point that freeze-intolerant insects can survive exposure to (Carrillo et al. 2004). A second common index to measure insect cold tolerance is the measure of time-temperature effect on insect mortality (Bale 1996, Denlinger and Lee 2010). Insect survival at colder temperature is dependent on intensity of exposed cold temperature and exposure time. Lethal exposure time (LT) at different sub-zero temperature has been used to assess cold tolerance in insects (Watanabe 2002). The low temperature threshold of chill coma onset or critical thermal minimum at which insect enters into reversible state of neuromuscular dysfunction is also used as a measure of cold resistance by insects (Terblanche et al. 2007, Findsen et al. 2014). Winter survival is another good indicator of insect cold tolerance evaluated by observing winter mortality under field conditions (Elsey 1993, Watanabe 2002).

The cold tolerance of *P. guildinii* is an important trait that would allow it to establish in northern parts of the U.S., despite its tropical origin. Similarly, low temperature endurance is an

essential feature for insect populations in overwintering survival and is highly relevant in developing pest management strategies (Bale 1991a). Thus, in this study, I studied the cold tolerance and supercooling capacity of *P. guildinii*. The objectives were to: (1) determine the supercooling capacity and seasonal variation in supercooling capacity of *P. guildinii*, (2) determine the chill coma temperature (critical thermal minimum, CTmin) of *P. guildinii*, (3) determine the lethal exposure time of *P. guildinii* at subzero temperatures, and (4) determine the winter survival of *P. guildinii* under field conditions.

2.2. Materials and methods

2.2.1. Measurement of supercooling points

Adult *P. guildinii* were collected monthly from May 2015 to April 2016 using a standard (0.38 m diameter) sweep net from the Ben Hur Research Farm (30º 22'12.2''N, 91º10'11.6"W), Baton Rouge, Louisiana. Insects were collected from soybean during the growing season and from white clover when soybean was absent. Insects were observed for any injuries and only healthy and active individuals were sexed and weighed before use in experiments. All experiments to calculate the supercooling points were conducted within 48 hours of insect captivity. Insects were kept at room temperature and no food or water were provided during this period as food and water consumption may alter supercooling points (Salt 1958, Baust and Rojas 1985). Supercooling points for each individual insect were measured through surface contact thermocouple thermometry. Each insect was placed in 2 ml plastic tube (Thermo Scientific™, Nalgene™). A small amount (< 50 mg) of high vacuum grease (DOW CORNING®, Dow Corning Corporation, Midland, MI) was applied at the base of the tube to immobilize the insect.

A thermocouple was attached to the abdomen of each individual immobilized in the tube using the high vacuum grease. A type T (copper/constantan) thermocouple (24-gauge teflon wrap, 0.91m long, Teflon®-coated, DATAQ Instruments, Akron, OH) was used in all experiments. The insect-thermocouple arrangements were retained in a Nalgene™ Cyro 1°C freezing container (Cat No. 5100-0001). The freezing container was filled with 250 ml of isopropanol alcohol and placed in a -20°C freezer, allowing the insects to cool at the rate of approximately 1°C/min. Temperatures were logged every 0.5s through a multi-channel data logger (DATAQ Instruments, Model DI-1000TC-Y, Akron, OH). The data logger used in this experiment was a four-channel data logger that allowed recording supercooling points for four insects in each trial, we conducted 25 trials each month of study. The SCP was defined as the lowest temperature attained before a sharp rebound visible on the thermal curve owing to the release of the latent heat of freezing. Seasonal changes in supercooling points of field collected *P. guildinii* were measured throughout the year from May 2015 to April 2016. In each month, supercooling points for 50 male and 50 female adult insects was determined, except in December of 2015 in which 33 male and 33 female insects were used because of the rarity of insects in the field. The monthly change in supercooling points and effect of month, sex, and their interactions were analyzed with analysis of variance (ANOVA). Tukey's Studentized Range Test (Honestly Significant Difference, HSD) was performed to make multiple comparisons at $P < 0.05$ (PROC Mixed, SAS Institute 2016).

2.2.2. Measurement of chill coma temperature (critical thermal minimum, CT_{min})

Chill coma temperature is the critical temperature at which insect movement completely stops due to neuromuscular dysfunction resulting from cold temperature (Hazell and Bale 2011,

Sinclair et al. 2015). Chill coma temperature was measured for adult (female = 21, male = 21) and nymph *P. guildinii* (n = 36). The adult *P. guildinii* used in this experiment were one month old and the nymphs consisted of pooled third, fourth, and fifth instars. These insects were taken from a lab colony maintained at 25°C, RH 45%, L: D 14:10. The thermoelectric temperature controller (Model no: TC-720, TE® Technology INC, MI, USA) that provide desired heating or cooling rate to the Peltier thermoelectric device (Model no: CP-200HTTT, TE® Technology INC, MI, USA) was used to induce chill coma. Each insect was placed on its dorsal side and immobilized using vacuum grease on the Peltier plate such that the insects were still free to move their legs and antennae. The Peltier plate was cooled at the rate of 0.5ºC/min from the initial plate temperature of 25ºC. Two thermocouples (type-K) were used to measure the temperature, one at the junction of plate and insect body touching the plate and one at the top of the insect body. Each insect was directly observed during the entire cooling process. The chill coma temperature was determined for each insect as the temperature at which there was complete arrest of legs and antennae movement. The average of the two recorder temperatures at the body- plate junction and upper body of the insect was used as the chill coma temperature. The effect of sex on adult as well as stage of *P. guildinii* on chill coma temperature were analyzed separately with one-way analysis of variance (ANOVA). The Tukey's Studentized Range test (HSD) was performed for separating means at $P < 0.05$ (PROC GLM, SAS Institute 2016).

2.2.3. Determination of the lethal exposure time (LT) of the redbanded stink bug at different subzero temperatures

Adult *P. guildinii* were collected from soybean fields at the Ben Hur Research Farm form late planted soybean in the month of October of 2013 and 2014 as described above. Collected insects were kept in the lab for 12h at room temperature and provided green beans, raw peanut,

and water in a rearing container. Insects were observed for any kind of physical injuries and only insects that were active and in good physical shape were used in the experiment. The critical exposure time $LT_{50,}$ (time required to kill 50% of the test specimens) and LT_{90} (time required to kill 90% of the test specimens) for *P. guildinii* was determined at three sub-zero temperatures. Based on supercooling points and preliminary tests; 0°C, -2°C, and -5°C were chosen. Adult *P. guildinii* were exposed to these temperatures for 2h to 108h depending upon the temperature chosen. For each exposure time, 30 adult insects were placed individually in 20 ml glass vials without food, and these vials were then incubated in a low temperature incubator (Precision™ model no. 815, Thermo scientific™) at different temperatures. Control samples of 20 adult individuals were held individually in 20 ml glass vials without any food at room temperature for each subzero temperature treatment. Three trials were conducted for each subzero temperature treatment. After exposure, insects were held at room temperature for 24h before assessing mortality. Insects that failed to show coordinated movement were consider dead. LT_{50} and LT_{90} were calculated through probit analysis (PROC PROBIT, SAS Institute 2016).

2.2.4. Winter survival of the redbanded stink bug in field conditions

This study was carried out during the winters of 2014 and 2015 at the Ben Hur Research Farm. Adult *P. guildinii* were collected from late planted soybean in the last week of October. Small ground cages were made out of PVC pipe measuring 15 cm in diameter and 12cm in height (Fig. 2.1). The lower end of the pipe was dug into the ground about 5 centimeter while the top end was covered with fine fiberglass screen wire mesh of size 24 cm × 24 cm secured by a 15.24 cm inside diameter galvanized full clamp. Thirty cages with six adults in each cage (3

male and 3 female) were deployed on the last week of October around the edges of the field within mixtures of grasses and clover species, which are potential overwintering sites for *P. guildinii* (Smaniotto and Panizzi 2015, Zerbino et al. 2015). Ten cages were randomly chosen to be destructively sampled on the first week of each month of each year starting in December to February to determine survival of *P. guildinii* during winter field conditions. Cages were taken to the laboratory where soil and foliage were manually processed and examined thoroughly for live *P. guildinii*. Numbers of live and dead *P. guildinii* and sex of those insects were recorded. A logistic regression was used to predict the mortality of *P. guildinii* using years, months, and sex (PROC LOGISTIC, SAS institute 2016).

In this analysis, odds ratios were used to compare the effect of each factor with their respective reference. The odds ratio represents the odds of mortality occurring for a given factor compared to odds of mortality occurring at their respective reference factor. The coldest recorded soil temperature from 10cm depth and air temperature and total chill hours (numbers of hours when the temperature is less than 7.2°C) for the months of November, December, and January in 2013-2014 and 2014-2015 from Ben Hur Research Farm were obtained from Louisiana Agriclimatic Information System website (LSU AgCenter 2016).

Figure 2.1. Ground cages made out of PVC pipe and fiberglass screen wire mesh for the study of winter survival of redbanded stink bug at Ben Hur Research Farm, Baton Rouge, Louisiana.

2.3. Results

2.3.1. Seasonal variation in supercooling ability of adult redbanded stink bug

There were significant differences in the observed mean SCP of *P. guildinii* over time ($F = 14.08$; $df = 11, 1142$; $P < 0.0001$). The mean SCP (± SE) of adult *P. guildinii* ranged from the highest (-8.3 ± 0.2°C) in March to the lowest (-11.0 ± 0.2°C) in January (Table 2.1). The mean SCP of adult *P. guildinii* for the month of March was significantly higher than the months of May, August, September, November, December, and January. Similarly, the mean SCPs from

April, June, and July were significantly higher than winter months of November, December, and January. In addition, mean SCPs from May, August, and October were significantly higher than SCPs in January (Table 2.1).

2.3.2. Effect of sex on supercooling ability of adult redbanded stink bug

There was significant effect of sex on the mean SCP**s** of adult *P. guildinii* ($F = 4.04$*; df* =1, 1142*;* $P = 0.0446$). Combined over all seasons, the mean SCP of male adult *P. guildinii* was significantly lower than female adult *P. guildinii* (Table 2.2). In addition, there was **a** significant interactions between sex and month *(*$F = 2.25$; *df* =11, 1142*;* $P = 0.0106$) for SCPs of adult *P. guildinii* (Table 3). The mean SCP of female *P. guildinii* from March, June, and September was significantly higher than male *P. guildinii* from December and January. Similarly, the mean SCP of male *P. guildinii* from March, April, and July was significantly higher than female *P. guildinii* from December, January, and February (Table 2.3).

Table 2.1. Mean supercooling points (°C ± SE) of field collected *P. guildinii* from Ben Hur Research Farm, Baton Rouge, Louisiana in 2015-2016.

Month	n	Means ± SE
March	100	-8.3 ± 0.2a
July	100	-8.9 ± 0.2ab
April	100	-9.2 ± 0.2abc
June	100	-9.3 ± 0.2abc
September	100	-9.4 ± 0.2bc
October	100	-9.9 ± 0.2bcd
August	100	-10.0 ± 0.2cd
May	100	-10.0 ± 0.2cd
November	100	-10.4 ± 0.2de
February	100	-10.6 ± 0.2de
December	66	-10.9 ± 0.3de
January	100	-11.0 ± 0.2e

Means followed by different letters are significantly different by Tukey's Studentized Range (HSD) test with $P<0.05$.

Table 2.2. The mean SCP (°C ± SE) and mean weight (gm ± SE) of *P. guildinii* combined over the year.

Sex	SCP	Weight
Female	-9.71 ± 0.09b	0.063 ± 0.001a
Male	-9.96 ± 0.09a	0.051 ± 0.001b

Means followed by different letters are significantly different by Tukey's Studentized Range (HSD) test with $P<0.05$.

Table 2.3. Mean supercooling points (°C ± SE) of field collected male and female *P. guildinii* from Ben Hur Research Farm, Baton Rouge, Louisiana in 2015-2016.

