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Incorporation of Biorationals and Trap Crop for Stink Bug Management in Soybeans

Kukuh Hernowo
Louisiana State University and Agricultural and Mechanical College, kherno1@lsu.edu

INCORPORATION OF BIORATIONALS AND A TRAP CROP FOR STINK BUG MANAGEMENT IN SOYBEAN

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Kukuh Hernowo
B.S., Jenderal Soedirman University, Purwokerto, Indonesia, 1991
M.Phil., University of Queensland, St. Lucia, Australia, 2004
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ABSTRACT

Stink bugs are one of the most important seed sucking pests impacting soybean production in Louisiana. Exploration of chemicals that exhibit attraction or repellent activities toward this major pest would be beneficial to develop a push-pull strategy against this pest complex. Spinosad and neem are two commercially available natural insecticides that were claimed to have attractant and repellent activities against stink bugs. To test the potential of these chemicals, a series of experiments was conducted to investigate the olfactory, tactile, feeding, and oviposition preference of stink bugs toward different commercial spinosad products and neem. Additionally, two years of field experiments were conducted to evaluate the effect of the chemicals in combination with early maturing soybean as components of a "push-pull strategy" on stink bug populations and soybean damage. Insecticides were tested at concentrations equivalent to recommended field applications. The results showed, in Y-tube assays, the southern green stink bug (SGSB) was significantly repelled by neem and significantly attracted to spinosad. In tactile tests, SGSB consistently avoided media treated with neem, but inconsistently was attracted to media treated with spinosad. In feeding preference experiments, Entrust® showed the strongest feeding stimulant compared to the other spinosad products, while neem (Azatin XL®) showed a considerable feeding deterrence. In oviposition preference tests, SGSB did not show a significant preference or avoidance to oviposition media treated with the tested substances. Field experiments showed early maturing soybean significantly attracted stink bug populations as shown by Cumulative Insect Days (CID), indicating that it has potential to be used as pulling component of the push-pull strategy. Meanwhile, in field conditions, the tendency of stink bugs towards Entrust® and the repellency of neem was not strong enough to give

statistical differences. The difference in stink bug populations affected seed production and quality as indicated by the parameters seed weight, percentage of undersized seed, and the percentage of good seed.

CHAPTER 1: INTRODUCTION & LITERATURE REVIEW

1.1. General Introduction

Soybean (*Glycine max* Merr.) is an important agronomic crop worldwide. Based on 2013 data, US is the leading soybean producer in the world, followed by Brazil, Argentina, China and India (FAO, 2015). In terms of planted acreage, there has been an increasing trend in the US soybean industry in the past 5 years. Soybean was grown on 75 million to 86 million acres and yielded roughly 3 to 4 billion bushel valued at $US 35 billion to 45 billion (NASS, 2015)

In the US, soybean is used indirectly through industrial processes for human consumptions; livestock feed or industrial inputs. Soybean seed is processed into margarine, mayonnaise, salad dressing, soybean meal and livestock or poultry feed (Goldsmith et al., 2015; Hymowitz, 2008). With the growing demand for healthier food, soybean is also processed into cooking oil that contains lower saturated fat as an alternative to palm oil (Goldsmith, 2008). In other countries, especially in Asia, soybean is consumed directly as tofu, soymilk, soy paste, soy sprout, soy sauce, natto, etc. (Liu, 2008). In Indonesia, soybean is consumed as a fermented product called “tempe”; a high protein, easily digestible cheap meat substitute (Nout & Kiers, 2005). Despite being a nutritious food, this fermented product is claimed to be able to prevent various degenerative diseases (Utari, 2010), including the ability to prevent the growth of myeloma cells or tumor cells (Kiriakidis et al., 1997). Additionally, soybean is recognized as a nutritious plant. Compared to other crops, soybean contains relatively higher fat and protein (Liu, 2012), two essential nutrients for growth.

The usage and demand of soybean seed and its products is projected to grow in coming decades. The prospects of the soybean industry are very promising, both for business expansion and profit projection. However, growers have to confront various obstacles, including unpredictable climatic change and the invasion of destructive biotic contenders. Little can be done to control the weather, but we have the ability to reduce the destructive impacts of biological factors such as insects.

Many insects utilize soybean as their food source, although factually few pose serious threats to the crop (Kogan & Turnipseed, 1987). In Louisiana, the major soybean pests differ by spatial and phenological plant stages. Based on 2013 report, the important pests that attacked soybean in Louisiana were threecornered alfalfa hopper (*Spissistilus festinus* Say), armyworm complex (*Spodoptera spp*.), corn earworm (*Helicoverpa zea* Boddie), green cloverworm (*Plathypeana scabra* F.), soybean looper (*Pseudoplusia includens* Walker), velvetbean caterpillar (*Anticarsia gemmatalis* Hübner), and stink bugs (Musser et al., 2014). Other insects, such as banded cucumber beetle (*Diabrotica balteata* LeConte), spotted cucumber beetle (*Diabrotica undecimpunctata howardi*, bean leaf beetle (*Cerotoma trifurcate* Forster), dectes stem borer (*Dectes texanus* LeConte), colaspis (*Colaspis brunnea* Fab), grasshopper, and some others were also caught from soybean field, but they were considered as minor pests and therefore, were not treated with insecticides.

The development of pest management strategies that rely on insecticides is continuously being challenged by the ability of insect pests to develop resistance. Nevertheless, the race between the development of new chemicals and the ability of pests to build resistance to new poisons seemingly will never end. However, utilizing more sustainable approaches of pest management through the employment of multiple tactics will slow the speed of resistance development.

The use of resistant varieties in soybean production is an effective and ideal way to minimize the excessive use of synthetic insecticides. Several attempts have been conducted to screen soybean varieties for resistance or tolerance to stink bugs. In 1973-1974, twenty seven lines were selected from 654 lines, and then tested for their resistance to stink bugs in the field; but none of those lines showed high resistance to stink bugs (Jones & Sullivan, 1979). Similarly, 894 introduction lines and 26 cultivars were tested in the year between 1975-1979 and all showed high susceptibility to stink bug feeding and only PI 171444 showed higher tolerance to stink bugs (Gilman et al., 1982). In the situation where resistant varieties are not available commercially, deployment of cultural control tactics can be chosen.

Cultural control or modification of cropping practices is a relatively user friendly tactic, as the method does not require specialized equipment or skill. In the field, this control tactic can be implemented through changing the time of planting to maximize pest avoidance; intercropping to increase biodiversity; and trap cropping to lure potential pests (Hill, 1983). For trap cropping in particular, a system must be developed to manipulate pest behavior; deployment of attractant; and diversion, interception or retention of targeted pests in order to reduce damage to the main crop (Shelton & Badenes-Perez, 2006).

In addition to the development of cultural tactics, scientists are also searching and developing chemicals that act not merely as toxicants, but also as repellents, anti-feedants and anti-ovipositional compounds. In pest management programs, compounds that pose such modes of action can be utilized as pest control material.

Plants have long been seen as a potential source of active compounds (Prakash & Rao, 1996). Over 2000 species of plants from more than sixty families are believed to have insect control properties (Dev & Koul, 1997). In some traditional agricultural practices, plants with insecticide properties have been utilized by farmers to kill or to deter pests (Belmain & Stevenson, 2001; Mihale et al., 2009; Mugisha-Kamatenesi et al., 2008). In the history of insecticide invention, crop protection practice thrived with botanical insecticide products such as derris, nicotine, quassin, and ryania (Jacobson, 1989; Singh et al., 2016). These have been replaced by more powerful synthetic chemicals (Isman, 2006).

Just as in the study of botanical insecticides, exploration of potential microorganisms as pest control agents has become an important in the development of alternative biorational insecticides. Among microorganisms that have been explored, *Bacillus thuringiensis* is one that has been studied extensively, manufactured commercially and considered as an entry point for the development of transgenic resistance crops (Sanchis, 2010; Starnes et al., 1993). Actinomycete *Saccharopolyspora spinosa* is another success story in the utilization of a microorganisms in pest control program (Kirst, 2010). Spinosad, the active compound found in the fermentation product of *S. spinosa*, has been successfully isolated, mass produced and marketed worldwide (Thompson et al., 2000). To date, these products are still being used in pest control programs in the US and worldwide.

1.2. Stink bugs as a major pest in Louisiana soybean production

Phytophagous stink bugs are considered a major pest of soybean in the southern United States. Surveys conducted between the year 2004-2008 showed that stink bugs were the most destructive pest in Alabama, Louisiana, Mississippi, Tennessee and Virginia (Musser et al., 2009). In Louisiana, the dominant stink bugs species has varied temporally. A survey conducted in 1991-1992 showed southern green stink bug (*Nezara viridula* L.) was the most abundant stink bug species (Boyd et al., 1997). In 1996-1997 experiments, southern green stink bug and brown stink bug were the most common stink bugs species caught from the experimental plots (Baur et al., 2000). In the last decade, however, species numbers have shifted significantly. Redbanded stink bug is now the most abundant stink bug in Louisiana (Temple et al., 2013) and Texas (Vyavhare et al., 2014) soybean.

Stink bugs usually arrive in soybean fields when the crop is at the R3 stage, when the plants are starting to develop pods with at least one pod in four upper nodes has reached 3/16 inch long (McWilliams et al., 1999). Stink bug populations generally increase along with pod development and reach a peak when the pods are fully developed and starting to mature (Bundy & McPherson, 2000; Smith et al., 2009a). On soybean, stink bugs feed mainly on pods. Their feeding activity directly causes seed and pod injury. The severity of seed damage may varies; depending on plant stage, stink bug species, pest life stage, pest density and other environmental conditions (Corrêa-Ferreira & De Azevedo, 2002; McPherson et al., 1979; Musser et al., 2011; Tood & Turnipseed, 1974; Yeargan, 1977). In general, pest attack at early stages of seed formation usually causes more severe damage (Miller et al., 1978). At R4 to early R5, stink bugs can suck up all the seed fluid, causing unfilled pods; whereas at later stages feeding can cause seed deformity and reduced seed quantity (Daugherty et al., 1964; Tood & Turnipseed, 1974). A study conducted by Todd and Turnipseed (1974) revealed that a single stink bug was able to cause 63.9 to 78.5% seed damage to 40 pods within 4 dates of infestation (Tood & Turnipseed, 1974).

Quantitative losses can be very significant and qualitative damage caused by stink bugs can also be economically detrimental. In a cage experiment, 2 stink bugs/0.3 m plot can cause

57% seed damage and 8% yield loss (McPherson et al., 1979). Commercially, the value of seed attacked by stink bugs can be rejected or downgraded (Musser et al., 2011; Tood & Turnipseed, 1974). In 2013 report, economic loss and cost spent to control stink bugs in southern US was estimated to be more than US $ 78.6 million annually, which was 24% of total loss and cost due to pests; making it the second most costly insect pest in Mid-south soybean field (Musser et al., 2016). Additionally, stink bug feeding also alters seed chemical composition and seed viability (Jensen & Newsom, 1972) and causes delayed maturity (Boethel et al., 2000).

Various control tactics have been developed to manage stink bug populations and to minimize yield loss. Chemical control is by far the most used method to control stink bug populations. Public concern has risen as intensive application of synthetic insecticides has led to pest resistance, pest resurgence, food and ground water contamination, pollution and killing of non-target organisms (Pimentel et al., 1992). Alternative tools are needed.

1.3. Spinosad Insecticides

Spinosad is a relatively new natural based insecticide. The active ingredient was isolated from colony of the actinomycete *Saccharopolyspora spinose*, taken from soil samples near an abandoned sugar rum house in the Caribbean. Dow AgroSciences is the company that screens, develops and mass produces the metabolites of this actinomycete (Thompson et al., 2000). Spinosad insecticide mainly contains the mixture of two active compounds; spinosyn A and spinosyn D. DowAgroSciences formulates these active compounds into several commercial products which are marketed as different trade names, including: Audienz™, Biospin™ , Boomerang™ , Caribstar™ , Conserve™ SC, Entrust™ Naturalyte®, Flipper™, GF-120™ NF Naturalyte, Laser™ , Mozkill™ , Musdo Gold™ , Naturalure™ Naturalyte, Spinoace™, SpinTor™

2SC Naturalyte, Success™ Naturalyte, Syneis™ Appat, and Tracer™ Naturalyte (AgroSciences, 2001).

Structurally, spinosyn is a tetracyclic macrolide with two different sugars, forosamine and tri-O-methylrhamnose (Salgado, 1998). The compound of Spinosyn D differs structurally from spinosyn A, the -H atom of the R1 is replaced with -CH3 (fig 1) (West, 1997). Under the IUPAC system, spinosyn A is named 2-((6-Deoxy-2,3,4-tri-O-methyl-α-L-mannopyranosyl)oxy)-13-((5-(dimethylamino)tetrahydro-6-methyl-2H-pyran-2-yl)oxy)-9-ethyl-2,3,3a,5a,5b,6,9,10,11,12,13, 14,16a,16b-tetradecahydro-14-methyl-1H-as-indaceno(3,2-d)oxacyclododecin-7,15-dione; while spinosyn D has the IUPAC name 2-((6-Deoxy-2,3,4-tri-O-methyl-α-L-mannopyranosyl)oxy)-13-((5-(dimethylamino)tetrahydro-6-methyl-2H-pyran-2-yl)oxy)-9-ethyl-2,3,3a,5a,5b,6,9,10, 11,12,13,14,16a,16b-tetradecahydro-4,14-dimethyl-1H-as-indaceno(3,2-d)oxacyclododecin-7,15-dione.

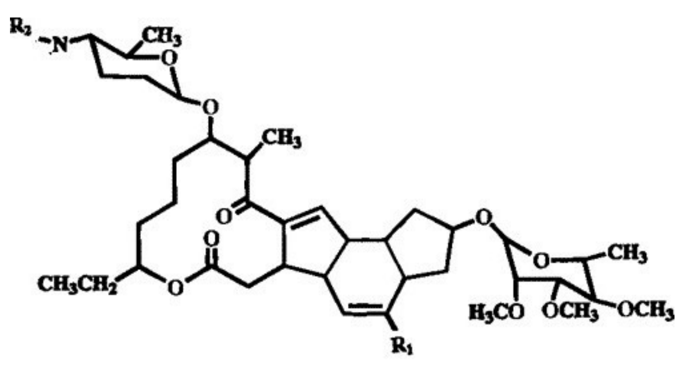


Figure 1.1. Chemical structure of spinosad (redrawn from West, 1997).

The mode of action of spinosad is claimed to be unique. It did not show toxicological effects when tested in assays of commonly known insecticidal-relevant target sites (Orr et al., 2009). Spinosad affects the central nerve system, causing prolonged hyper-excitation, spontaneous muscle contraction, and death (Salgado, 1998). Based on observable symptoms, spinosad also causes involuntary muscle contractions, prostration with tremors, and paralysis. These symptoms indicated that spinosad may activate the nicotinic acetylcholine receptor and GABA receptor function (Thompson et al., 2000).

As a natural product, spinosad has relatively shorter persistence in the environment compared to existing synthetic insecticides. Several factors play important roles on fate of spinosad residue in the environment. Spinosad dissipates in the environment through several routes including photolysis, hydrolysis and biotic degradation. Under aqueous photolytic conditions, spinosyn molecules lose their forosamine sugar and undergo reduction at the 13,14-bond on the macrolide ring; while hydrolytic degradation causes the loss of the forosamine sugar and water and reduction on the macrolide ring to a double bond at the 16,17-position (Cleveland et al., 2002). Biotic degradation, primarily involving soil microorganisms, is important for compound degradation in the absence of light. In the soil, microbial degradation of spinosyn occurs through demethylation on the forosamine sugar and possibly hydroxylation on the aglycone portion (Hale & Portwood, 1996).

As a nerve poison, spinosad shows a considerable range of targetable species. At early commercial application, spinosad was mainly recommended to control lepidoptera larvae. Later investigation showed that it was effective against mosquitoes (Bond et al., 2004; Cetin et al., 2005), houseflies (Scott, 1998), fruit flies (Yee & Alston, 2006), thrips (Eger et al., 1998), beetles (Elliot et al., 2007; Kowalska, 2010; Nayak et al., 2005; Toews & Subramanyam, 2003), tick (Cetin et al., 2009), and lice (Cole & Lundquist, 2011; Cueto et al., 2006).

From the stand point of safety, there is still a dispute concerning the negative impacts of spinosad, particularly to non-target species. Some studies support the claim that spinosad is relatively safer compared to synthetic insecticides for non-target species, including honey bee (Spencer et al., 2003), the predatory mosquitoes *Toxorhynchites sp.* (Garza-Robledo et al., 2011), and predatory mite *Phytoseiulus persimilis* (Holt et al., 2006). Other studies showed the opposite outcome, where spinosad affected non-target species negatively (Cisneros et al., 2002; Lim & Mahmoud, 2008; Rimoldi et al., 2012; Tillman, 2008; Williams et al., 2003). Notwithstanding the controversy, spinosad is generally believed to be more selective toward target insects and less harmful to many beneficial predators as well as mammals, aquatic and avian animals; to which it was awarded with U S Presidential Green Chemistry Challenge Awards (Kirst, 2010).

In spite of being a broad spectrum insecticide, spinosad is not recommended against hemipterans. It is not surprising since there are only a few studies that have attempted to investigate the effect of spinosad on hemipteran species. Toxicity of spinosad was inferior compared to other products recommended to control stink bugs (Brown et al., 2012). Spinosad may weaken targeted stink bugs and increase efficacy when it is applied in combination with other insecticides (Santos et al., 2016). An unusual outcome was obtained by Kamminga and her colleagues, in which, despite not significantly killing nymphs and adults of green stink bug (*A. hilare*) and brown stink bug (*E. servus*), spinosad induced feeding (Kamminga et al., 2009). Further study is required to examine the consistency of spinosad as a phagostimulant for stink bugs, since recommendations of spinosad to control lepidopteran pests in soybean field can be counterproductive due to the risk of increasing stink bugs populations. From a different standpoint, attractiveness of stink bugs toward spinosad may also be integrated into stink bug control strategies.

1.4. Neem

Neem (*Azadiracta indica* A. Jussifu) is one of the botanical insecticides widely known and promoted as an alternative to synthetic insecticides. The term “botanical insecticides” or “plant insecticides” refers to toxic compounds that occur naturally in plants and are extracted from them. Neem extracts contain many compounds that apparently have a wide range of activities on the biochemistry and physiology of many organisms (Schmutterer, 1990).

The insect repellent properties of neem were first reported in the scientific literature from India in 1928 (Ma, 2001). In 1959, Dr. Heinrich Schmutterer noticed that neem trees were the only plants that remained green and healthy when all other vegetation had been completely destroyed by a locust plague in Sudan (Schmutterer, 1995) and such experiences led to neem being investigated for its specific impact on insects.

Azadirachtin was found to be the major active compound in neem. The compound was first isolated from neem seed by Butterworth and Morgan in 1968 (Butterworth & Morgan, 1968). Since then, the interest in the study of its insect control properties and modes of action have significantly grown (Jacobson, 1989; Rembold, 1989; Schmutterer, 1990). Along with other compounds in neem extract, azadirachtin exhibits various bioactivities on insects including antifeedancy, growth inhibition, fecundity suppression, oviposition repellency, and toxic effects (Lowery & Isman, 1994; Saxena et al., 1981; Seljåsen & Meadow, 2006).

Hundreds of insects species were reported to be affected by neem extracts; including those that are highly resistant to almost all synthetic pesticides (Singh & Saxena, 1999). Negative impacts were also observed when neem was tested on stink bugs. Several scientific works confirmed this claim; for instance: at a dose 200-500 ng/insect, all southern green stink bug nymph tested by Riba et al. (2003) were dead during molting. Meanwhile, Seymour and his colleagues found neem seed extract capable of reducing more than 70% of feeding activity of *N. viridula* (Seymour et al., 1995). Additionally, field applications of commercial neem products in cowpea significantly reduced stink bug populations, seed damage, and yield loss (Abudulai et al., 2003b).

The promotion of botanical insecticides derived from neem, particularly in developing countries, as a substitute for synthetic chemical insecticides comes with optimism as the plant is able to grow fast even in infertile soil in arid regions and can also be easily propagated (Radwanski, 1977). More significantly, the active materials associated with neem can be extracted from the seed using locally accessible materials at the farm. Thus, the concept of producing "one's own pesticides" through neem cultivation is feasible for farmers in developing countries. In addition to traditional extraction methods, commercial neem-based insecticide formulations have been available in Europe, the Middle East, Africa, Asia and the USA.

1.5. Push-pull strategy of pest management.

Agroecosystems are dynamic environments, in which components interact with each other in an intimate interconnection and dependency. Compared to a natural ecosystem, an agroecosystem is relatively more fragile and unstable (Altieri & Letourneau, 1982). This instability is primarily associated with the domination of commercial crops over the landscape that provide unlimited food sources for adapted herbivorous insects. When natural limiting factors are absent or inadequate, the population of those herbivorous insects can grow exponentially causing massive injury and yield loss.

Studies on the mechanism and factors involved in the interaction between plant and insects revealed that semiochemicals play an important role. Phytophagous insects are known to use volatile secondary plant metabolites to locate their potential hosts (Bruce & Pickett, 2011). On the other hand, plants are also known for their mastery in producing metabolites that are used as self-protectants or to disorient their potential enemies (Baldwin, 2010). From the standpoint of plant protection, these attributes can be used to develop pest control tactics.

Repellents and attractants are gaining more attention in pest management program as insect resistance cases increasingly occur among agricultural pests (Deletre et al., 2016). In pest management, a repellent is a substance that causes an insect to make oriented movements away from its source (Nordlund, 1981), while an attractant is defined as a substance that causes an insect to perform directive responses toward the source (Dethier et al., 1960). The utilization of repellents or attractants to control agricultural pests have been used for some time. The concept of push-pull strategies in pest control by simultaneously utilizing attractants and repellents to manipulate pest abundance in agroecosystems, however, has only been promoted in the last few decades (Cook et al., 2006). Similar concepts with different terminology were used by Miller and Cowles, where they proposed using the term “stimulo-detterent diversion” or SDD (Miller & Cowles, 1990). This concept works by preventing potential pests from invading or by repelling potential pests away from the protected crop using repellent substances, antifeedants, or oviposition deterrents. At the same time, the pests are lured out into specific areas in the agroecosystem using aggregative substances or by applying trap crops.