Month	n (Female)	Means ± SE (Female)	n (Male)	Means ± SE (Male)
March	50	-7.6 ± 0.3a	50	-8.9 ± 0.3abc
September	50	-8.9 ± 0.3abc	50	-9.8 ± 0.3bcdef
June	50	-9.0 ± 0.3abcd	50	-9.5 ± 0.3bcde
July	50	-9.4 ± 0.3bcde	50	-8.5 ± 0.3ab
April	50	-9.4 ± 0.3bcde	50	-8.9 ± 0.3abc
October	50	-9.5 ± 0.3bcde	50	-10.3 ± 0.3cdef
May	50	-9.8 ± 0.3bcdef	50	-10.1 ± 0.3cdef
August	50	-9.9 ± 0.3bcdef	50	-10.1 ± 0.3cdef
November	50	-10.0 ± 0.3cdef	50	-10.9 ± 0.3ef
February	50	-10.8 ± 0.3ef	50	-10.4 ± 0.3cdef
December	33	-10.8 ± 0.4def	33	-11.1 ± 0.4ef
January	50	-11.2 ± 0.3f	50	-10.9 ± 0.3ef

Means followed by different lower case letters in columns and different upper case letters in rows are significantly different by Tukey's Studentized Range (HSD) test with $P < 0.05$.

2.3.3. Chill coma temperature (critical thermal minimum, CT_{min})

The chill coma temperature (CT_{min}) for female and male *P. guildinii* ranged from 6.9°C - 9.5°C and 7.3°C - 9.6°C respectively. The mean chill coma temperature for male *P. guildinii* was slightly higher (8.3 ± 0.2°C) than female *P. guildinii* (7.9 ± 0.2°C). The chill coma temperature range for *P. guildinii* nymph was 7.2°C -11.0°C.The mean chill coma temperature

between adult and nymph *P. guildinii* (F=10.35; df=1, 70; P=0.0020) was significantly different (Fig. 2.2).

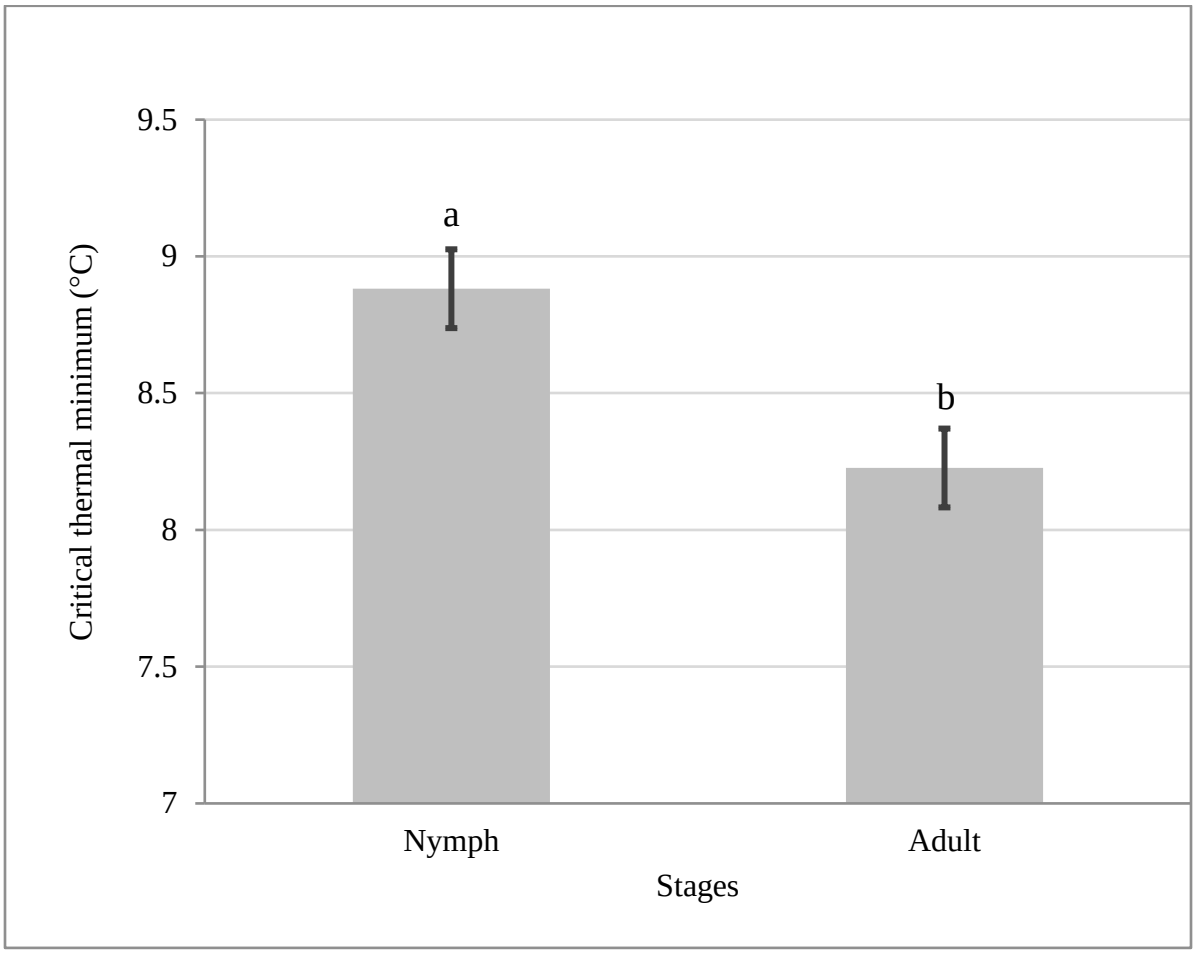

Figure 2.2. Chill coma temperature (critical thermal minimum, CTmin) of adult and nymph redbanded stink bugs. Means followed by different letters are significantly different by Tukey's Studentized Range (HSD) test with $P < 0.05$.

2.3.4. Lethal exposure time

The lethal exposure time to kill 50% and 90% of adult *P. guildinii* populations at subzero temperatures 0°C, -2°C, and -5°C were determined (Table 4). LT_{50} for 0°C, -2°C, and -5°C was 53.4 hrs, 37.4 hrs, and 6.8 hrs respectively. Likewise, the LT_{90} at 0°C, -2°C, and -5°C was 75.4

hrs, 56.81 hrs, and 10.36 hrs respectively. There were significant differences in lethal exposure time for tested subzero temperatures as indicated by the 95% fiducial limits (Table 4).

Table 2.4. Lethal exposure time (hrs.) required for 50% and 90% mortality of adult *P. guildinii* from Ben Hur Research Farm, Baton Rouge, Louisiana to subzero temperatures.

Temp (ºC)	n	LT_{50} (hr)	95% FL	LT_{90} (hr)	95% FL	Slope ± SE	χ^2
0	810	53.41	51.18 - 55.64	75.44	72.32 - 79.17	0.058 ± 0.003	23.27
-2	720	37.41	35.93 - 40.00	56.81	53.93 - 60.30	0.068 ± 0.004	15.69
-5	630	6.77	6.25 - 7.29	10.36	9.59 - 11.24	0.363 ± 0.031	32.51

Non-overlapping 95% fiducial limits (FL) shows the significant differences in lethal exposure time at given temperatures.

2.3.5. Winter survival in field conditions

There were significant differences ($\chi 2 = 23.74$; $P < 0.01$) in the mortality of *P. guildinii* between the winter months of 2013-2014 and 2014-2015 (Table 5). Likewise, the mortality was significantly different ($\chi 2 = 75.84$; $P < 0.01$) among the winter months tested. However, differences were not detected ($\chi 2 = 2.75$; $P < 0.11$) in the mortality between sex. The estimated odds ratio of mortality of *P. guildinii* for the winter of 2013-2014 was 3.61, which suggests that the mortality of *P. guildinii* was 3.61 times higher than in the winter of 2014-2015, all other things being constant. Similarly, when compared among winter months, the mortality of *P. guildinii* was 0.04 times and 0.11 times lower in November and December than in January, respectively (Table 5).

Table 2.5. Odds ratio estimates (95% CI) for the winter mortality of adult *P. guildinii* from Ben Hur Research Farm, Baton Rouge, Louisiana.

Effect		Odds ratio	95% *CI*	P value
Year	2013-2014	3.61	2.15 - 6.06	<0.01
	2014-2015 (Reference)			
Month	Nov.	0.04	0.023 - 0.092	<0.01
	Dec.	0.11	0.062 - 0.227	0.02
	Jan. (Reference)			

Note: Year = 2014-2015, Month = Jan. are used as reference category for the calculation of odds ratio.

Table 2.6. Temperature records from Ben Hur Research Farm, Baton Rouge, Louisiana.

Year	Month	Chill hours*	Coldest recorded soil temp (ºC) (10 cm)	Coldest recorded air temp (ºC)
2013-14	Nov.	148.3	8.3	-3.3
	Dec.	267.9	8.3	-1.6
	Jan.	362.9	3.3	-7.7
2014-15	Nov.	160.5	10	-2.2
	Dec.	82.4	10.5	1.1
	Jan.	318.4	6.6	-1.1

Climatic data source: Louisiana Agriclimatic Information System, *Chill hours = number of hours exposed to temperature < 7.2°C.

2.4. Discussion

Piezodorus guildinii is a stink bug species of tropical origin that is expanding its range into cooler regions probably due to warmer winters (Panizzi 2015). This study provides the firsthand informations on the cold tolerance of this species. This study shows that there is seasonal variation in the supercooling capacity of field populations of *P. guildinii*. The SCPs for *P. guildinii* were higher in summer months and decreased with the progression of fall and winter months. Depression of SCPs as insects enter fall and winter months has been documented in a number of insects (Baust 1972, Bale 1980, Sømme 1982, Carrillo et al. 2005, Hou et al. 2009, Bale and Hayward 2010, Cira et al. 2016). This observation is also an evident that *P. guildinii* undergo seasonal acclamation possibly due to change in temperature regimes in their surroundings. The lowest mean SCP of *P. guildinii* (-11.0 ± 0.2°C) and the lowest minimum recorded temperature from the sampling location (-2.2°C) both occurred in January. The cold tolerance study on the southern green stink bug from South Carolina has reported that the SCP of this species is approximately -11°C (Elsey 1993). The comparable cold tolerance study of the invasive brown marmorated stink bug, *Halymorpha halys* (Stål) from Minnesota and Virginia has also reported significant difference in the mean SCP over the seasons (Cira et al. 2016). The mean SCP of brown marmorated stink bug occurring in those regions was -9.43°C in summer, -15.4°C in fall, and -16.11°C in winter (Cira et al. 2016) which is lower than *P. guildinii* and southern green stink bug.

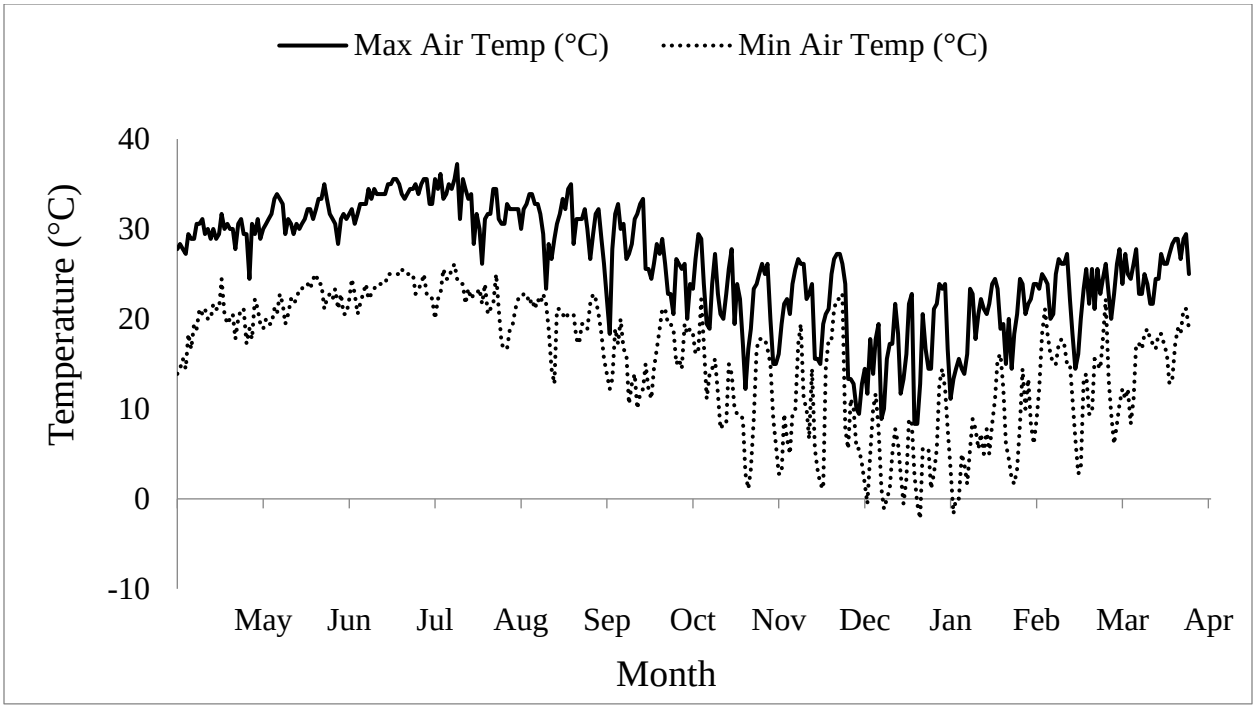

Figure 2.3. Seasonal change in minimum and maximum air temperatures at sampling location Ben Hur Research Farm, Baton Rouge, Louisiana, from May 2015 to April 2016 (Source: Louisiana Agriclimatic Information System).

In many insects, depression of SCP is associated with accumulation of cryoprotectants such as glycerol and antifreeze compounds like alcohols and sugars (Sømme 1982, Bale 1987, Atapour and Moharramipour 2009, Hou et al. 2009). We found lowest SCP in the month of January and the highest in the month of March. Similar observations were reported for the Asiatic rice borer larva, *Chilo suppressalis* (Walker) with lowest SCP in winter and highest in March (Hou et al. 2009). It is also reported that body water content of Asiatic rice borer larva significantly increased and the hemolymph glycerol significantly plummeted from winter to March (Hou et al. 2009). Therefore, comparable phenomena may also be associated with adult *P. guildinii*. It is shown that in fall and winter, adult *P. guildinii* has reduced body size, under developed reproductive organs, and excess lipids reserve (Zerbino et al. 2015). It is possible that the lowest

mean SCP for field acclimated population of *P. guildinii* observed in the month of January may be due to higher lipids reserves. On the other hand, the lowest mean SCP in the month of March may have resulted from the depletion of lipid reserve as insect become active during early spring.

I found that there were significant differences in SCP between sexes. The difference between sexes may be due to difference in body weights. As males weigh less than females and are usually smaller, differences in total body water content might affect SCP (Salt 1956, Miller 1969, Sømme 1982). Based on this study, *P. guildinii* cold tolerance strategies appears to be freeze intolerant because no survival was found once the SCP was reached and the insect body froze. The SCP represents the lowest temperature at which a freeze intolerant insect could stay alive without body freezing but insect mortality can occur well above the SCP due to chilling injuries as well (Bale 1987). For this reason, I cannot rule out the possibilities of *P. guildinii* as chill-intolerant, which need further investigations.

Non-lethal low temperatures can also have profound effects on insect that can directly affect their foraging, reproduction, and defense against predation and parasitism (Hazell and Bale 2011). The critical thermal minimum represents non-lethal temperatures at which an insect enters into a chill coma characterized by reversible state of complete lack of movement (Hazell and Bale 2011, MacMillan and Sinclair 2011). Our data indicates that adult *P. guildinii* has lower critical thermal minimum than nymphs do. Difference in thermal tolerance has been reported in insects based on their life stages (Bowler and Terblanche 2008, Marais et al. 2009). Lower critical thermal minimum of adult *P. guildinii* means that adults may be more capable to endure the effects of non-lethal cold temperature compared to nymphs. In our experiment, critical thermal minimum is evaluated only from laboratory colony. It has been reported that

acclimatization to colder temperatures can increase critical thermal limits in insects (Colhoun 1960). Therefore, further experiment with field acclimatized insect population will elucidate the importance of critical thermal minimum in overwintering biology of *P. guildinii*.

The lethal exposure time to kill 50% of *P. guildinii* at -5°C in our experiment is 6.8hrs, which is more than 5 times shorter than the diapausing adult southern green stink bug as reported by Elsey (1993). Similarly, the LT_{50} at 0°C is just over 2 days and the LT_{50} at 2°C of a day and half. The results from lethal exposure time indicates that the mortality of *P. guildinii* collected in fall season is rapid under sub-zero temperatures. Our study shows that winter survival of *P. guildinii* under field conditions depends upon the severity of low temperature during the winter. The predicted mortality of *P. guildinii* was 3.61 times higher in the winter of 2013-2014 compare to the winter of 2014-2015. The unusually severe cold spell of the winter of 2013-2014 in Louisiana was responsible for the higher mortality. The lowest recorded air temperature was -7.7°C in the month of January and this observation was comparable to the lethal exposure time study in the laboratory conditions. The lowest soil temperature of 3.3°C also occurred during January of 2013-2014 (Table 6). However, during the mild winter of 2014-2015, the predicated mortality of the *P. guildinii* was lower. The lowest air temperature recorded was -2.2°C in November and lowest soil temperature recorded was 6.6°C in January. The mortality significantly increased as the winter month progressed. This may be the result of accumulation of chilling injuries, as exposure to chill hours increased with time. However, other stressors like starvation and desiccation may also directly affect the winter survival (Koštál et al. 2007, Terblanche et al. 2011). The incidents of rise and successive fall of *P. guildinii* populations may best be explained by the severity of winter temperatures occurring in those regions. The

favorable winter conditions in this time of climate warming may be a probable cause of its recent range expansions. As for future studies, I would suggest to use this data into temperature models to predict the range of expansion of this insect with temperatures predicted for future years.

In conclusion, this study provides the first insights into the cold tolerance of *P. guildinii* in the U.S. Information on cold tolerance of *P. guildinii* is critical for understanding tits possible geographic range, their relative abundance, and their seasonal activity and distribution patterns. This information will form the basis for developing a model to forecast overwintering survival and potential pest population numbers. Further investigation on ecophysiological bases of the *P. guildinii* will elucidate the underlying mechanism of cold tolerance.

CHAPTER 3: PREFERENCE OF *PIEZODORUS GUILDINII* (WESTWOOD) TO SELECTED SPRING HOST PLANTS

3.1. Introduction

Stink bugs (Hemiptera: Pentatomidae) are one of the most important economic soybean pests in the southern region of the U.S. (Funderburk et al. 1999, McPherson and McPherson 2000a). The 2014 survey of soybean losses due to insects across the southern U.S. showed that the soybean stink bug complex was the most expensive in yield loss plus control in four out of the seven states surveyed (Musser et al. 2015). Traditionally, the predominant species of phytophagous pentatomids damaging soybeans in this region included the southern green stink bug *Nezara viridula* (L.), the green stink bug *Chinavia hilarus* (Say), and the brown stink bug *Euschits servus* (Say) (Funderburk et al. 1999, McPherson and McPherson 2000). Nevertheless, in the last decade a new species of stink bug, the redbanded stink bug, *Piezodorous guildinii* (Westwood), has established as a yield limiting pest of soybean in the southern regions of the U.S. *Piezodorous guildinii* was reported from the U.S.as early as the 1960s but was never an economic concern to U.S. agriculture (McPherson and McPherson 2000b). This species was reported only from Florida, Georgia, and South Carolina (McPherson et al. 1993, McPherson and McPherson 2000a). However, *P. guildinii* was documented as a soybean pest in the year 2000 in Louisiana (Baldwin 2004). Since then, it has become the dominant species, comprising 54% of the stink bugs species composition found in Louisiana soybean (Temple et al. 2013a). Likewise, *P. guildinii* has established as a major stink bug species in the upper Gulf Coast regions of Texas (Vyavhare et al. 2014). *Piezodorous guildinii* has also been reported from southern Arkansas in 2005 (Smith et al. 2009) and from Mississippi during 2007 (Catchot 2009) and from southeastern

Missouri in 2009 (Tindall and Fothergill 2011). Thus, *P. guildinii* has emerged as an economic threat to soybean enterprise in the southern as well as the mid-south areas of the U.S.

Stink bugs are polyphagous pests that devour a wide range of farmed and wild plants with fruiting structures as their primary feeding sites (Panizzi and Slansky 1985a, Panizzi 1997). *Piezodorous guildinii* host range includes an array of cultivated and wild plants, but prefers leguminous host plants (Panizzi and Slansky 1985c). It is an economic pest of soybean, *Glycine max* (L.) Merrill and many other legumes like common bean *Phaselous vulgaris* (L.), pea *Pisum sativum* (L.), and alfalfa *Medicago sativa* (L.), in South America (Panizzi and Slansky 1985b, Panizzi 1997). It is also reported to feed on legumes in the genus *Cajuns*, *Sesbania*, and *Crotalaria* (Panizzi and Slansky 1985a, Panizzi 2000). In Uruguay, it was found that the abundance of both nymph and adult *P. guildinii* was higher in alfalfa, compared to other plant species (Zerbino et al. 2014). It is reported that white clover *Trifolium repens* (L.), acts as alternative host of *P. guildinii* during early summer in Texas when soybean is not yet attractive (Vyavhare et al. 2016). It is also a minor pest of coffee *Coffea arabica* (L.), cotton *Gossypium hirsutum* (L.), guava *Psidium guajava* (L.), and sunflower *Helianthus annus* (L.) (Panizzi and Slansky 1985b). An important *P. guildinii* wild hosts include *Indigofera* spp. in Columbia, Brazil, and in the southern U.S. (Panizzi and Slansky 1985c, Panizzi 1992), and privet, *Ligustrum lucidum* (Ait.) in Brazil **(**Panizzi and Grazia 2001).

In the spring, stink bugs come out from their overwintering habitats and feed and reproduce on numerous non-crop hosts (McPherson et al. 1994). Subsequent stink bug generations migrate into crops coinciding with senescence of spring hosts and development of crop hosts (Panizzi and Meneguim 1989). It is suspected that the first and second generations

from the overwintered stink bug populations develop on available spring host plants with subsequent third generation adults moving into soybean fields with onset of bloom and pods (Baur and Baldwin 2006, Vyavhare et al. 2016). Knowledge of seasonal host plant sequence utilization by stink bugs and their suitability for reproduction and nymphal development is of great importance in stink bug management (Panizzi 1992, Panizzi 1997). Since *P. guildinii* is a relatively new species in the existing southern U.S. stink bug complex, there is a lack of information about this pest compared to other stink bug species in the U.S. agroecoscape. One of the gaps in our present understanding is *P. guildinii* potential spring hosts on which its population can develop before migrating into soybean. In addition, there is no concrete information on *P. guildinii* preference for particular spring hosts, which can be valuable in developing its management strategies. Field observations in Louisiana have revealed that *P. guildinii* were present in higher numbers in leguminous hosts like medic, clovers, and vetch along roadsides, rights-of-way, ditches, and field margins early in the spring. The objective of this study was to identify the relative preference of *P. guildinii* to selected spring hosts.

3.2. Materials and methods

Six Leguminous host plants; crimson clover *Trifolium incarnatum* (L.), cardinal red clover *Trifolium pretense* (L.), Austrain winter pea *Pisum sativum* (L.), berseem clover *Trifolium alexandrium* (L.), hairy vetch *Vicia villosa* (Roath), and white clover *Trifolium repens* (L.), were evaluated for suitability as potential spring hosts of *P. guildinii* in Louisiana. The authors choose these leguminous host plants for three reasons; (1) some are reported to be suitable reproductive hosts of *P. guildinii* in the Neotropics (Smaniotto and Panizzi 2015), (2) these legumes are also popular cover crops in Louisiana that are primarily used to manage soil erosion and soil fertility

in the farmland during the spring season (Boquet 2012), and (3) extensive amounts of some of these host plants are planted for erosion control by Louisiana Department of Transportation & Development (LaDOTD) after road construction.

This study was carried out at four different agriculture research stations located in Louisiana; New Iberia Research Station (southwest, 29º 57’35.7’’N, 91º42’57.3”W), Ben Hur Research Station (southeast, 30º 21’48.5’’N, 91º10’11.6”W), Burden Research Station (southeast, 30º 24’28.8’’N, 91º06’31.4”W), and Dean Lee Research Station (central, 31º 10’08.2’’N, 92º24’47.7”W) during 2013-2015 (Fig. 3.1). The selected six leguminous host plants were established in a randomized complete block design (RCBD) with four replications at each location. The plots for each host plant were established by sowing the seeds into the experimental plots during each fall of 2012 to 2014 with exception that in 2013 insect samples were collected from voluntarily growing white clover around the margins of the experimental plots at each location. Individual plot size was 7.62 m × 3.04 m with 3.04 m alley between plots. When all the host plants attained adequate foliage growth to cover the ground, sampling was initiated. Samples were obtained by randomly placing a square frame of size 0.38 sq. m on an individual plot and D-vacing the area inside the frame for one minute at maximum air speed (73.76 m/sec). The Agricultural Backpack 2-cycle Aspirator-Model 1612 (available from John W. Hock Company, Gainesville, Florida) with a maximum air speed of 73.76 m/sec was used as a vacuum sampler.