Some agricultural pests are naturally aggregated (Davis, 1994). In a push-pull strategy, pest aggregation is strengthened or intentionally manipulated using attractant and repellent factors (Foster and & Harris, 1997). By pushing out potential pests from vast areas of protected crop into much smaller patches of sacrificial plants, selective area applications or site specific applications of insecticides can be more efficiently carried out.

1.6. Study objectives.

Integration of cultural and chemical control in pest management strategies requires strong fundamental baseline data on the potential of each control strategy. In order to gather the baseline information, a series of experiments were designed and conducted both in the lab and in the field. In general, these experiments were grouped into three different projects: 1) To evaluate olfactory response of redbanded stink bug and southern green stink bugs to spinosad products and azadirachtin; 2) To evaluate the tactile response and feeding preference of redbanded stink bug and southern green stink bugs to selected spinosad products and azadirachtin; and 3) to evaluate the effectiveness of spinosad and azadirachtin as elements of a push-pull strategy to manage stink bugs in soybean fields.

CHAPTER 2: OLFACTORY RESPONSE OF SOUTHERN GREEN STINK BUG AND REDBANDED STINK BUG (HEMIPTERA: PENTATOMIDAE) TO SPINOSAD AND AZADIRACHTIN

2.1. Introduction

A semiochemical is defined as natural language used by plants and animals to communicate. In the floral world, it is well documented that plants release various volatile substances for different purposes (Baldwin, 2010), with some of the volatile compounds released to lure beneficial counterparts, such as pollinators (Pichersky & Gershenzon, 2002; Reinhard et al., 2004). Others directly protect against herbivores (Ryan & Jagendorf, 1995), or indirectly by inducing third trophic insect activity (Birkett et al., 2000; Kessler & Baldwin, 2001). Moreover, these airborne messages can also reach neighboring plants and be perceived as alarming signals (Farmer & Ryan, 1990). Unfortunately, plant volatile compounds are also utilized by herbivorous insects to recognize and locate their position (Bruce & Pickett, 2011).

Insects utilize different arrays of volatile compounds to communicate with other individuals of the same species, to locate potential mates, to find potential hosts or prey, or to deter potential competitors or enemies (Dicke & Sabelis, 1988). In relation to host finding, once a volatile cue is detected by sensory systems, this long distance signal will consecutively guide them into specific patches where their potential host is located. The ability of insects to discriminate specific odors related to their potential host within the blend of odors in the air enable insects to find their preferred host (Bruce et al., 2005).

The tendency of an insect to choose particular crops, cultivars, or plant stages among a range of potential host plants, has been recognized and utilized to formulate pest control tactics. In the field, this tactic is incorporated into cultural practices known as trap crops (Hokkanen, 1991). In a similar way, insect preference toward compounds emitted by non-host plants can be manipulated for the same purpose (Borges & Aldrich, 1994). An effective trap crop strategy can greatly minimize insecticide application, reduce production costs, and lessen environmental risk from chemical exposure.

Implementation of trap crop tactics in soybean fields, particularly in managing stink bug populations, has not been widely adopted (Todd, 1989). The uncertainty of pest suppression is possibly the reason underlying farmers' decision not to gamble on this tactic. For scientists, the challenge arose from the difficulty to find non-soybean plants, or compounds that are highly attractive to stink bugs. Currently, planting of an early maturing soybean variety is still the only feasible way of attracting stink bugs populations in soybean (Kobayashi & Cosenza, 1987; Smith et al., 2009b).

Several investigations on natural or synthetic attractants or repellents for stink bugs have been carried out targeting an effective compound that can be utilized for stink bug monitoring or to support cultural control tactics against the pest (Abudulai et al., 2003b; Mcbrien et al., 2001; Seymour et al., 1995; Tillman et al., 2010). One contentious issue emerged in relation to the application of spinosad in soybean fields as this insecticide was claimed to induce feeding of nymph and adult of green stink bug (*A. hilare*) and brown stink bug (*E. servus*) (Kamminga et al., 2009), which in the end may increase stink bug populations. From a different perspective, this preliminary information may be worthwhile in investigating spinosad as a luring substance for stink bugs. Likewise, neem shows repellent activity to various insects (Isman, 2002), including stink bugs (Abudulai et al., 2003b; Seymour et al., 1995) and may be employed complementary with the attractant to drive stink bugs population into the sink zone in soybean fields. To assess the prospect of using these chemicals in such pest control tactics, a Y-tube bioassay was carried out to examine the olfactory response of southern green stink bug (*N. viridula* L.) and the redbanded stink bug (*P. guildinii* Westwood) to several different spinosad products and neem.

2.2. Material and Methods

Southern green stink bug (*N. viridula*) used in this experiment was obtained from a lab colony that has been cultured and established for several years in the soybean laboratory, LSU Agcenter. The stink bug is maintained in 15 cm diameter x 25 cm high carton containers, fed with fresh green bean and raw peanut. Cotton paper is placed on plastic petri dishes and soaked with water to serve as drinking reservoirs for the stink bug. The redbanded stink bug (*P. guildinii*) was collected from soybean fields in Louisiana. This field specimen was acclimatized and maintained in similar way as the southern green stink bug for at least 24h prior to the experiment.

Materials

The spinosad products (Entrust® : 80% spynosin A & D, Tracer® :44.2% spynosin A & D, Radiant®SC : 11.7% Spinetoram J & L, and technical grade spinosad; Dow AgroSciences, IN) and neem (Azatin XL®: 3% azadirachtin; OHP Inc. Mainland, PA) were diluted into distilled water to achieve concentrations equivalent to recommended field applications in soybean fields

(Entrust®: 599 ppm a.i., Tracer®: 690 ppm a.i., Radiant® SC: 365 ppm a.i. and Azatin XL® : 375 ppm). The concentration for technical grade was made at 600 ppm a.i.

Methods

A Y-tube olfactometer (ARS Inc: stem 16 cm; arms 8 cm at 60^0 angle between arms; ID 2.4 cm) was used in this experiment. Medical-grade compressed air was filtered with active carbon and left to flow through both arms at the speed of 300 ml/min per arm, regulated by flowmeters. The temperature in the bioassay room was maintained at 26° ± 1°C. The bioassay was conducted by placing 1.5 µl of insecticide suspension onto filter paper (Whatman No.1; Ø 15 mm) using a microsyringe. It was then placed into one of the odor source adapters of the Y-tube olfactometer. As a paired control, a piece of filter paper was wetted with distilled water only and placed into the other odor source adapter. A thin strap of paper (0.5 cm X 15 cm) was placed inside the main arm (the base) of the Y-tube to facilitate stink bug movement on glass surface of the Y-tube. After all parts were installed, fresh adults of tested insects were placed individually into the insect inlet adapter at the basal edge of the Y-tube and left to crawl inside the tube. To stimulate insect crawling toward odor sources and to minimize the effect of light, a paper box design purposely for this experiment was used to cover the main arm of the Y-tube.

Observations for each replicate lasted 5 minutes. Scoring was done by determining which direction the insect was crawling. A choice was considered to have been made when the stink bug passed a line about 3 cm from the base of the arm junction toward the source odor and remained there for at least 20 s. Insects that did not pass the base line during the duration of 5 minutes were discharged from the experiment. Each insecticide was tested using at least

100 individual (50 males and 50 females). To reduce environmental bias, the position of control and treatment arms were switched after every other time and a new Y-tube was used after 4 individuals were tested. The apparatus was cleaned with fragrance-free detergent, rinsed with acetone, distilled water and then oven dried prior to subsequent use. Chi-square analysis (SAS Institute 2010) was used to determine the goodness of fit of the data.

2.3. Results

Table 2.1. The proportion of Southern green stink bug and Redbanded stink bug selected treatment and control arms, and the value of chi-square and probability for each treatment.

	Frequency			
	Treatment	Control	Chi- Square	P-value
Southern Green Stink Bug				
Tech Grade	62	38	5.76	0.016*
Entrust	64	36	7.84	0.005*
Tracer	50	50	0	1ns
Radiant	47	53	0.36	0.548ns
Azatin XL	37	63	6.76	0.009*
Redbanded Stink Bug				
Tech Grade	52	48	1.44	0.726ns
Entrust	57	43	1.88	0.178ns
Tracer	55	45	0.94	0.3454ns
Radiant	53	47	0.22	0.585ns
Azatin XL	39	61	5.53	0.032*

Values compared using Goodness of fit (χ^2). * Significant difference (P <0.05); *** significant difference (P <0.001); ns, not significant

Results of our test showed that there were significant differences in the response of southern green stink bug toward Entrust® ($\chi 2$ = 7.84 P value = 0.005, technical grade spinosad ($\chi 2$ = 5.76 P value = 0.016) and azadirachtin ($\chi 2$ = 6.76, P value = 0.0093). The number of tested insects that went to the arm with the odor of those two spinosad products was significantly higher compared to those that went to the control arm, indicating that southern green stink bug was attracted to those two treatments. On the other hand, the number of tested insects that went to the arm with the odor of azadirachtin was significantly lower compared to those that went to control arm; this indicated that azadirachtin acted as a repellent to the southern green stink bug. The redbanded stink bug responded significantly only to azadirachtin ($\chi 2$ = 5.53, P value = 0.0187); indicating that azadirachtin was repellent to the redbanded stink bug. Meanwhile, although there were no significant differences, there was a numerical tendency for the redbanded stink bug to be attracted to spinosad; this can be seen from overall number of tested insects that were higher toward the treated arm (table 2.1.). This is a good sign that the tested insects positively responded to the odor from the treated source. In addition, further investigation through different tests such as feeding, direct contact, as well as a field trial, may strengthen or clarify this preliminary result.

2.4. Discussion

Overall results of our study suggest spinosad products, particularly Entrust®, and azadirachtin (Azatin XL®) have the potential to be used in a push and pull strategy to manipulate stink bug populations. The deployment of these insecticides can be adjusted with the whole pest management program designed for the soybean field. Practically, they can be used in separate ways to aggregate stink bugs into particular areas in the field or be used to strengthen the effectiveness of the trap crop tactic. Azadirachtin (Azatin XL®) was proven to act as a repellent to both species in this experiment and has potential to be used as a push element to restrain the stink bugs invading protected main plots. This insecticide may not kill stink bugs in the field, but it still can effectively prevent the crop from becoming infested. To become an effective element of a push-pull strategy, azadirachtin does not have to be toxic to targeted species. The main function of a push element is to create unfavorable or unattractive environments for the pests (Cook et al. 2007). Despite several claims that azadirachtin is generally less toxic to tested insects compared to the synthetic counterparts, the repellency and antifeedant activity posed by this natural compound is appropriate with ecological based management tactics. Natural aggregation patterns of the redbanded stink bug (Temple 2011), which is now dominating stink bug species in soybean field in Louisiana, will potentially increase the effectiveness of this precision pest management strategy.