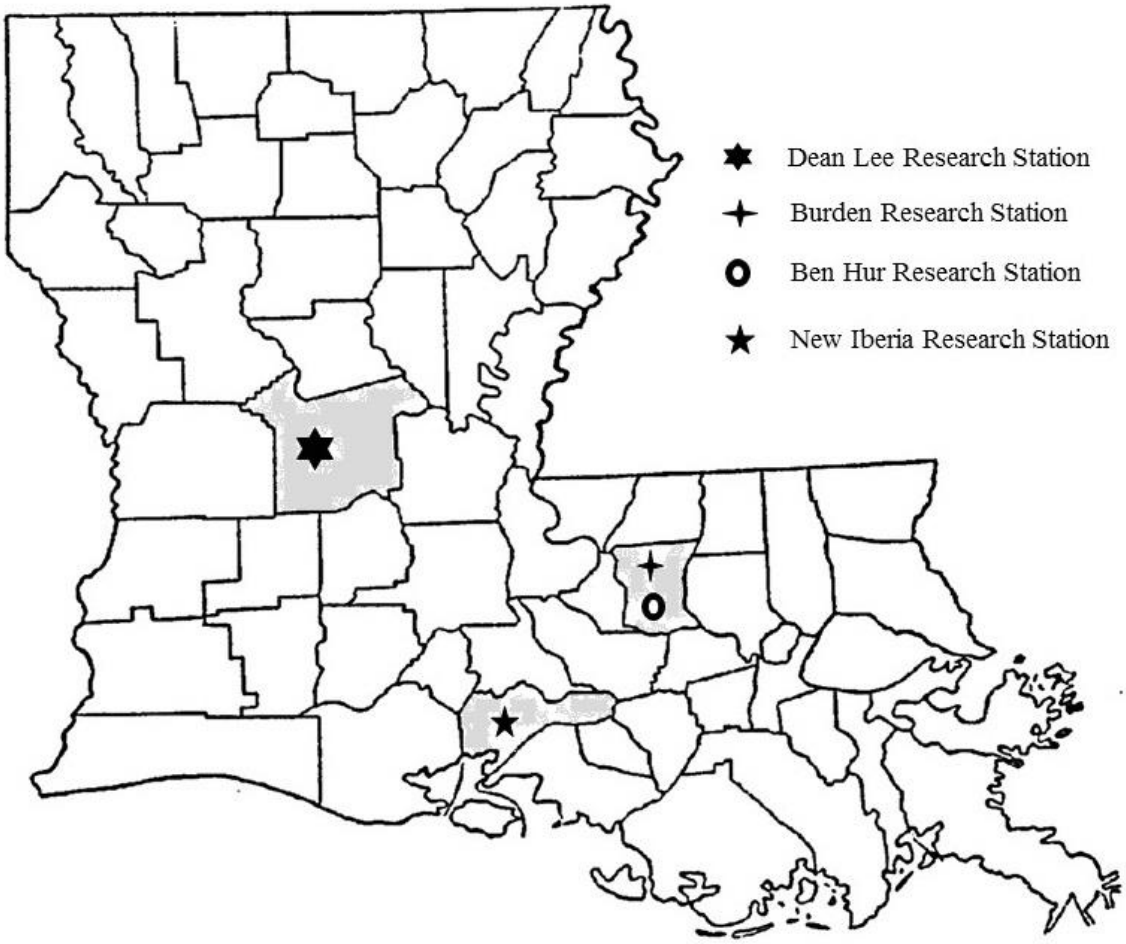

Figure 3.1. Study sites in Louisiana during 2013-2015.

Sampling was taken at biweekly intervals (with the exception that samplings were not taken due to inclement weather conditions) on six different dates from February to early May in each year expect at the Dean Lee Research Station in 2015 where samples were obtained for 5 sampling dates (Table 3.1). Three random samples were taken from each individual plot on each sampling date. Obtained samples were retained in Zip-Loc® bags and stored at -20°C until proceed. Stink bugs from the samples were identified to species according to keys provided by McPherson (1982) and Paiero et al. (2013) and their numbers were recorded.

Table 3.1. Sampling dates for *P. guildinii* in Louisiana during 2013-2015.

Year	Location	Sampling dates					
2013	Ben Hur	20-Feb	7-Mar	19-Mar	3-Apr	16-Apr	6-May
	Burden	28-Feb	14-Mar	28-Mar	12-Apr	23-Apr	8-May
	Dean Lee	26-Feb	12-Mar	26-Mar	9-Apr	23-Apr	16-May
	New Iberia	22-Feb	8-Mar	21-Mar	4-Apr	18-Apr	7-May
2014	Ben Hur	14-Feb	28-Feb	14-Mar	1-Apr	15-Apr	5-May
	Burden	18-Feb	13-Mar	27-Mar	11-Apr	24-Apr	12-May
	Dean Lee	10-Feb	26-Feb	19-Mar	2-Apr	17-Apr	7-May
	New Iberia	5-Feb	19-Feb	11-Mar	25-Mar	10-Apr	2-May
2015	Ben Hur	4-Feb	18-Feb	6-Mar	18-Mar	22-Apr	7-May
	Burden	12-Feb	27-Feb	18-Mar	7-Apr	22-Apr	7-May
	Dean Lee	10-Feb	-	20-Mar	1-Apr	24-Apr	11-May
	New Iberia	2-Feb	16-Feb	4-Mar	18-Mar	4-Apr	20-Apr

3.2.1 Statistical Analysis

The sporadic presence of both adult and nymph *P. guildinii* in low numbers (4% presence) in selected host plant for each location, sampling date and year altered analyses. Due to the sparse nature of data, count data of adult and nymph stink bugs was converted into presence or absence and analyzed by logistic regression (Proc logistics, SAS institute 2016). In

the logistic regression, the inclusion of either two or three way interactions in the model increased the full parameterization and reduced the validity of the model. So, logistic regression with treatments, location, and sampling dates as three main effects were selected via stepwise logistic regression. Odds ratios (OR) were calculated for a given factor with respect to a reference. The odds ratio represents the odds of insect presence for a given factor compared to odds of insect presence given a respective reference category (Agresti 1996). OR is the probability that an outcome will occur divided by the probability that an outcome will not occur. Thus, the odds ratio compares the odds of an outcome with respect to a reference.

3.3. Results

A total of 1,135 adult stink bugs belonging to 11 different species and 1,230 nymphal stink bugs belonging to 10 different species (Table 3.2) were captured during the whole study period. Adult and nymph *P. guildinii* accounted for 63% and 55% of the total adult and nymph stink bugs captured (Table 3.2). The list and count of all major arthropod taxa (other than stink bugs) found in Louisiana during 2013 to 2015 is given in (Appendix B).

Table 3.2. Summary of stink bug species composition, total count and percent combined over locations, treatments, and sampling dates in Louisiana during 2013-2015.

Species	Count		Percent	
	Adult	Nymph	Adult	Nymph
Piezodorus guldinii	716	685	63	55.7
Podius maculiventris	106	202	9.3	16.4
Euschistus servus	84	18	7.4	1.4
Nezara virdula	81	267	7.1	21.7
Euschistus quadrator	51	7	4.5	0.6
Euschistus ictericus	33	12	3.0	1.0
Oebalus pugnax	25	6	2.2	0.5
Thayanta accerra	22	0	2.0	0.0
Hymenarcys nervosa	11	27	0.9	2.2

Acrosternum hilare	3	6	0.3	0.5
Mormidea lugens	3	0	0.3	0.0
Total	1135	1230	100	100

The presence of adult *P. guildinii* was significantly affected by treatment, location, and sampling dates (Table 3.3). The OR for the New Iberia location was 2.72, which suggests that the presence of adult *P. guildinii* in this location was 2.72 times higher than that from the Dean Lee location keeping all other factors constant. When compared to the Dean Lee location, the presence of adult *P. guildinii* in Ben Hur and Burden locations was not significantly different ($P < 0.05$) (Table 3.4). When compared within the selected host plants, the odds of finding adult *P. guildinii* in Austrian winter pea was 0.01 times lower than that of white clover keeping all other things constant. Likewise, when compared to white clover, the odds of finding adult *P. guildinii* were 0.04 times lower than berseem clover, 0.07 times lower than cardinal red clover, and 0.05 times lower than hairy vetch respectively keeping all other things constant. In contrast, the odds of finding adult *P. guildinii* was 1.8 times higher in crimson clover compared to white clover. In the same way, the odds of finding adult *P. guildinii* were 0.5 times lower during the first sampling date compared to the third sampling date. Odds of finding adult *P. guildinii* on second and third sampling dates were not different. The odds of finding adult *P. guildinii* increased with later sampling dates in the season (Table 3.4).

Table 3.3. Type III fixed effects of factors for presence of adult *P. guildinii* in Louisiana.

Effect	*df*	Wald Chi-square	*Pr* > ChiSq
Location	3	34.76	<0.01
Treatment	5	207.81	<0.01
Sampling date	5	47.76	<0.01

Table 3.4. Odds ratio of parameters for presence of adult *P. guildinii* in Louisiana.

Parameters		Odds ratio	95% *CI*	*P* value
Location				
	Burden	1.44	0.88 - 2.33	0.1463
	Ben Hur	0.64	0.37 - 1.11	0.1165
	New Iberia	2.72	1.7 - 4.34	<0.0001
	Dean Lee (Reference)			
Treatment				
	Austrian winter pea	0.01	0.004 - 0.07	<0.0001
	Berseem clover	0.04	0.01 - 0.10	<0.0001
	Crimson clover	1.80	1.22 - 2.63	0.0025
	Cardinal red clover	0.07	0.03 - 0.16	<0.0001
	Hairy vetch	0.05	0.27 - 0.12	<0.0001
	White clover (Reference)			
Sampling date				
	First	0.50	0.25 – 0.98	0.0453
	Second	0.62	0.32 - 1.22	0.1711
	Fourth	2.39	1.34 – 4.26	0.0029
	Fifth	2.14	1.20 - 3.83	0.0095
	Sixth	2.60	1.46 - 4.63	0.0011
	Third (Reference)			

Note: Location = Dean Lee, Treatment = White clover, and Sampling dates = Third are used as reference category for the calculation of odds ratio.

The presence of nymph *P. guildinii* was also significantly affected by treatment, location, and sampling dates (Table 3.5). Odds of finding nymph is 2.87 times higher in New Iberia compared to Dean Lee keeping all other things constant. No difference was found on presence of nymph *P. guildinii* in either Ben Hur or Burden locations when compared to Dean Lee. Within the selected host, compared to white clover, the odds of finding nymphal *P. guildinii* was 0.03 times lower in

berseem clover and 0.01 times lower in cardinal red clover and hairy vetch respectively keeping all other things constant. Whereas, the odds of finding nymphal *P. guildinii* was 1.92 fold greater in crimson clover compared to white clover keeping all other things constant. In addition, the odd of finding nymph was lower in early sampling dates and the odds increased with later sampling dates (Table 3.6). The probability of finding adult *P. guildinii* increased with progression of season while the probability of finding nymph remain low throughout sampling period (Fig.3.2)

Table 3.5. Type III fixed effects of factors for presence of nymph *P. guildinii* in Louisiana

Effect	*df*	Wald Chi-square	*Pr* > ChiSq
Location	3	10.78	0.00130
Treatment	5	63.54	<0.001
Sampling date	5	52.43	<0.001

Table 3.6. Odds ratio of parameters for presence of nymph *P. guildinii* in Louisiana.

Parameters		Odds ratio	95% *CI*	*P* value
Location				
	Burden	1.87	0.86 - 4.06	0.1126
	Ben Hur	0.95	0.4 - 2.22	0.9070
	New Iberia	2.87	1.34 - 6.11	0.0063
	Dean Lee (Reference)			
Treatment				
	Austrian winter pea	<0.001	-_	-
	Berseem clover	0.033	0.008 - 0.14	<0.001
	Crimson clover	1.92	1.087 - 3.41	0.0247
	Cardinal red clover	0.018	0.002 - 0.13	<0.001
	Hairy vetch	0.017	0.002 - 0.12	<0.001
	White clover (Reference)			
Sampling date				
	First	<0.001	-	-
	Second	<0.001	_-	-
	Fourth	10.12	1.24 - 82.09	0.0302
	Fifth	45.26	15.96 - 343.60	0.002
	Sixth	124	16.39 - 938.53	<0.001
	Third (Reference)			

Note: Location = Dean Lee, Treatment = White clover, and Sampling dates =Third are used as reference category for the calculation of odds ratio.