CHAPTER 3: ARRESTANT RESPONSE, FEEDING PREFERENCE, AND OVIPOSITION PREFERENCE OF SOUTHERN GREEN STINK BUG (HEMIPTERA: PENTATOMIDAE) TO SPINOSAD AND NEEM

3.1. Introduction

Olfactory and visual stimuli play very important roles in insects finding their potential host plants. The combination of these stimuli is crucial for long range exploration (Reeves, 2011) that will guide them into specific patches where their potential host is located. In this long range examination, insects have to be able to discriminate specific odors related to their potential host within the blend of odors in the air (Bruce et al., 2005). Once an insect has landed on the surface of a potential host, in situ olfactory assessment will be carried out to identify the suitability of the host (Thorsteinson, 1960). An insect is equipped with a number of sensory receptors on antennae, mouthparts, tarsi, and ovipositors that are actively involved in detecting volatile and non-volatile plant compounds (Renwick, 1989). Host plant acceptance is confirmed when herbivorous insects feed or oviposit on the selected host plant. The ability of an insect to discriminate acceptable host plants from non-acceptable vegetation will determine their survival (Rausher et al., 1983), especially when the selection is to find an oviposition site, in which the newly hatched offspring will depend on for suitable food (Singer, 1986). A proper oviposition site selection will ensure a successful growth, development, and reproduction potential of the offspring.

Notwithstanding the importance role of volatile compounds in finding a potential habitat, the foraging behavior of some species tends to be random, and accurate cue detections occur only at relatively close distances (Thorsteinson, 1960). Hence, it is plausible that insects sometimes fail to respond positively toward a cue; even if the intensity of the odor is sufficient. Therefore, insect preference should not be determined by only one technique (Hoffmann, 1985). It is necessary to use different indicators to complement olfactory experiments in determining the attractant or repellent activities of a substance. For this purpose, feeding preference, tactile response, or oviposition preference are commonly applied.

Insects conduct in situ host assessment with different approaches, involving all sensory receptors necessary for this purpose. Some behavioral patterns have been observed related to this host selection process, although some variation may occur among different groups of insects. In general, the pattern of host selection begins with walking or standing over a potential host. In this initial step, chemical stimuli is perceived through chemoreceptors located on their tarsi. At the same time, the receptors on antennae may actively detect short range volatile compounds released by the plant. More detailed information on the nutritional value of the plant, the existence of phagostimulants or the absence of feeding deterrents is believed to be perceived through chemoreceptors in their mouthpart and foregut. To get this information, insects commonly perform palpation, piercing, or biting tests (Bernays & Chapman, 2007; Chapman & Bernays, 1989; Simmonds, 2001).

A “phagostimulant” is a substance that elicits feeding (Dethier et al., 1960). Sugar, especially sucrose and fructose, are the most common phagostimulants found in plant tissue, (Bernays & Chapman, 2007; Thorsteinson, 1960). Some other compounds include amino acids, vitamins, salts, phospholipids and sterols (Cook, 1977; Thorsteinson, 1960). Certain concentrations of flavonoids also induce feeding (Simmonds, 2001). For stink bugs, spinosad was suspected to induce feeding nymphs and adults of green stink bug (*C. hilare*) and brown stink bug (*E. servus*) (Kamminga et al., 2009).

In contrast to phagostimulats, many plant secondary metabolites are known to have negative impacts on insects. Terpenoids, phenolics, and alkaloids have long been associated with plant defense and act as repellents, deterrents, or suppressants (Beck, 1965). In pest management programs, many of these compounds have been utilized as natural pesticides (Dayan et al., 2009), or are being used as references or models to create synthetic versions (Elliott & Janes, 1978). These have included pyrethrins, nicotin, and rotenone (Casida, 1980). Neem is one of the natural products that has been promoted as a natural insecticide that poseses an array of bioactivity, including antifeedancy, growth inhibition, fecundity suppression, oviposition repellency, and toxic effects (Lowery & Isman, 1994; Saxena et al., 1981; Seljåsen & Meadow, 2006). Neem has been reported to deter stink bugs in the field (Abudulai et al., 2003b).

In integrated pest management practice, the application of a push-pull strategy, the attraction activity of spinosad and repellent activities of neem could potentially be used simultaneously to manipulate stink bug behavior; targeted to artificially aggregate stink bug populations for site specific insecticide applications. To determine the potential of these chemicals as an attractant and repellent for stink bugs, Y-tube bioassays, feeding preference bioassays, and oviposition bioassays were conducted in laboratory.

3.2. Materials and methods

Materials

Insects. Southern green stink bug (*N. viridula*) used in this experiment was obtained from lab colony that has been cultured and established for several years in the Soybean Laboratoy, LSU Agcenter. The stink bug is maintained in 15 cm x 25 cm carton containers, fed with fresh green bean, and raw peanut. Cotton paper is placed on plastic petri dishes and soaked with water; serving as a drinking reservoir for the stink bugs.

Chemicals. The spinosad products: Entrust® (80% spinosin A&D), Tracer® (44.2% spinosin A&D), Radiant®SC (11.7% Spinetoram J&L), and Azatin XL® (3% azadirachtin) (OHP Inc. Mainland, PA) were firstly diluted into distilled water to achieve concentration equivalents to recommended field applications in soybean fields (Entrust®: 599 ppm a.i., Tracer®: 690 ppm a.i., Radiant® SC: 365 ppm a.i. and Azatin XL® : 375 ppm).

Methods

Arrestant response assay. A choice test method was used to evaluate arrestant and repellency effects of spinosad and neem on southern green stink bug. Tested chemicals were applied by dipping half of a piece of filter paper into the solution until the entire surface was evenly saturated. It was then air dried in a hood prior to being used in the experiment. As a control, the other half of the filter paper was dipped in distilled water only; it was then air dried in the same way. Plastic petri dishes (0.9 x 1.5 cm) was used as the experiment arena by placing 1 half piece of treated filter paper and another half piece of the untreated filter paper into the base of the petri dish. Adult southern green stink bug (≤ a week old) were then placed individually into the arena by letting it walk on the inner surface of the petri dish lid just before the lid was placed to cover the arena. Observations were conducted by determining the position of tested insects, either on the treated (T) or untreated (U) portion of the filter paper at 30’, 1h, 2h, 4h, 6h, 8h, 10h, and 24h after treatment. Each replicate of the test was conducted using 40 individuals (20 males and 20 females).

Feeding preference assay. A feeding choice assay was used to determine feeding preference of the tested insects on soybean seeds treated with the insecticides. In this experiment, treatments were applied to soybean seeds by dipping the seeds into tested insecticides solutions and stir them for a minute to homogenize the seed coating. As standard control, seeds was dipped into distilled water only. Prior to the coating process, the dry seed was immersed in distilled water for 2 to 3 hours. Petri dishes (15) cm were used as the arena of the experiment. The stink bug used for this experiment was starved for 24 hours prior to the test. The test was carried out in two arrangements, pair test and complete choice test. In pair test, an individual stink bug was placed into the arena and exposed to treated seed and untreated seed (control) and left to feed for 24 hours. In the complete choice test, an individual stink bug was placed into the arena that contained all treatments and control seed and left to feed for 24 hours. The seeds were arranged randomly in the arena in circular formation. Each seed was placed into a marked plastic cup lid. After 24 hours of exposure, seeds with different treatments were dipped separately into fuchsin stain to dye stylet punctures (stylet sheaths). The number of stylet sheaths on the seed was used as the parameter of feeding preference. 30 individual stink bugs (male and female) were tested for each experiment.

Oviposition preference test. A choice test was used to determine the oviposition preference of the southern green stink bug on oviposition media (muslin cloth) that was treated with spinosad (Entrust®, Tracer®), neem (Azatin XL®), or untreated (control). Treatments were applied to muslin cloth (5 x 15 cm) by dipping the cloth into the tested insecticides solution until it was evenly wet. As a control, muslin cloth was dipped into distilled water only. The wet treated clothes were then air dried in the hood prior to being used in the experiment. Carton containers (15 cm diameter, 25 cm height) were used as oviposition arenas. During the duration of the test, fresh green bean and crushed raw peanut were put inside the container to serve as a food source for the stink bug. A cohort of stink bugs (± 1-2 week old) was maintained in the rearing container and allowed to mate prior being used in the experiment. The experiment was carried out in two different arrangements. Test 1 (pair test), treated cloth and untreated cloth (control) were placed alternately on the inside wall of the container; each arena contained 4 pieces of cloth (2 treated and 2 control). For test 2 (complete choice test), 3 pieces of cloths that were treated with different tested insecticides + 1 piece that was not treated were placed randomly on the wall inside the arena. 10 pairs of mated stink bugs were placed into each of the oviposition arenas and allowed to oviposit for 7 days. The number of egg masses and total number of eggs deposited on each cloth was recorded daily.

Statistical analysis. Chi-square analysis was used to determine the goodness of fit of the data taken from arrestant response assay; pooled as binomial proportion that was significantly different from the probability 50% using the standard normal approximation. T-pair test was applied to analyze data from pair test, and ANOVA was used to analyze data from the complete choice test.

3.3. Result

Table 3.1.: Proportion (%) of *N. viridula* seated on the treated portion and the Chi-square (χ^2) value at different check times.

Obsv. Time	Azatin XL®		Entrust®		Tracer®	
	%	χ^2	%	χ^2	%	χ^2
30"	4.76	81.9	40.00	4.0	52.00	0.2
1	17.39	42.5	31.25	14.1	52.00	0.2
2	17.24	42.9	20.00	36.0	48.00	0.2
4	16.67	44.4	23.08	29.0	45.83	0.7
6	13.33	53.8	25.00	25.0	50.00	0.0
8	6.67	75.1	43.75	1.6	53.85	0.6
12	6.67	75.1	47.06	0.3	54.55	0.8
24	10.71	61.7	70.83	17.4	55.56	1.2

Feeding preference assay. Result of the feeding test showed a consistent performance of neem as a feeding deterrent. In the pair test, both adult and nymphs of SGSB pierced untreated seeds more frequently than neem treated seeds, as seen in much higher numbers of stylet sheaths on that untreated seeds (t = 7.89, P < 0.0001; t = 6.49, P < 0.0001 respectively). In pair test using spinosad, there was a tendency for SGSB to feed more on spinosad treated seeds than on untreated seeds. Statistically, however, only nymphs were significantly different (Table 3.3). In complete choice tests, adults of SGSB prefer to feed on spinosad treated seeds and avoided neem treated seeds, as it can be seen in the number of stylet sheaths (F = 5.9 df = 5, P < 0.0001). Based on Tukey's HSD test, adults of SGSB showed clearer preference toward spinosad treated seeds and avoided neem treated seed. This is appeared in the highest number of stylet sheaths on spinosad treated seeds and the lowest number on neem treated seeds (Table 3. 2).

Fig 3.1. Arrangement of feeding bioassay and the appearance of stylet sheaths on soybean seed.

Table 3.2. Mean number ± SE of stylet sheaths on treated and untreated seeds punctured by adult and nymph *N. viridula* in pair feeding choice tests

Treatment	Adult SGSB		*t*	*P*	5th instar SGSB		*t*	*P*
	Treated	Untreated	value	value	Treated	Untreated	value	value
Entrust	4.35±0.45	4.15±0.57	0.29	0.7759	6.65±0.66	3.77 ± 0.56	3.66	0.0005
Tracer	4.43±0.54	4.27±0.60	0.20	0.8434	6.57±0.79	4.13 ± 0.69	2.14	0.0368
Radiant SC	4.00±0.52	5.23±0.76	1.33	0.1884	5.06±0.58	5.55 ± 0.84	0.49	0.6226
Azatin XL	1.53±0.26	8.00±0.72	7.89	<0.0001	2.52± .45	8.11 ± 0.88	6.49	<0.0001

Table 3.3. Mean number of stylet sheaths and the number of seed punctured by the adult of *N. viridula* in complete feeding choice test

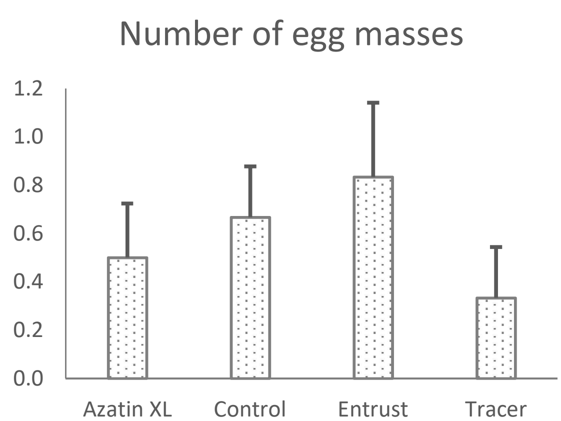
Treatment	Mean ± SE*	Number of seed with stylet punctured
Entrust	1.22 ± 0.26 a	24 out of 30
Tracer	0.85 ± 0.19 ab	22 out of 30
Tech. grade	0.42 ± 0.12 bc	13 out of 30
Control	0.40 ± 0.18 bc	12 out of 30
Radiant SC	0.33 ± 0.12 bc	8 out of 30
Azatin XL	0.08 ± 0.04 c	5 out of 30

* Mean in the same column which share a lowercase letter are not significantly different (P>0.05; Tukey HSD test).

Oviposition preference assay. Results of the data analysis showed female southern green stink bugs did not show particular preference in selecting oviposition sites, both in pair test and in choice test, indicating by the P value that is higher than 0.05; although the tendency to choose Entrust® (spinosad) treated media still appeared (Table 3.4, Fig 3.2).

Table 3.4. The average number of egg mass and average number of egg per arena deposited by *N. viridula* on treated or untreated (control) clothes in paired choice test.

Treatment	No. of Egg masses	Ratio	t value	P value	No. of Egg	Ratio	t value	P value
Entrust	2.8	1.2	0.32	0.769	152.5	1	0.04	0.97
Control	2.3				149.0			
Tracer	2.8	1.1	0.52	0.638	144.8	1.1	0.21	0.844
Control	2.5				135.5			
Azatin XL	4.3	1.1	0.42	0.703	169.3	0.8	0.52	0.641
Control	3.8				201.8			



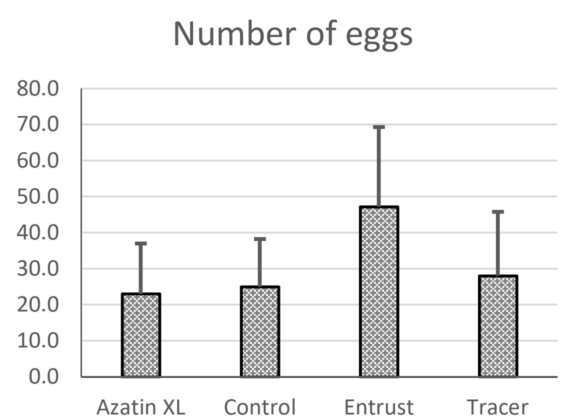


Fig 3.2. The average number of egg masses and average number of eggs deposited by *N. viridula* on clothes treated with different selected insecticides, in complete choice test.

3.4. Discussion

Neem and spinosad are natural insecticides that are attributed with different bioactivities to targeted insect pests. Neem is known to have active ingredients that pose a strong antifeedant and an insect growth regulator (Isman et al., 1990). For this reason, some scientists believe that neem is a potential candidate as an alternative for synthetic neuro-toxin type insecticides. On the other hand, spinosad is an organic insecticide that is claimed to pose a unique mode of action (Salgado, 1998). Spinosyn, the active ingredient of spinosad, disrupts nicotinic acetylcholine receptors and is claimed to be more selective toward targeted insects and less harmful to beneficial insects, mammals, bird or aquatic animals (Kirst, 2010).

Generally, the result of this study demonstrated the potential of neem as a repellent and feeding deterrent to SGSB. In Y-tube assays, tested insects significantly avoided Y-tube arms blown with neem volatile. This indicated that stink bugs were able to detect neem volatiles and responded negatively by moving toward the control arm (Chapter 2). The result of this olfactory experiment is supported with the data from tactile tests that showed avoidance of tested insects to sit on neem treated media within 24 hour observations. This repellency effect of neem has been demonstrated in an extensive study against various insects, including whiteflies (Krzyzaniak et al., 2016), mosquitoes (Caraballo, 2000), stored pest beetles (Xie et al., 1995), and many others. Feeding tests in this study showed feeding deterrent activity of neem on nymph and adult SGSB. In both paired and choice tests, tested stink bugs significantly pierced seed treated with spinosad. This antifeedancy of neem has been demonstrated extensively, including against stink bugs (Abudulai et al., 2003a; Kamminga et al., 2009;

Seymour et al., 1995). In this study, however, neem did not show an effective oviposition deterrent for SGSB. Although many studies demonstrated this effect on various insects from different genera, some studies reported that it did not effectively deter oviposition (Charleston et al., 2005; Naumann & Isman, 1995; Weathersbee III & McKenzie, 2005), or the oviposition deterrent effect rapidly declined (Webb et al., 1983).

Spinosad is a macrocyclic lactone insecticide that was originally isolated from the fermentation of soil actinomycetes *Saccharopolyspora spinosa*. At early development, spinosad was recommended to control lepidopteran larvae and thrips (Salgado, 1998). Later, it was shown to have a broader spectrum; including dipteran (Stark et al., 2004), coleopteran (Toews et al., 2003), orthopteran (Thompson et al., 2000), isopteran (Scheffrahn & Thoms, 1999), as well as acarina (Villanueva & Walgenbach, 2006).

When it was applied to control stink bugs, spinosad was not effective compared to synthetic insecticides such as dicrotophos, cyfluthrin, lambda-cyhalotrin and pyrethroid (Greene & Capps, 2002). Additionally, spinosad was found to attract *E. servus* and appeared to act as a feeding stimulant to *A. hilare* (Kamminga et al., 2009). The result of bioassays in our study showed inconsistent bioactivity of spinosad against SGSB. In olfactory tests, adult SGSB showed a significant preference toward spinosad (Entrust® and technical grade).

In the tactile test, we found inconsistence attraction or repellence of SGSB adult to spinosad; in which in some observations the proportion of tested insects that sat on spinosad treated portions was either significantly lower, higher or did not differ significantly compared to those that sat on control portions. In the paired test feeding bioassay, 5th instar nymphs of

SGSB significantly pierced spinosad treated seeds more than untreated seeds; indicating that spinosad possibly acted as a feeding stimulant to nymphal SGSB. The same test with adult SGSB did not give a significant preference, although the number of stylet punctures tended to be higher on spinosad treated seeds. A more noticeable feeding preference of adult SGSB to spinosad was seen in compete choice tests, where the number of stylet sheaths on spinosad (Entrust®) treated seeds was significantly higher compared to other treatment. In oviposition bioassays, SGSB females did not show significant preference for oviposition media treated with spinosad; both in paired tests and in complete choice tests.

In summary, our results show promise for neem as a repellent and deterrent that can be used as a pushing component in a push-pull strategy to control stink bugs in the soybean field. To a lesser extent, spinosad might be used to lure stink bugs to an area. Our research also suggests that the application of Entrust (spinosad) in soybean fields during pod filling stage might increase seed damage as it may induce stink bugs to feed. Therefore, small plot field studies to test the implementation of a push-pull strategy using this two chemicals will help us to examine the feasibility of this strategy, as well as to quantify the potential effect of both chemicals on seed damage and quality.

CHAPTER 4: FIELD APPLICATION OF AN EARLY MATURING SOYBEAN VARIETY, AN ATTRACTANT, AND A REPELLENT IN SOYBEAN PRODUCTION TO CONTROL STINK BUGS.

4.1. Introduction

The trap cropping tactic has been developed based on the mechanism of pest host preference (Hokkanen, 1991). The effectiveness of a trap crop is not solely dependent on the differential attractiveness of trap crops and cash crops, but is also influenced by insect pest behaviors and movement patterns (Shelton & Badenes-Perez, 2006). Although it has not been widely adopted, several records present success stories on the application of the trap crop tactic, such as the use of alfalfa to manage *Lygus* bug in cotton (Godfrey & Leigh, 1994) and the use of squash that attracted >90% of the total estimated squash bug population in cantaloupe and watermelon fields (Pair, 1997).

Investigation of trap crop tactics to minimize stink bug impacts on cash crops have been conducted. Scientists noticed the challenge in the deployment of a trap crop to control stink bugs. One challenge is that stink bugs are polyphagous and highly mobile (Mizell et al., 2008). Sorghum (*Sorghum bicolor* L.), when planted adjacent to cotton, significantly suppressed the dispersal of *Nezara viridula* and *Euschistus servus* into cotton fields (Tillman, 2006; Tillman & Cottrell, 2012). Similarly, stink bug densities were higher in soybean plots when it was grown adjacent to cotton (Bundy & McPherson, 2000; Tillman et al., 2015) and adjacent to corn (Rea et al., 2002); indicating that soybean potentially could be used as trap crop for stink bugs in cotton fields or corn fields.

Implementing trap crops to control stink bugs in soybean fields is constrained due to the lack of a trap crop more attractive to stink bugs than soybean. In soybean fields, stink bug preference was predominantly driven by soybean developmental stage (Schumann & Todd, 1982), in which the pests prefer to feed on filling pods. Hence, there is a narrow window of opportunity. Some attempts have been carried out to exploit this critical period of stink bug behavior for pest management purposes. For instance, soybean that was grown 4 to 6 weeks earlier than conventional planting season harbored much higher stink bugs population during mid-season (McPherson et al., 2001). Similarly, in a 2 year field experiment (2004-2005) Smith and his colleagues found that early soybean production systems were proven to attract stink bugs which colonized and oviposited, but this early system could not protect the full-season soybean crop (Smith et al., 2009b). A more optimistic result was obtained when the trap crop was applied in combination with an insecticide application (Baur et al., 2000).

Trap cropping success in pest management can be improved when the tactic is combined with the deployment of an attractant or sex pheromone (Hokkanen, 1991; Mizell et al., 2008). Alternatively, chemicals that act as repellents, antifeedants, or oviposition deterrents can be used to prevent pest invasion. The deployment of substances that act as attractants in combination with substances that act as repellents or deterrents to control agricultural pests is the basic principle of a "push-pull" strategy.