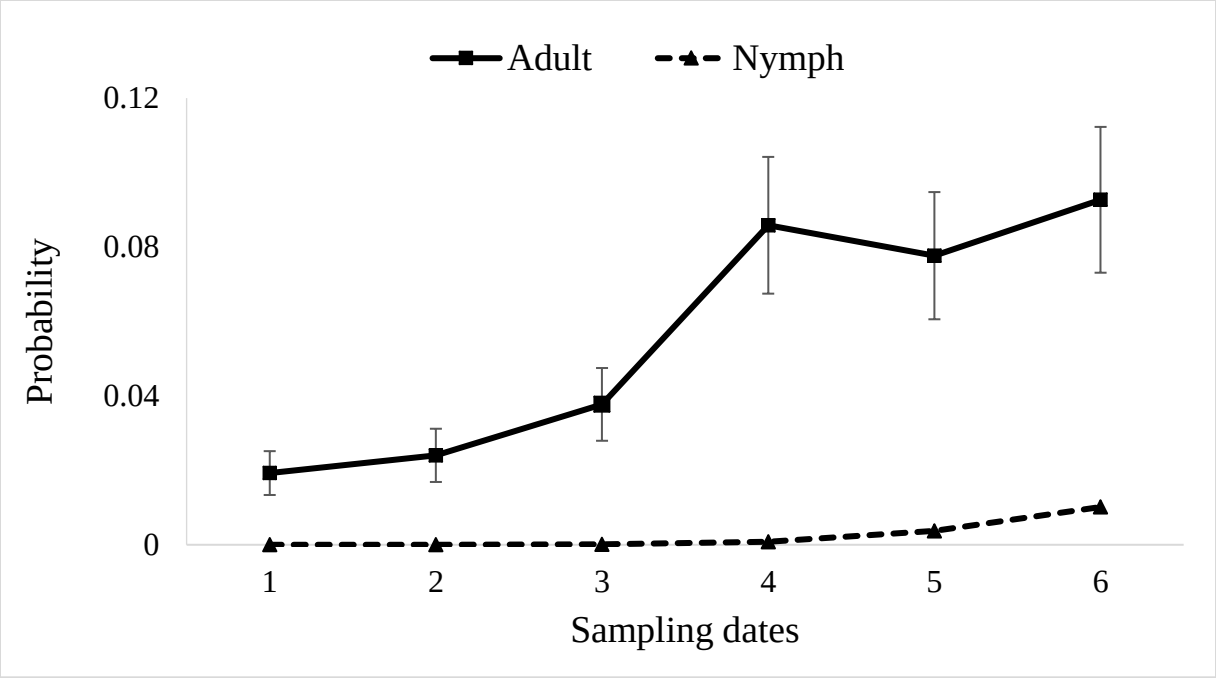

Figure 3.2. Probability of finding *P. guildinii* over dates in Louisiana. The listed sampling dates are bi-weekly intervals from first week of February to May first week.

3.4. Discussion

Piezodorus guildinii is established as an economic pest in southern U.S. soybean production (Temple et al. 2013b, Musser et al. 2015). The understanding of host plant preference of this insect pest early in the spring can be useful in predicting its incidence and future population build up in the soybean field. Most stink bug species spend considerable time in their life history in non-cultivated hosts which provide food, breeding sites and overwintering sites (Panizzi 1992, Panizzi 1997, Jones and Sullivan 1982). Knowledge of these host plants utilized by herbivore insects to complete life history can be exploited in developing pest management tactics (Kogan and Turnipseed 1987, Van Emden and Dąbrowski 1994).

This study shows that different species of stink bugs utilize selected hosts during the spring season in Louisiana. The scant presence of the stink bug was expected as sampling was conducted from the early spring season when climatic conditions were not favorable for the insect population to build up to higher numbers. This data showed that the most encountered stink bug species in these selected host plants is *P. guildinii,* both as adults and nymphs. This suggests that *P. guildinii* can exploit one or more of these hosts during spring. The occurrence of *P. guildinii* was significantly affected by the location, which may be contributed by many factors including local climatic conditions (see Chapter 2) and proximity of winter refuse site to the experimental field. The incidence of both adult and nymph *P. guildinii* was significantly different among selected hosts. The odds of finding both adult and nymph *P. guildinii* was highest in crimson clover followed by white clover. This suggest that crimson clover is the most attractive spring host in the presence of all selected hosts and white clover is the second most attractive host. Thus, these two hosts are preferred hosts of *P. guildinii* in the spring and probably in the early summer when soybean is not yet attractive to *P. guildinii*. In addition, higher odds of finding nymph in these two hosts make them most attractive reproductive hosts of *P. guildinii* in Louisiana as well. The probabilities of finding insects increased with later sampling dates suggesting possible population build up as the weather conditions became favorable.

Based on our data, crimson clover and white clover appear as promising bridging host for *P. guildinii* in Louisiana. Once *P. guildinii* becomes active, this species can exploit these hosts, leading to population buildup prior to moving to soybean. Therefore, avoidance of crimson clover and white clover as a cover crop in the winter, as well as controlling the volunteer crimson

clover and white clover around the farm is advisable as important management tactics to prevent *P. guildinii* population build up in soybean fields. Future studies looking at the nymphal in understanding the role of selected hosts on overall biology and nutritional ecology of this species.

CHAPTER 4: MONITORING THE DISPERSION OF THE REDBANDED STINK BUG, *PIEZODORUS GUILDINII* (WESTWOOD) IN SOYBEAN USING A PROTEIN BASED MARK-CAPTURE METHOD

4.1. Introduction

Stink bugs (Hemiptera: Pentatomidae) are key soybean insect pests in southern U.S. soybean production (McPherson and McPherson 2000a). The economically important stink bugs infesting soybean in the region are the southern green stink bug *Nezara viridula* (L.), the green stink bug *Chinavia hilaris* (Say), and the brown stink bug *Euschistus servus* (Say) (Funderburk et al. 1999). Nonetheless, a new species of stink bug, redbanded stink bug, *Piezodorus guildinii* (Westwood), has invaded and become an economic pest in this complex during the last decade. *Piezodorus guildinii* is established as a dominant species in soybean grown across Louisiana (Temple et al. 2013) and Texas (Vyavhare et al. 2014). The ecological and biological information of this emerging pest species is critical in developing its management strategies (Akin et al. 2011). However, key information is still lacking in regards to its biology and ecology in the U.S. Stink bugs are highly polyphagous and move regularly among their host plants making their monitoring and management difficult (McPherson and McPherson 2000a). Redbanded stink bug movement among different non-crop and crop hosts and their host utilization patterns over time and space in Louisiana agroecosystems largely remains unexplained.

The dispersal of an insect pest is directly related to crop damage as the abundance, colonization, and distribution of an insect in a crop field is impacted by its dispersal ability (Stinner et al. 1983, Irwin 1999, Petrovskii et al. 2014). Therefore, knowledge of insect pest dispersion in an agroecosystem is indispensable in developing effective management practices

(Irwin 1999, Mazzi and Dorn 2012, Codling 2014). Stink bugs move within the agro-landscape to find food, mates, and oviposition sites (Lamp and Zhao 1993, Panizzi 1997, Tillman 2011). The ability to disperse quickly among suitable hosts enables stink bugs to exploit many host plants (Lamp and Zhao 1993). Insect movement is a highly dynamic process, determined by the interplay of many features like age specific stadia, climatic conditions, and habitat allocations (Kennedy and Storer 2000). Insect movement can be categorized at three spatial levels: long range migration, inter-habitat movement, and within habitat movement (Clark et al. 1967, Rabb 1978). Stink bug movements are usually localized but during the period of emerging from overwintering sites until mating, southern green stink bug showed movements up to 1000 meters per day (Kiritani and Sasaba 1969).

After emerging from overwintering sites in the spring, stink bugs use an array of plants through the time of the year for oviposition, nymphal development, and adult survival (Nakasuji et al. 1965, Toscano and Stern 1976, Jones and Sullivan 1982, Reay-Jones 2010). Plant use by stink bugs varies with plant phenology and maturity (Schumann and Todd 1982). Stink bugs are most attractive to plants during fruiting stages (Velasco and Walter 1992, Bundy and McPherson 2000b). When plants reach maturity, they become less suitable and stink bugs move to younger plants that are rich in nutritional value (Bundy and McPherson 2000, McPherson and McPherson 2000b).

Stink bugs exhibit edge-mediated dispersal in their inter-habitat movement (Panizzi et al. 1980, Ehler 2000, Tillman 2011, Tillman et al. 2014). It has been reported that in farmscapes with corn, cotton, and peanuts, stink bug density was highest at crop interfaces, first infesting the crop edge when colonizing (Tillman et al. 2009, Tillman et al. 2014). It is also reported that

density and damage of stink bugs is highest at the edge of corn and soybean fields adjacent to crops or wooded habitats compared to open habitats (Venugopal et al. 2014). Clumped spatial distribution patterns are documented in *N. viridula* in soybean (Todd and Herzog 1980) and rice (Nakasuji et al. 1965). Clumped distribution patterns are also observed in several other stink bugs in tomato (Zalom et al. 1996). It is believed that an aggregation pheromones are responsible for such aggregation and it can greatly reduce the dispersion of stink bug within the field (Zalom et al. 1996). In a dispersal study carried out with the redbanded stink bug nymphs and southern green stink bug nymphs within Brazilian soybean fields, it was found that they move more lengthwise in the direction of row than transversely (Panizzi et al. 1980). It has been reported that adult *P. guildinii* dispersed 142 m in soybean fields compared to 121 m by adult *N. viridula* (Costa and Link 1997), which indicates that *P. guildinii* may be a more mobile pest. It is suspected that, in some cases, the failure of chemical control targeted to this species may be due to the high mobile nature of this pest and their spatial distribution within plant canopy (Baur and Baldwin 2006). However, studies looking at the dispersion and movement of this highly mobile pest are lacking.

Various marking systems can be used to determine insect spatial distribution, density, and migrations (Lavandero et al. 2004). Marking methods used are dependent on the insect of question (Hagler and Jackson 2001). Common markers used in insect movement study include paints (Southwood 1978), tags (Gray 1971), dust (Prasifka et al., 1999, Hagler et al., 2011), dye (Schellhorn et al., 2004), and various trace elements (Prasifka et al., 2001, Qureshi et al., 2004). Protein based markers with vertebrate specific proteins is a relatively a new marking method used in insect movement studies (Hagler 1997, Hagler and Jackson 2001). Moreover, recent

developments of inexpensive protein based markers with crude food proteins have made it possible to mark large numbers of insects in the field effectively with little expense (Jones et al. 2006). These inexpensive protein markers have been employed to study movement in many insects including *Hippodamia convergens* (Guérin-Méneville) (Hagler and Naranjo 2004, Horton et al. 2009, Bastola et al. 2014), *Musca autumnalis* (DeGeer) (Peck et al. 2014), *Scaphoideus titanus* (Ball) (Lessio et al. 2014), and *Drosophila suzukii* (Matsumura) (Klick et al. 2014). Among commonly used crude food proteins, chicken egg albumin is the most persistent and efficient marker (Jones et al. 2006, Klick et al. 2014).

A protein marking method may be an effective technique to study *P. guildinii* dispersion in a soybean field. Information on dispersal of *P. guildinii* in soybean can improve our capability to forecast and apply multiple control strategies to manage *P. guildinii* populations. An understanding of *P. guildinii* dispersal can even help in reducing pesticide use, therefore saving growers money and minimizing negative impacts to the environment. Thus, a mark-capture study on *P. guildinii* dispersion in a soybean field was conducted in which an insect population was directly marked in their natural habitat and later captured to detect the applied marker. Chicken egg albumin was used as a marker because it has been shown to be the most persistent and easy to detect food based protein markers (Hagler and Jones 2010). The objectives of this study were: 1) to evaluate the efficacy of the protein (chicken egg albumin) marking for stink bugs in a soybean field under field conditions, and 2) to assess the dispersal of *P. guildinii* (adult and nymph) in the soybean field.