Push-pull strategies in pest control have been used in IPM over the last few decades. In this approach, attractants and repellents are used simultaneously to manipulate pest abundance in agroecosystems (Cook et al., 2006). Similar concepts with different terminology were used by Miller and Cowles, where they proposed using the term “stimulo-detterent diversion” or SDD (Miller & Cowles, 1990). This concept works by preventing potential pests from invading or by repelling potential pests away from the protected crop using repellent substances, antifeedants, or oviposition deterrents and at the same time, the pests are lured out into specific areas in the agroecosystem using aggregative substances or by applying trap crops (Cook et al., 2006; Foster and & Harris, 1997). By pushing out potential pests from vast areas of protected crop into much smaller patches of sacrificial plants, selective area applications or site specific applications of insecticides can be more efficiently carried out.

Push-pull strategies can be applied to manage stink bugs in soybean fields, because they are known to have preferences for soybean growth stages (Smith et al., 2009b) or certain compounds (Kamminga et al., 2009) and also are deterred by neem (Abudulai et al., 2003b; Seymour et al., 1995). Theoretically, the dispersed stink bugs can potentially be pooled into certain patches where the soybean is at a preferable development stage and strengthen this with the application of attractants. At the same time, the cash crop is treated with a deterrent or repellent compound.

4.2. Materials and Methods

Soybean variety: Two soybean varieties were used in this experiment; Pioneer 93Y92 (Maturity Group III) and variety Asgrow AG5332 (Maturity Group V).

Chemicals: Entrust® (80% spinosin A&D), Tracer® (44.2% spinosin A&D), Radiant®SC (11.7% spinetoram J&L), technical grade spinosad, Azatin XL® (3% azadirachtin), Endigo® (λ-cyhalothrin: 9.48% + thiometoxam: 12.6%), and Karate® (λ-cyhalothrin 22.8%) were tested in this experiment. The chemicals were diluted with tap water to achieve concentration equivalents to recommended field applications in soybean (Entrust®: 599 ppm a.i., Tracer®: 690 ppm a.i., Radiant® SC: 365 ppm a.i., Azatin XL®: 375 ppm a.i., Endigo®: 773 ppm a.i., and Karate: 342 ppm a.i.). Meanwhile the concentration of technical grade was: 600 ppm a.i.

Experiment Design and Procedure: This experiment was carried out in three locations: Ben Hur Research Station (30°22’00.01”N 91°09’53.26”W), Dean Lee Research Station (31°10’11.49”N 92°24’38.85”W), and Crowley Research Station (30°10’32.87”N 92°24’38.85”W). Plots were arranged in a split plot layout. Each plot (6 x 7.5 meter area) was fit to plant 20 rows of soybean and was separated from each plot with 2.5 meter fallow alley. In block I, 4 rows in each edge were planted with Pioneer 93Y92 variety (MG-III soybean); while 12 rows in the center of the plot was planted with Asgrow AG5332 variety (MG-V soybean); Meanwhile, for block II all 20 rows was planted with Asgrow AG5332 variety (MG-V soybean). The chemical treatments (6 different insecticides + control) were arranged randomly into experiment units (plot) in each block and replicated 4 times. Spraying was conducted 2 times at the interval of 2 to 3 weeks. First spray was carried out when the plant was at R4 stage, while the next application was applied approximately when the plant at R5-R6 stage. Spraying was conducted using a boom plot sprayer on a tractor with TwinJet flat spray tips, with 40 psi pressure, at a rate of 280 L/ha. For manual application, the solution was sprayed using a portable CO_2 spraying system, with 30 psi pressure, at a rate of 320 L/ha. Spraying was applied onto the first 4 rows on each edge of the plot, leaving the remaining 12 rows in the center unsprayed. In particular to technical grade spinosad, due to limited amount of chemical available, it was sprayed onto 2 rows in each of the plot. Sweeping was conducted weekly, starting from the time when soybean was at R1 and was stopped when the plants had reached R7. For the purpose of data collection, each experimental unit (plot) was divided into 4 sub-units (2 sub-units of 4 rows on the edges and 2 sub-units of 6 rows in the center of the plot). Sweeping was carried out on each sub-unit using a standard heavy duty sweep net (38 cm) with 25 sweeps per plot. After being identified and counted, the stink bugs were then released back into the same plot. At the end of field experiment, ten soybean plants were randomly taken from each sub-unit (sub-plot). The samples were then oven dried (38°C) for 1-2 weeks. After the pods were dry, the sample was then processed manually to minimize seed loss. Machine harvest was carried out on 2 rows of each sub-plot when all soybean plants reached R8. About 0.5 – 1 kg seed was taken from each sub-plot sample, brought to the lab, and processed. The seed obtained from either manual sampling or machine harvest was measured for total seed weight, undersize seed weight, and normal-size seed weight. From each sub-unit sample, 100 seeds were randomly selected using a "seed counting board" and used to measure seed damage parameters, which included split seeds, shriveled seeds, fungal damaged seeds, and seeds with stink bug punctures. In particular to stylet punctured seed, the samples were immersed overnight in water to make the seed swollen, which enabled the stylet puncture on the seed surface to be more obvious.

Statistical Analysis: Cumulative Insect-days (CID) were used as the parameter to evaluate insect abundance. Insect-days was calculated based on the formula:

Insect-days $= (X_{i+1} - X_i)[(Y_i + Y_{i+1}) \div 2]$;

where X_i and X_{i+1} are adjacent points of time, and Y_i and Y_{i+1} are the corresponding points of insect counts (Ruppel, 1983). Two way ANOVA was applied to analyze data for parameters: CID, total seed weight, the number of good seed, and the number of seed with punctures. Repeated measure ANOVA was applied to determine the difference in stink bug populations on a weekly basis using SAS (SAS Institute 2010).

4.3. Results

Stink bug populations. Stink bugs started to appear in experiment plots when the soybean was at R3-R4 stage. The population gradually increased and reached a peak when the plant was at R6-R7 stage (Fig. 4.1). Seven stink bug species were found during this two-year study. Of the seven, six were considered pest species capable of doing economic damage. These economically damaging stink bugs included redbanded stink bug (*Piezodorus guildinii* Say), green stink bug (*Chinavia hilaris* Say), southern green stink bug (*Nezara viridula* L.), redshouldered stink bugs (*Tyanta custator*), brown stink bug (*Euschistus servus* Say.), *Euschistus quadrator* Rolston, and the dusky stink bug (*Euschistus tristigmus* Dallas). Consperse stink bug (*Euschistus conspersus* Uhler), and Rice stink bug (*Oebalus pugnax* F) were also captured on the field, although their populations were very low. The predatory spined soldier bug (*Podisus maculiventris* Say) was found in the field, hunting softbodied insects, especially caterpillars. During the experiment period, the most common stink bug species found in the experiment plots were *P. guildinii*, *C. hilaris, N. viridula* L. and *E. servus* and *E. quadrator,* which represented more than 97 % of the stink bug species captured in 2014 and 2015 planting seasons (Table 4.1). The population dynamics of these species during weekly sweeping schedule is presented in Fig. 4.1.

Table 4.1. Composition (%) of major stink bug species captured during 2014 and 2015 planting season on experiment plots at different locations.

Stink Bug Species	2014		2015		
	Ben Hur	Dean Lee	Ben Hur	Dean Lee	Crowley
P. guildinii	23.9	36.0	59.3	88.38	42.4
C. hilaris	17.8	27.4	18.8	1.83	14.4
N. viridula	35.7	6.3	6.1	1.71	30.4
E. servus	1.4	15.3	2.5	3.13	3.4
E. quadrator	19.0	12.8	10.0	4.37	7.2
E. tristigmus	2.0	0.2	2.9	0.00	1.7
T. custator	0.1	1.4	0.1	0.35	0.3
Other species	0.2	0.6	0.3	0.18	0.2

Stink bug populations in experiment plots were much higher during 2015 compared to 2014 (Fig. 4.1). Statistical analysis of cumulative insect days (CID) that represent stink bugs population confirmed that during 2015 planting season the population was significantly higher compare to 2014 planting season (Table 4.2) (Ben Hur: (F = 73.14, df = 1, P < 0.0001; Dean Lee (F = 99.39, df = 1, P < 0.0001).

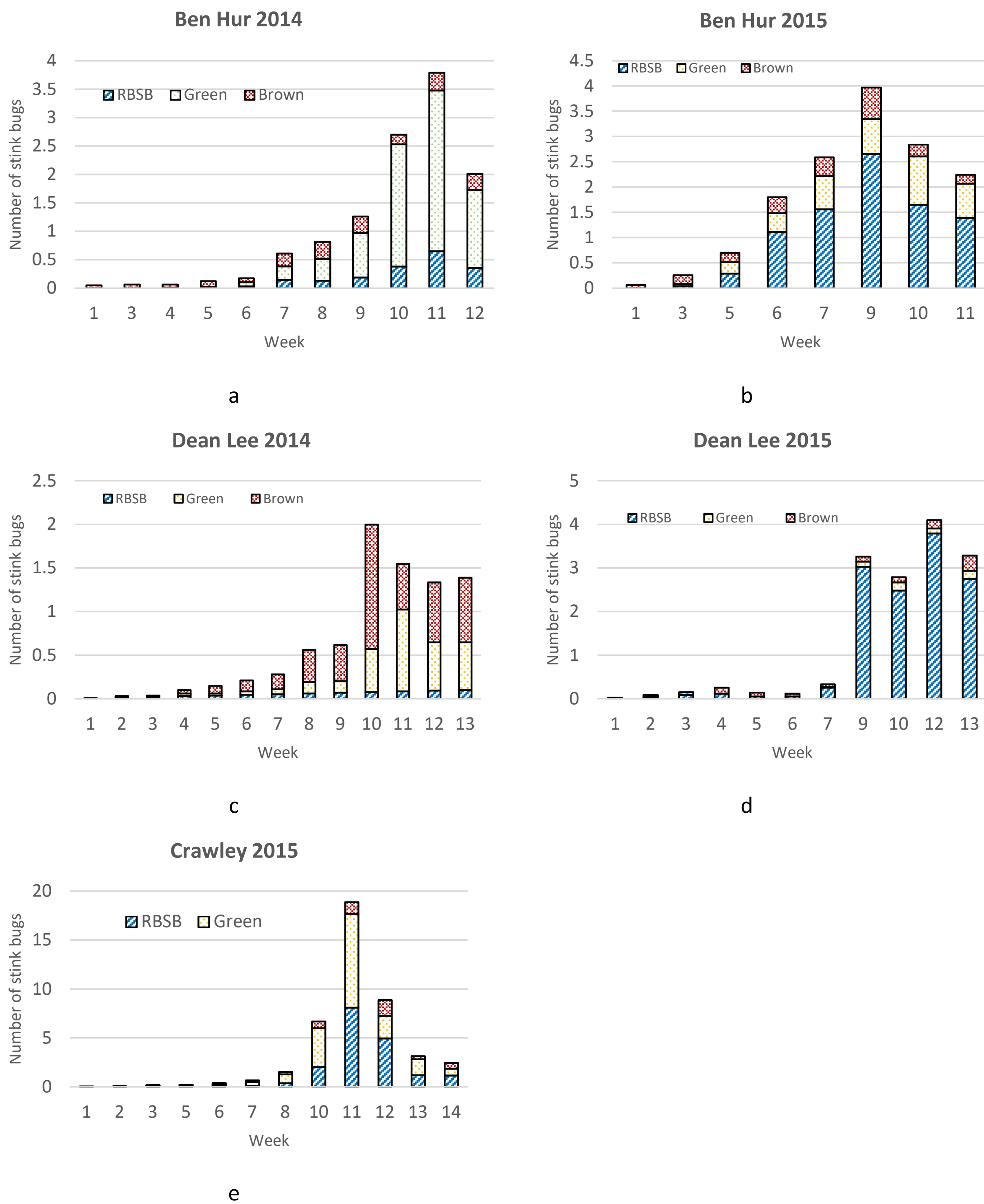


Fig. 4.1. Weekly average number of three major groups of stink bug captured/25 sweeps at three different experiment locations.