4.2. Materials and methods

4.2.1. Plot layout

Mark-capture studies were conducted during 2015 and 2016 at Ben Hur Research Farm (30º 22'12.2''N, 91º10'11.6"W), Baton Rouge, Louisiana in soybean production fields under standard agronomic practices. In 2015, a soybean production field of size 304.8 m ×76.2 m was selected for the study (Figure 4.1). At the north end of the field, the first six rows of soybean from the edge at the middle of the field with dimension of 91.4 m × 4.5 m of the field were reserved for spraying with markers. In 2016, a soybean field of size 304.8 m ×42.6 m was selected for the study (Figure 4.2). Likewise, at the north end of the field, the first six rows of soybean from the edge at the middle of the field with dimension of 91.4 m × 4.5 m of the field were reserved for spraying markers.

4.2.2. Field marking

Field marking was carried out with 10% chicken egg white follow methods as described in Jones et al. (2006). All whites® 100% liquid egg whites distributed by Crystal farm, Minnetonka, MN as the chicken egg albumin marker was used. The 100% liquid egg white was mixed with tap water to obtain 10% chicken egg white. The marker was applied with a hand held 2 gallon lawn and garden sprayer ((RL FLO-Master®, Lowell, MI). The 10% chicken egg white marker was carefully applied on both sides of the soybean rows covering all top, middle, and lower plant strata at the rate of 720 L/ha in both years. In 2015, marking was carried out on July 7 when soybean was at the pod fill (R5) stage while in 2016 marking was carried out late on August 30 due to inclement weather issue when soybean was at the beginning of seed maturity stage (R7) (Fehr et al. 1971).

4.2.3. Insect sampling

Following the marker application, insect sampling was carried out each day for five days after marker application. Sweep samples were taken with a standard sweep net (0.38 m diameter) at each sampling time rotated between 3 different sweep nets to minimize contamination. In 2015, sweep samples were taken from soybean rows every 3.04 m along the sprayed rows at both directions up to 30.48 m. Samples were taken strictly along only one row in one day of sampling to avoid interference in insect movement. Samples were also taken from every 3.04 m across the sprayed area of the field to 30 m to middle of the field. A section was reserved 15.24 m of sampling rows across the field for each day of sampling and sampling was restricted to reserved portions of the rows only (Figure 4.1). In 2016 total sampling distance was increased to 91.4 m along the sprayed rows from the end of the sprayed area. Samples were taken on every 15.24 m along the rows (Figure 4.2). Samples were also taken from every 3.04 m across the sprayed area of the field to15 m to middle of the field. The sampling plan in 2016 was similar to that of 2015. Insects from sweeps were collected in Ziploc® bags. They were freeze killed in the laboratory by keeping samples overnight at -20°C**.** Then insects were stored individually in 1.5 µl centrifuge tubes at -20°C for further analysis by indirect ELISA.

4.2.4. Marking efficacy test

4.2.4.1. Soybean leaf tissue test from the sprayed field

The detectability of the egg white marker in the soybean leaf tissue under field conditions was evaluated. Following each marker spraying event, twice in 2015 (June 25 and July 7) and once in 2016 (August 30), soybean leaves were randomly collected daily from the sprayed areas daily from the day of spraying until 8 days after spraying of markers. The collected leaves were brought back to the lab and a leaf disc of 6 mm diameter was bored from each leaf using a plated

brass cork-borer. The cork borer was washed and dried with a paper towel each time before its use to avoid cross contamination. The leaf discs were stored individually in 1.5 µl centrifuge tube at -20°C until further analysis via ELISA.

4.2.4.2. Insect sample test from the sprayed field

The detectability of the egg white marker on the insect body from the sprayed area was evaluated after each marker spraying event, twice in 2015 (June 25 and July 7) and once in 2016 (August 30). Insects were collected from the marker sprayed area starting from the day the marker was applied until 8 days after marker application. Insects were collected with a standard sweep net (0.38 m diameter) and rotated between three sweep nets to minimize possible cross contamination. Insect samples were collected in Ziploc® bags. Insects were then freeze killed after they were brought back to lab. Freezed killed insects were individually stored in 1.5 µl centrifuge tubes at -20°C until further analysis via ELISA. For the movement study, adult and only 4^{th} and 5^{th} nymphal stages of *P. guildinii* were used as other younger nymphal stages are reported to be gregarious and show little dispersal (Panizzi et al. 1980).

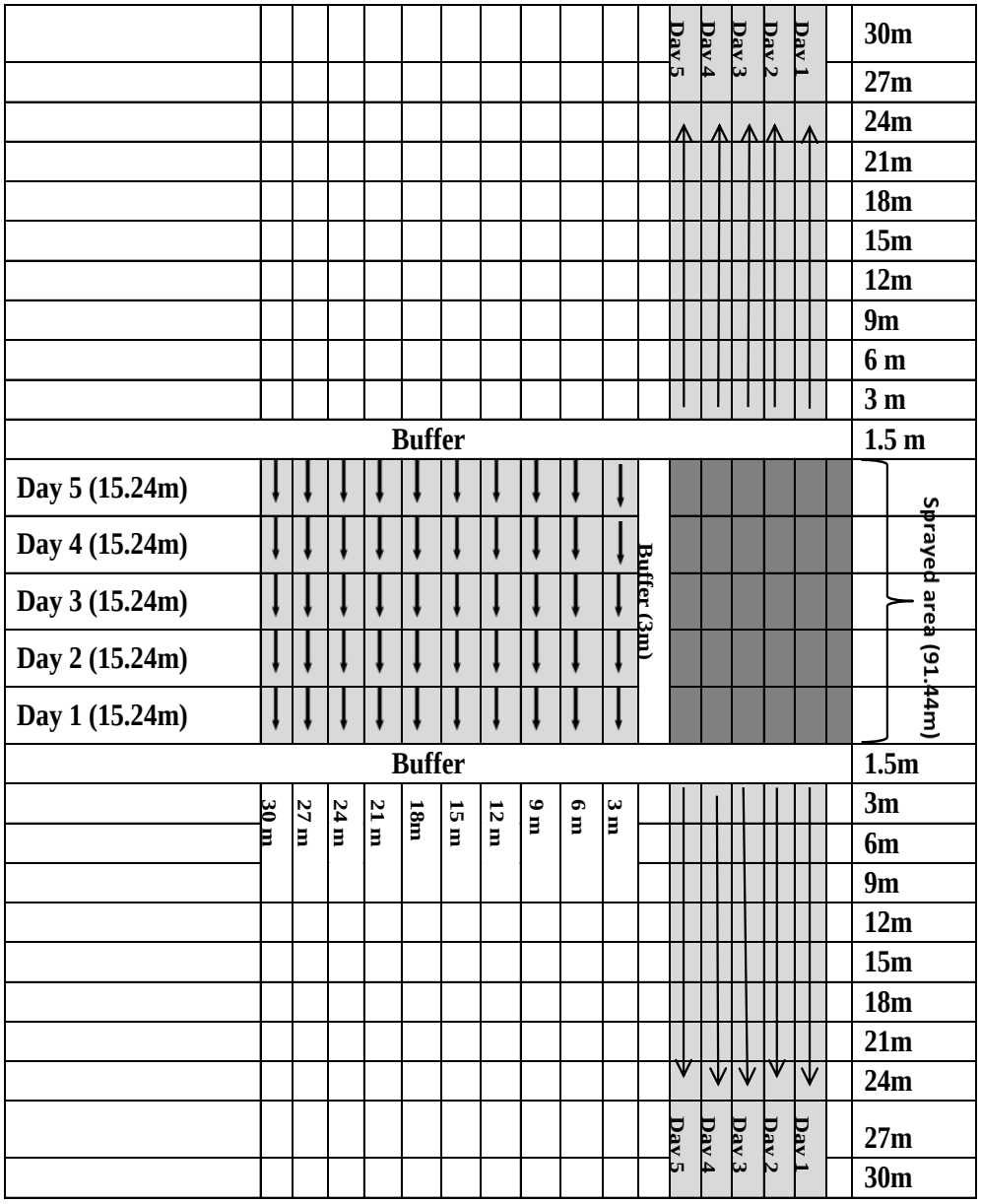

Figure 4.1. Plot layout and sampling plan for 2015 in the Ben Hur Research Farm Baton Rouge, Louisiana.

Note: The dark gray area is the marker sprayed area in the soybean field. The light gray area represent the actual soybean row along which samples are taken on the given day along and across the sprayed rows. The arrow represents the direction of sampling. The sampling distance is given in numeric values and is not to scale.

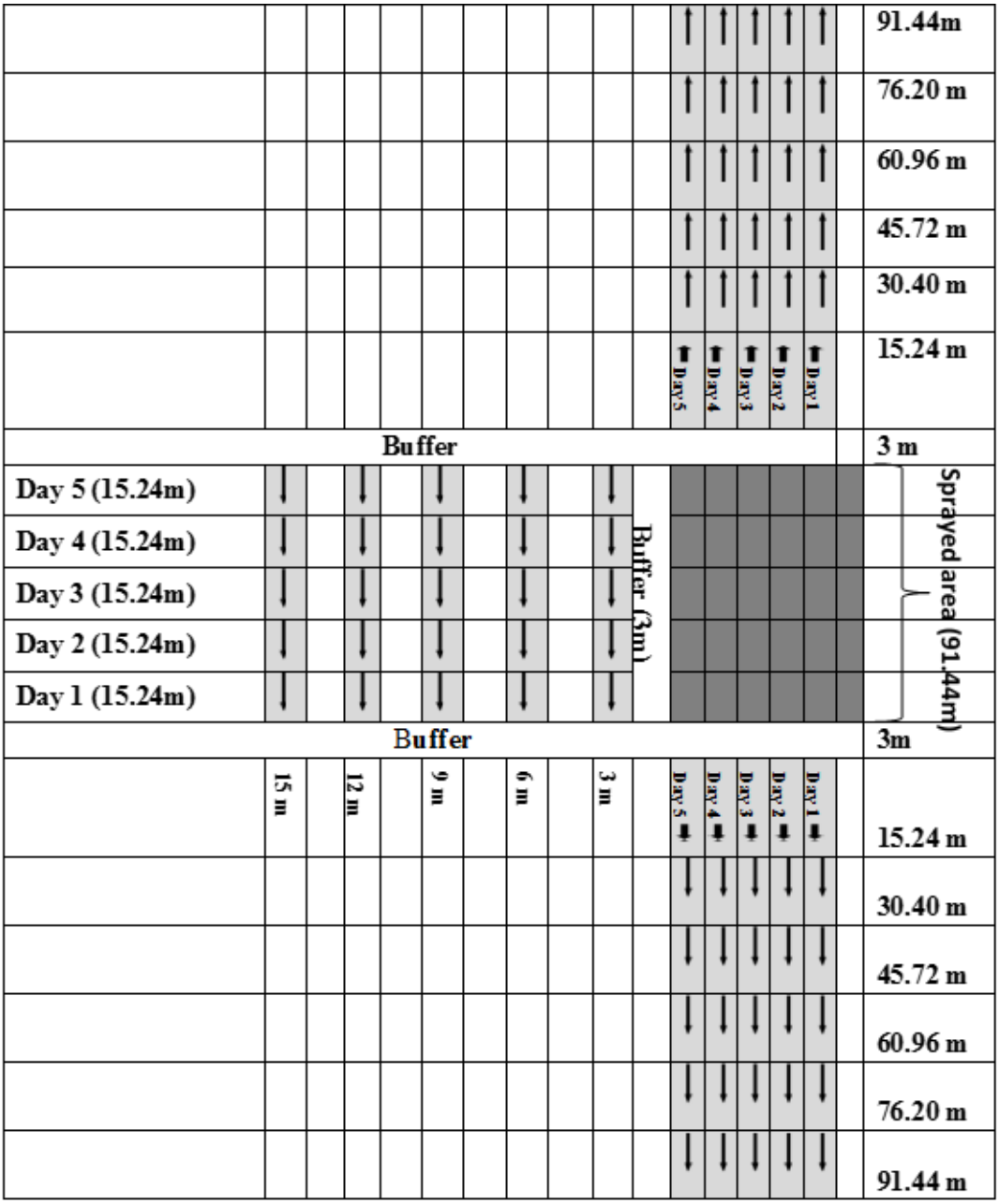

Figure 4.2. Plot layout and sampling plan for 2016 in the Ben Hur Research Farm Baton Rouge, Louisiana.

Note: The dark gray area is the marker sprayed area in the soybean field. Light gray area is actual soybean row along which samples are taken on the given day along and across the sprayed

rows. The arrow represents the direction of sampling. The distance is given in numeric values and is not to scale.

4.2.4. Marker detection

Indirect enzyme-linked immune sorbent assay (ELISA) was carried out for each individual insect captured (adult, 5^{th} and 4^{th} nymphal stages of *P. guildinii*) at each sampling dates and to detect the marker in the insect body. The same procedure was also used to detect the marker presence in the soybean leaf tissue samples. A modified protocol for chicken egg albumin from Jones et al. (2006) was used for the indirect ELISA procedure. To each insect sample or leaf disc (6 mm diameter) placed in a 1.5 µl centrifuge tube, 500 µl of TBS buffer (Tris-buffered saline) was added and then vortexed for 30 seconds. The samples were then held at 4°C overnight. Next, 80 µl of solution from each sample was placed into individual wells of a 96-well micro-plate (BRAND plates®, REF 781720, Wertheim, Germany). On each plate, serial dilutions (1000 ppm to1 ppb) of albumin from chicken egg white (A7641, Sigma-Aldrich, St. Louis, MO, USA) were used as positive controls. In addition, for each plate the negative controls from washing of unsprayed insect or unsprayed leaf tissue (80 µl) and blank containing nothing was employed. The micro-plate was incubated at 37°C for 2h in a mini incubator (Stellar Scientific, Baltimore, MD). The plate was then washed with 200 µl of 5X PBST (phosphate-buffered saline + 0.5% Tween-20) wash buffer 3 times. Next, 200 µl of PBS solution containing 1% BSA (bovine serum albumin, P3688 Sigma-Aldrich, St. Louis, MO, USA) as a blocking solution was added to each well using a multi-channel pipet. The plate was incubated for 1h at 37°C. Each well was then washed 3 times with 200 µl of 2X PBST wash buffer. Next, 80 µl of primary antibody (1:8000 in 1% PBS-BSA, rabbit antiserum to chicken egg albumin, C55298, MP Biomedicals, Solon, OH, USA) was added to each well. The plate

was again incubated at 37°C for 1h. After incubation, the plate was washed 3 times with 200 µl of 5X PBST wash buffer. Thereafter, 80 µl of secondary antibody (1:2000 in 1%PBS-BSA, Alkaline Phosphatase Anti-Rabbit IgG (H+L) made in goat (AP-1000, Vector Laboratories Inc., Burlingame, CA, USA) was added to each well. The plate was then incubated for 1h at 37°C. Following incubation, each well in the plate was washed 3 times with 5X PBST wash buffer. Subsequently, 50 µl of alkaline phosphatase yellow (pNPP) liquid substrate system for ELISA (P7998, Sigma-Aldrich, St. Louis, MO, USA) was added to each well. The plate was incubated at 37°C for 30 minutes to facilitate the reaction. Following incubation, 30 µl of 3M NaOH solution was added to stop the reaction. Absorbance was read using a photometric microplate absorbance reader (Multiskan® EX, Cat no. 51118170, Thermo Electron Corporation, Vantaa, Finland) at 405 nm wavelength. The insect samples with reading of ELISA optical density value (OD values) above mean plus 4 times standard deviation (mean + 4 SD) of OD values of negative control were described as positive samples for the marker for both insect samples and leaf tissue samples tested in the experiment (Jones et al. 2006). Based on the ELISA results, the movement of insect was confirmed when insect tested positive for the marker.

4.3. Data analysis

The (mean ± SE) ELISA optical density value and percentage of positively marked samples for leaf tissue and insects collected from the marked field was calculated for the day marker was applied to eight days after marking as the descriptive statistic. The data for the presence (positive) or absence (negative) of markers in the captured insect body was analyzed by logistic regression (Proc logistics, SAS institute 2016) with distance and date as the main effect.

4.3.1. Mean distance travelled

The mean distance travelled was estimated with the relationship provided by Fletcher (1974). To estimate mean distance traveled by adult and nymphal *P. guildinii,* the proportion of positively marked insect captured in a given distance (annulus) F_i was calculated by:

$$\hat{F}_i = \frac{\text{ni}}{\text{gi}}(x_{i+1}^2 - x_i^2) / \sum_{i-1}^{y} \frac{ni}{gi}(x_{i+1}^2 - x_i^2) \qquad (1)$$

Where,

$\frac{\text{ni}}{\text{gi}}$ = total number of marked insects/number of samples

x_i= the distance of the inner radius of the *i*th annuals

x_{i+1}= the distance of the outer radius of the *i*th annuals

$y =$ total number of annuli

Then, mean distance (MD) travelled is estimated by:

$$MD = \sum_{i-1}^{y} \hat{F}_i \ \ \frac{1}{2}(x_i + x_{i+1}) \qquad (2)$$

The mean distance travelled in our experiment is the lowest estimate of insect dispersal as insects may have dispersed farther than our sampling distance.

4.4. Results

The leaf samples collected from the field from three different marking events (n = 24) for each day after marking showed that both mean OD value and percent marked leaf sample declined rapidly after just 3 days of marking. No leaf samples were positive 7 days after marker applications. The freely roaming insects collected from sprayed areas of the field at three different marking events (n = 24) showed sharp decline in the mean OD value from the day marker was applied. The mean OD values remained similar for 2 to 4 days after marking and declined after that.

In 2015, distance ($\chi 2$ = 9.5; P = 0.38) and days after marking ($\chi 2$ = 4.4; P = 0.35) for adult and distance ($\chi 2$ = 8.2; P = 0.50) and days after marking ($\chi 2$ = 1.7; P = 0.88) for nymphs did not significantly affect the presence or absence of markers on captured insects. Similar results followed in 2016 where distance ($\chi 2$ = 4.14; P = 0.52) and days after marking ($\chi 2$ = 6.33; P = 0.17) for adult and distance ($\chi 2$ = 4.81; P =0.43) and days after marking ($\chi 2$ = 3.06; P =0.54) for nymphs were not significant for the presence or absence of markers on the captured insects. The mean travelled distance calculated for adult *P. guildinii* in 2015 was up to 68 m along the soybean rows observed after 2 days of marking (Table 4.1). In the same way, adult dispersed up to 15 m across the soybean row (Table 4.1) was documented after 5 days of marking. In 2016, mean travelled distance calculated for adults was up to 137 m along the rows and 10.2 m across the rows observed after 5 days of marking. In 2015, mean distance calculated for nymphs along the rows was up to 56.4 m and across the row was 19.4 m (Table 4.2). Whereas, in 2016 mean distance calculated for nymphs was up to 122 m along the row and 11.7 m across (Table 4.2).

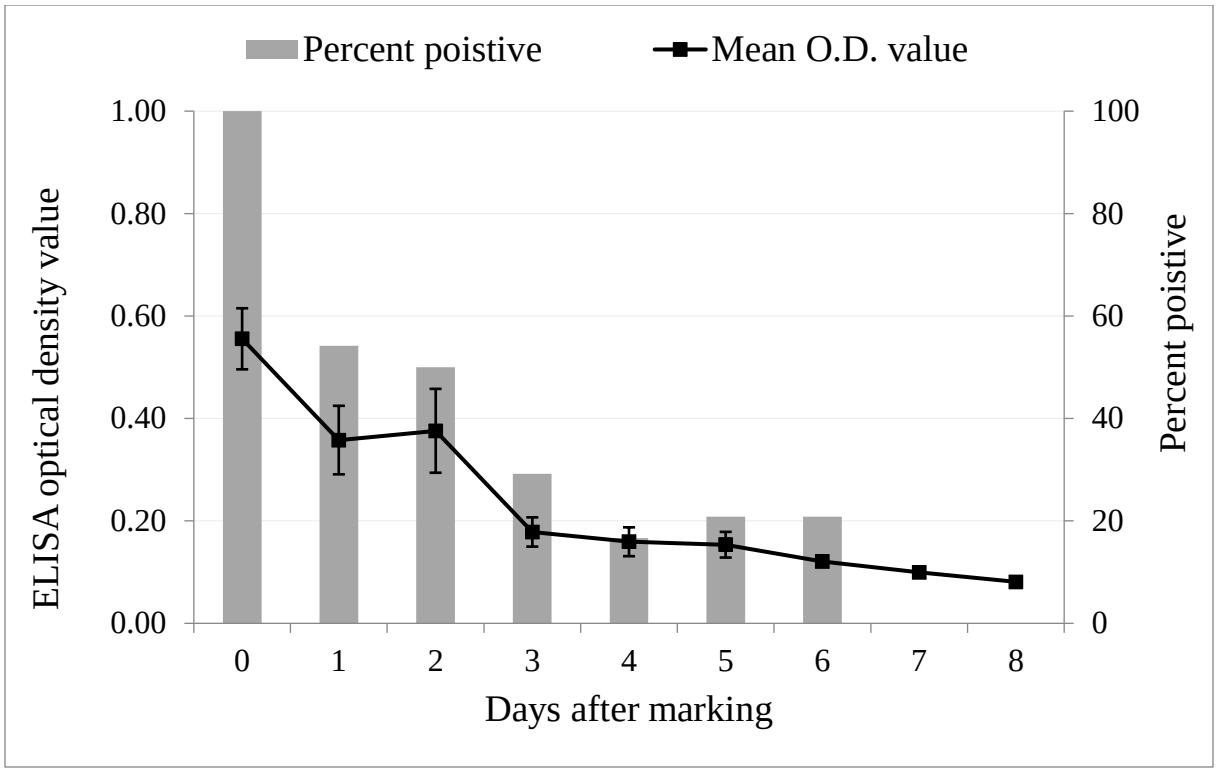

Figure 4.3. ELISA optical density values (mean ± SE) and percent of soybean leaf tissue (n = 24) found positive for the egg white marker from the sprayed soybean field.

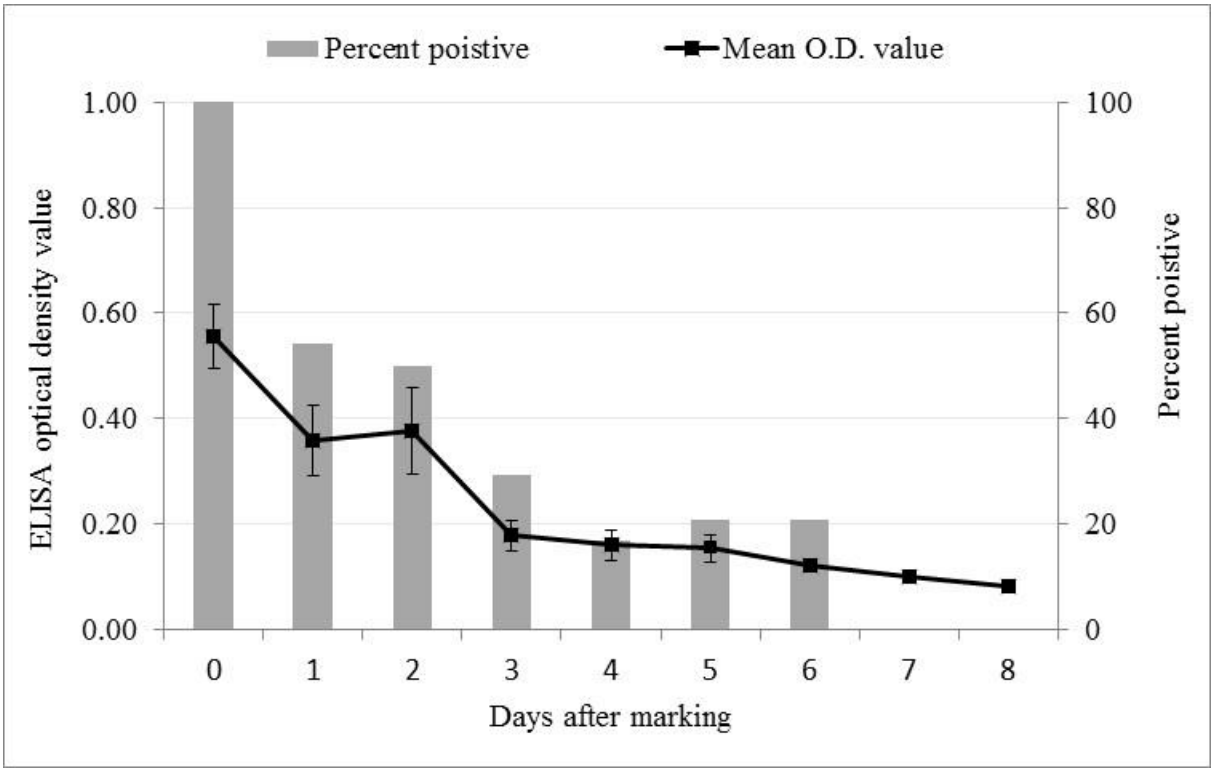

Figure 4.4. ELISA optical density values (mean ± SE) and percent of *P. guildinii* (n = 24) found positive for the egg white marker from the sprayed soybean field.