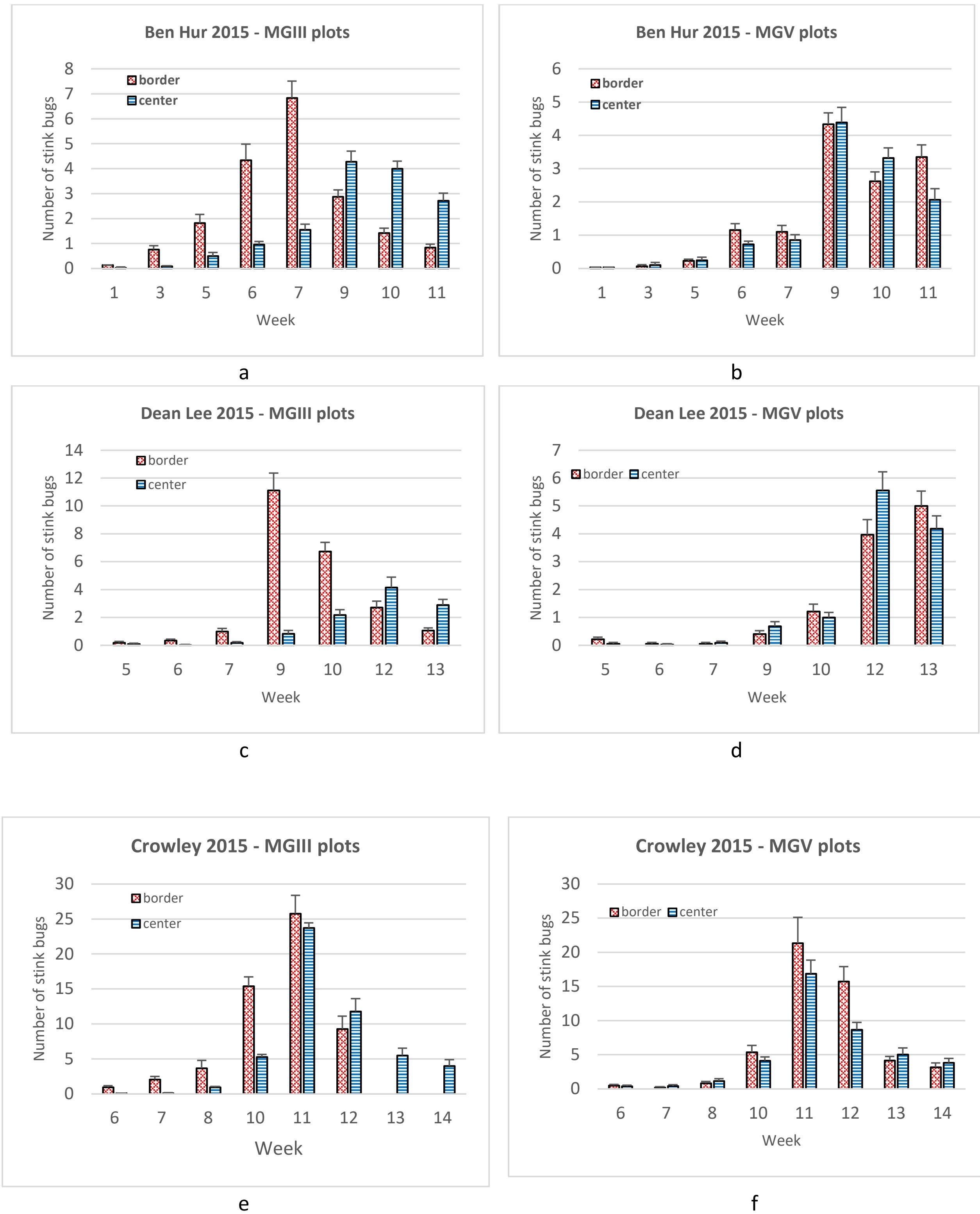


Fig. 4.2. Stink bugs population dynamic on center plots and border plots, presented as total stink bugs captured/25 sweeps during weekly sweeping.

During 2015, there was more stink bug movement into plots that were protected by the early maturingmaturing (MGIII) variety adjacent to the later maturingmaturing (MGV) variety. Stink bugs tended to occupy the MGIII plants before gradually moving to the later maturing (MGV) plants in the center plots as the crop. Meanwhile, in the plots without MGIII protection, the stink bug density between center and border plots was more balanced (Fig. 4.2).

Statistical analysis of CID from border (trap crop) plots showed that early maturing soybean (MGIII variety) was significantly higher compared to the later maturing (MGV) variety (Table 4.2.); indicating that the early maturing soybean variety harbored more stink bugs compared to the later maturing variety. Additionally, the interaction between year and variety were statistically significant (Ben Hur: $F = 28.52$, df = 1, $P < 0.0001$; Dean Lee: $F = 53.74$, df = 1, $P < 0.0001$). The CID of center plots for category 'variety' were not significantly different.

There was a tendency for Entrust® (spinosad) to attract stink bugs (Table 2 & Fig. 3). The effect, however, was not significantly different (Ben Hur: $F = 1.94$, df = 6, $P = 0.076$; Dean Lee: $F = 1.28$, df = 3, $P = 0.286$; Crowley: $F = 1.84$, df = 2, $P = 0.1756$). Similarly, insecticides had no effect on center stink bug CID. The mean value ± SE of cumulative insect days (CID) from the three experiment locations is presented in Table 4.2.

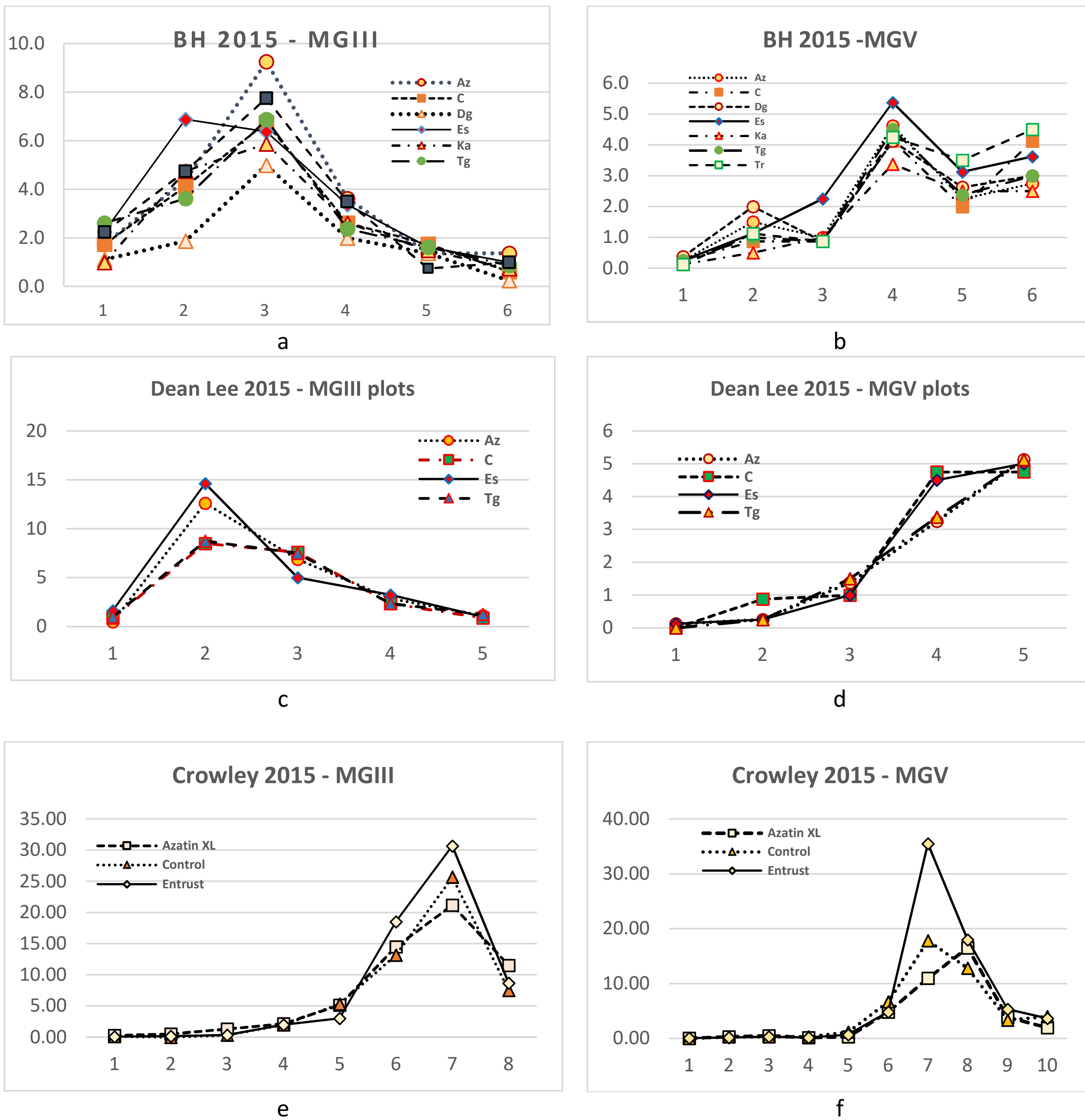


Fig. 4.3. Average number of stink bugs caught per 25 sweeps on the treated plots at week after treatment of different experiment locations

Table 4.2. Mean ± SE Cumulative Insect Days (CID) of total stink bugs caught on experiment plots from different experiment locations.

Fixed Effects	BEN HUR Mean ± SE		DEAN LEE Mean ± SE		CROWLEY Mean ± SE
BORDER PLOTS					
2014	57.71 ± 3.74	a	54.97 ± 4.23	a	
2015	118.11 ± 7.78	b	149.69 ± 12.28	b	420.33 ± 30.05
MGIII	106.86 ± 8.60	b	138.41 ± 13.23	b	468.55 ± 38.08
MGV	68.96 ± 3.24	a	66.25 ± 4.86	a	372.11 ± 44.67
Azatin XL	89.72 ± 14.99		104.06 ± 15.86		374.21 ± 57.34
Control	83.74 ± 12.78		104.40 ± 12.05		388.12 ± 58.39
Endigo	71.06 ± 9.22				
Entrust	109.85 ± 14.83		112.21 ± 18.78		498.66 ± 32.84
Karate	74.34 ± 11.17				
Tech. grade	91.15 ± 11.44		88.65 ± 14.69		
Tracer	95.52 ± 12.54				
CENTER PLOTS					
2014	89.42 ± 4.54		61.10 ± 3.86	a	
2015	81.30 ± 3.51		88.87 ± 6.66	b	330.44 ± 15.02
MGIII	84.08 ± 3.79		75.19 ± 6.39		368.81 ± 17.89
MGV	86.64 ± 4.34		74.78 ± 4.96		292.07 ± 20.87
Azatin XL	81.63 ± 6.89		81.43 ± 10.95		331.14 ± 25.84
Control	82.54 ± 7.53		75.33 ± 7.62		312.10 ± 21.92
Endigo	86.30 ± 6.83				
Entrust	82.25 ± 6.86		73.65 ± 5.48		348.07 ± 30.76
Karate	89.91 ± 7.58				
Tech. grade	82.76 ± 6.73		69.54 ± 7.44		
Tracer	92.13 ± 10.76				

Mean in the same column for each fixed effect which share a lowercase letter are not significantly different ($P>0.05$; Tukey HSD test).