Table 4.1. Estimates of mean distance travelled (meters) ± SE for adult *P. guildinii* adjusted for sampling number and sampling distance.

	Along the row				Across the row			
	Day after marking	Total captured	Total marked	Mean distance moved (meters) ± SE	Day after marking	Total captured	Total marked	Mean distance moved (meters) ± SE
2015								
	1	21	2	50.2 ± 3.3	1	6	0	NA
	2	55	3	68.2 ± 5.6	2	12	1	9 ± 0.9
	3	24	5	58.5 ± 2.4	3	17	0	NA
	4	23	6	55.7 ± 5.3	4	11	0	NA
	5	18	7	56.38 ± 2	5	26	4	15.3 ± 1.2
2016	1	34	1	83.8 ± 14	1	4	0	NA
	2	32	8	111 ± 11	2	7	1	6 ±1.5
	3	22	4	82 ± 10.5	3	9	2	10 ± 1.8
	4	40	2	122 ± 17	4	7	0	NA
	5	33	2	137 ± 21.5	5	6	2	10.2 ± 1.4

Table 4.2. Estimates of mean distance travelled (meters) ± SE for nymph *P. guildinii* adjusted for sampling number and sampling distance.

	Along the row				Across the row			
	Day after marking	Total captured	Total marked	Mean distance moved (meters) ± SE	Day after marking	Total captured	Total marked	Mean distance moved (meters) ± SE
2015								
	1	29	3	47.2 ± 4.7	1	13	0	NA
	2	34	8	52.1 ± 2.8	2	8	1	9.9 ± 0.9
	3	14	6	56.4 ± 2.4	3	3	1	6 ± 0.6
	4	14	7	54.5 ± 2.5	4	7	5	19.4 ± 1.0
	5	6	4	30.5 ± 6.3	5	4	3	14.4 ± 1.0
2016	1	10	3	59.3 ± 9.5	1	2	0	NA
	2	12	8	53.3 ± 8.8	2	8	2	7.8 ± 1.2
	3	17	3	80.4 ± 10.5	3	9	2	12 ± 3.0
	4	19	4	122 ± 7.0	4	8	3	11.7± 1.7
	5	34	14	96.1 ± 3.3	5	5	2	10 ± 1.8

Discussion

Insect movement behavior is a phenomenon of great importance in pest management as it is an important predictor in population dynamics for a highly mobile and polyphagous insect pest (Kennedy and Storer 2000). *Piezodorus guildinii* is a polyphagous and mobile pest that uses a sequence of hosts to complete multivoltine generations. In the past studies, stink bug movements are inferred indirectly via comparing sampling at different time points. The field marking study conducted allowed us to directly track *P. guildinii* movement in the soybean field. A reliable marker is very important in the study of any type of mark capture study (Hagler and Jackson 2001). The marking efficiency test based on the presence of residue marker on the leaf tissue in our study showed that less than 40% of tested leaf tissue scored positive with a sharp decline in mean optical density values just after 3 days (Figure 4.3). This is different from the observation by Hagler and Jones (2010) where they reported that the spraying of 10% egg white marker in the leaf tissue of caged cotton plants in the field score nearly 100% positive up to 12 days after marking. The differences observed may be due to differences in environmental conditions and target plant species evaluated. Plants were exposed fully to weather conditions and UV breakdown contrary to caged plants. The differences observed may also be due to the modified protocol where alkaline phosphatase yellow (pNPP) liquid substrate read at 405 nm compared to TMB Substrate read at 650 nm used in their experiments. The insect samples captured from the sprayed area showed less than 40% positive just after 5 days (Figure 4.4). In a closed system with cages in the field, it is reported that 90% of the arthropods tested positive up to 14 days after marking (Hagler and Jones 2010). In our study it is possible that low residue on the leaf tissue may have resulted in recovery of a lower percentage of marked insects. In addition, new

immigrant insects in the open system may have diluted the recovery of marked individuals**.** In a comparable study, it is reported that 9 to 19% of unmarked insects were recovered from sprayed field (Bastola et al. 2014).

Our data indicated that the presence or absence of markers on the captured insect was not affected by the distance and the days after marking which may be due to dilution of the marked insect population in the field. The maximum mean distance travelled by adult *P. guildinii* along rows was found in our study was 137 m and across rows was 15.3 m. Our estimation is comparable to Tillman et al. (2009) where it is reported that stink bug showed movement of at least 120 m. Huang (2012) documented that *Nezara viridula* and *Chinavia hilaris* travelled at least 49.4 m and 87.8 m in a mark recapture study within 2 days after marker application between peanut and soybean. The dispersal distances up to 450 m is reported for face fly, *Musca autumnalis* DeGeer (Peck et al. 2014) and up to 330 m for *Scaphoideus titanus* Ball (Lessio et al. 2014) in a mark-capture study. In some mark-capture studies, the mean distance flown is reported even farther for active flying insects such as *Lygus spp.* (1157 m) and *Hippodamia convergens* (1037 m) (Sivakoff et al. 2012). It is possible that adult *P. guildinii* can disperse beyond the distance sampled. Therefore, our estimates are the minimum estimate. The maximum mean distance travelled by fourth and fifth instars of *P. guildinii* in our study was 122 m along the row and 11.7 m across the soybean row. The dispersed distance shown by nymphs in our study was 10 times farther than the distance reported by Panizzi et al. (1980) where maximum moved distance recorded along the row was only 12 m and across the row was 7.2 m. The different approach used to assess the dispersal may have resulted in such disagreement. Few marked individuals captured at the farthest distance can influence the mean distance estimate.

However, mean distance travelled by *P. guildinii* in our study between years was consistent. The results of our study demonstrated that the protein marker is feasible in marking naturally occurring insect populations in the field under Louisiana climatic conditions. The direct evidence of insect movement is demonstrated in this study.

CHAPTER 5: SUMMARY AND CONCLUSION

Stink bugs (Hemiptera: Pentatomidae) are part of the major insect pest complex in the soybean production area of Southeastern U.S. Of this complex, redbanded stink bug *Piezodorus guildinii* (Westwood), has emerged as the most serious soybean pest in the last decade, resulting in increased insecticide applications and yield loss. This species has established as an annual pest in Louisiana and the rice belt area of Texas. Moreover, it is spread into the surrounding southern and Mid-southern states, with 2017 having the most insecticide applications for this pest ever occurring in AR and MS.

Management of this species is very challenging due to its tolerance of insecticides. Redbanded stink bug populations in soybean quickly reach above action thresholds and the damage it can cause is more sever compared to other stink bugs. Reliance on insecticide control is making this insect resistant to organophosphates. Additionally, intense spraying kills many beneficial insects. The cost effective management of this pest is only possible when multiple tactics based on its biology and ecology are utilized.

Since, redbanded stink bug is a recently encountered soybean pest in the U.S., very little is known about its biology and ecology. One of the little known areas is overwintering biology and cold tolerance ability of this species. Although its native range is neotropical, this stink bug has shown its range expansions into relatively cooler climatic regions of Mid-South. Information on cold tolerance of this species is therefore valuable in predicting its home range in northern limits.

The first research project undertaken in this dissertation was laboratory and field studies to look into the cold tolerance ability of redbanded stink bug. The different indices of cold tolerance like supercooling points, lethal exposure time at sub-zero temperatures, and chill coma

temperature as well as winter survival under field conditions were evaluated. The studied showed that supercooling points of field population decreased from summer months to fall and lowest supercooling points observed were from winter months. Exposure to sub-zero temperatures reveled that these insects die rapidly above their supercooling points. Chill tolerance of adult insect was significantly higher than insects from combined nymphal stages. Odds of adult insect surviving winter decreased as winter month progressed. In addition, winter survival differed between years depending on the severity of cold. Based on results from Chapter 2, the incidents of rise and successive fall of *P. guildinii* populations may best be explained by the severe winter temperatures occurring in those regions. Our result indicates that the *P. guildinii* is chill sensitive and low temperature during the winter is a critical determinant of its permanent home range. Based on our observations, RBSB range expansion greater than the current range is unlikely unless the insect becomes more cold adapted.

One of the knowledge gaps in the ecology of redbanded stink bug in the U.S. is its alternative hosts. Little is known about how redbanded stink bug utilizes available spring hosts to build up early spring populations before infesting soybean. Equally, their preference for spring hosts is not well documented. I designed the study to look at the preference of redbanded stink bug to selected spring hosts. Six different hosts of redbanded spring bug based on available literature and field observations were selected. These were crimson clover *(Trifolium incarnatum*), cardinal red clover (*Trifolium pretense*), austrain winter pea (*pisum sativum*), berseem clover (*Trifolium alexandrium*), hairy vetch (*Vicia villosa* Roath), and white clover (*Trifolium repens*). I conducted the redbanded host preference in four different research farms at three different geographic regions. Our data indicate that the presence or absence of redbanded

stink bug is significantly affected by the location, host, and sampling dates. Among hosts, the odds of presence of *P. guildinii* were greater in crimson clover followed by white clover. Similar result was observed in case of nymph too. Crimson clover and white clovers seems not only preferred host but also important reproductive hosts of redbanded stink bug during spring season in Louisiana. These host acts as bridging host once stink bug becomes active after winter leading to infestation into soybean production field in the summer. Management of these clovers around the farmscapes is highly recommended to reduce the redbanded stink bug buildup in the spring.

The third research project was to monitor the dispersal of redbanded stink bug in the soybean field using protein marker with mark-capture method. This study showed that protein marking of insect with chicken egg albumin is a practical method in studying within field dispersal of redbanded stink bug. Marking efficiency in the field condition was evaluated. The mean distance documented for adult was137 m along and across rows was 15.3 m across the soybean rows. For the combined fourth and fifth instars nymph mean distance estimated was 122 m along and 11.7 m across the soybean rows.

This dissertation has attempted to address some key biological and ecological aspect of redbanded stink bug. The future research looking at following aspect is recommended.

1. Investigation on ecophysiological basis and underlying mechanism of cold tolerance of redbanded stink bug.
2. Investigation on development and population growth of redbanded stink bug on selected spring host.

3. Investigation on movement of redbanded stink bug in a landscape level between non-crop and crop host.

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APPENDIX: MAJOR ARTHROPOD TAXA (OTHER THAN STINK BUGS) FOUND INSPRING HOSTS 2013-2015.

Taxa	Count
Nabidae	499
Nabidae nymph	432
Araneae	5529
Diptera	77829
Formicidae	5818
Cicadellidae	8454
Coccinellidae	1504
Coccinelidae larvae	877
Aphididae	63547
Coleoptera	8888
Hymenoptera	2615
Spissistilus festinus	1847
Spissistilus festinus nymph	161
Geocoris spp.	1557
Geocoris spp. nymph	450
Lygus spp.	3335
Lygus spp. nymph	2307
Diabrotica undecimpunctata	1888
Diabrotica balteata	406
Hemerobiidae	478
Chrysopidae	122
Neuroptera nymph	16
Orius spp.	3181
Lepidoptera	271
Hemiptera nymph	33
Hemiptera	1838
Curculionidae	3594
Carabidae	42
Apidae	23
Orthoptera	240
Orthoptera nymph	145
Tupiladae	279
Bibionidae	3344
Others	502

VITA

Anup Bastola, son of Mr. Kamal Prasad Bastola and Mrs. Devaki Bastola was born and raised in Bhaktapur, Nepal. He got his bachelor degree in agriculture in 2006 from the Institute of Agriculture and Animal Sciences, Tribhuvan University, Nepal. He received his M.S. in plant protection from Texas Tech University in 2011. His thesis entitled "Arthropod Community Structure and Convergent Lady Beetle Intercrop Movement Behavior in Adjacent Cotton and Alfalfa" was carried out at the Texas AgriLife Research and Extension Center in Lubbock, Texas under the guidance of Dr. Megha N. Parajulee, Professor of Entomology. He joined department of entomology at Louisiana State University in 2012 to pursue a PhD in entomology under the supervision of Dr. Jeffrey A. Davis. He is married to Rita Devkota and has a daughter, Aashna Bastola. Anup is a PhD candidate in the Department of Entomology at Louisiana State University.