Statistical analysis of seed weight of the center plots showed a significant difference in the category ‘year’ (Ben Hur: F = 30.12, df = 1, $P < 0.0001$; Dean Lee: F = 7.94, df = 1, P = 0.0058). For category ‘variety’, only data from Crowley was significantly different (F = 18.95, df = 1, P = 0.0002). For ‘insecticides’, all data was not significantly different. For border plots, seed production at Dean Lee in 2014 planting season was significantly higher compared to 2015 planting season (F = 6.54, df = 1, P = 0.012), while the data from Ben Hur did not significantly differ. For the category variety, there was a significance difference between MGIII and MGV for data from Ben Hur and Dean Lee (Ben Hur: $F = 106.44$, df = 1, $P < 0.0001$; Dean Lee: $F = 128.76$, df = 1, $P < 0.0001$), while the data from Crowley did not significantly differ. For category 'insecticides', only data from Ben Hur that was significantly different ($F = 2.43$, df = 6, $P = 0.0272$), while the data from other locations was not significantly different.

For percentage of undersize seed, analysis showed that 2015 samples had significantly higher proportions of undersize seed (border plot Ben Hur: $F = 371.46$, df = 1, $P < 0.0001$, Dean Lee: $F = 363.73$, df = 1, $P < 0.001$; center plot Ben Hur: $F = 627.49$, df = 1, $P < 0.0001$; Dean Lee: $F = 182.12$, df = 1, $P < 0.0001$). For category 'variety', analysis showed early maturing (MGIII) varieties planted as trap crops produced less normal sized seed compared to MGV variety (Ben Hur: $F = 106.71$, df = 1, $P < 0.0001$; Dean Lee: $F = 105.78$, df = 1, $P < 0.0001$). Meanwhile, seed data from Crowley did not differ. For seed data taken from protected (center) plots, only data from Ben Hur differed significantly ($F = 22.77$, df = 1, $P < 0.0001$). Meanwhile, applications of insecticides did not give a significant difference to the proportion of undersize seed of sacrificed plots and protected plots. The mean value ± SE of seed quantity data from the three experiment locations is presented in Table 4.3.

For the parameter "percentage of good seeds", there was a significant difference between year for border plots data taken from Dean Lee ($F = 7.31$, df = 1, $P < 0.0001$), while Ben Hur data did not significantly differ. For category 'variety', the percentage of good seed of the samples taken from the three experiment locations were significantly different (Ben Hur: $F = 447.46$, df = 1, $P < 0.0001$; Dean Lee: $F = 444.62$, df = 1, $P < 0.0001$; and Crowley: $F = 10.73$, df = 1, $P = 0.0027$). For category 'insecticides', only Ben Hur data that showed a significant difference ($F = 4.35$, df = 6, $P = 0.0005$). For the data that were taken from center plots, only Ben Hur data that showed a significant different for the category 'year' ($F = 67.18$, df = 1, $P < 0.0001$); while for category 'variety', all the data was not significant. For category 'insecticides', data from Ben Hur and Crowley showed a significant difference (Ben Hur: $F = 4.14$, df = 6, $P = 0.0008$; Crowley: $F = 4.05$, df = 2, $P = 0.0276$).

Statistical analysis of border plots data showed the seed from 2015 experiment had significantly higher proportion of shriveled seed compared to 2014 samples (Ben Hur: $F = 280.69$, df = 1, $P < 0.0001$; Dean Lee: $F = 265.32$, df = 1, $P < 0.0001$). For center plots data, only seed from Ben Hur that showed a significant difference ($F = 148.27$, df = 1, $P < 0.0001$). For category 'variety', border plots data taken from Ben Hur and Dean Lee showed a significant different (Ben Hur: $F = 106.69$, df = 1, $p < 0.0001$; Dean Lee: $F = 159.00$, df = 1, $p < 0.0001$), while data from Crowley did not significant. For center plots data, only seed from Ben Hur that showed a significant difference ($F = 5.02$, df = 1, $p = 0.0262$). For category 'insecticides, only data from Ben Hur that showed a significant different ($F = 3.99$, df = 6, $p = 0.0009$).

For parameter 'the percentage of seeds with stylet punctured', the border seed samples from Ben Hur and Dean Lee, were both significantly different for the category 'year' (Ben Hur: $F = 532.02$, df = 1, $p < 0.0001$; Dean Lee ($F = 270.28$, df = 1, $p < 0.0001$). For category 'variety', only Crowley data that was significantly different ($F = 19.15$, df = 1, $p < 0.0001$). For category 'insecticides, data from Ben Hur and Dean Lee were significant (Ben Hur: $F = 8.78$, df = 6, $P < 0.0001$; Dean Lee: $F = 9.29$, df = 3, $P < 0.0001$). For center plots data, only category 'year' that were differ significantly (Ben Hur: $F = 227.73$, df = 1, $P < 0.0001$; Dean Lee: $F = 107.61$, df = 1, $P < 0.0001$), while category 'variety' and 'insecticides' did not significantly different. The mean value ± SE of seed quality from the three experiment locations is presented in Table 4.

Correlation analysis showed that stink bug CID negatively correlated with yield (seed weight) ($R = -0.52$) and positively correlated with the percentage of undersize seed ($R = 0.44$). For qualitative parameters, stink bug density negatively correlated with the percentage of good seed ($R = -0.56$); but positively correlated with the percentage of shriveled seed and the percentage of punctured seed ($R = 0.57$ & $R = 0.52$ respectively) (Fig. 4).

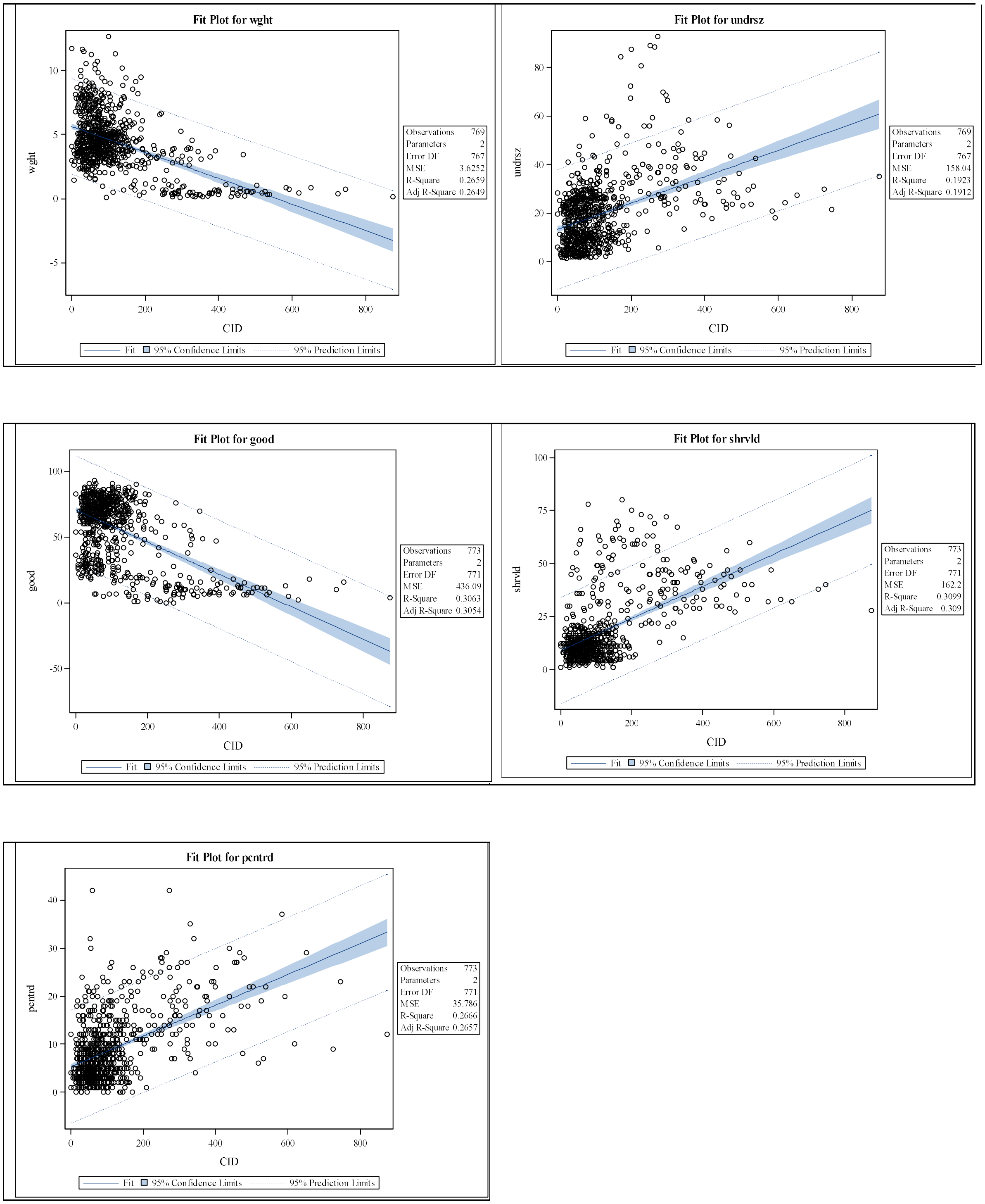


Fig. 4.4. Correlation plot of CID with seed weight, percentage of undersize seed, percentage of good seed, percentage of shriveled seed and percentage of seed with punctured

Table 4.3. Seed quantity (mean ± SE of seed weight (kg) per 2 rows and % undersize seed) of the samples taken from Border plots from different experiment locations.

	BEN HUR		DEAN LEE		CROWLEY	
	weight (kg)	% undersize	weight (kg)	% undersize	weight (kg)	% undersize
BORDER						
Year:						
2014	3.84 ± 0.11	15.76 ± 0.57 a	5.50 ± 0.22 b	5.31 ± 0.27 a		
2015	3.83 ± 0.08	29.26 ± 0.64 b	4.88 ± 0.32 a	35.29 ± 3.1 b	0.57 ± 0.04	27.63 ± 1.21
Variety:						
MGIII	3.25 ± 0.06 a	25.99 ± 0.88 b	3.62 ± 0.18 a	30.10 ± 3.56 b	0.47 ± 0.03	26.47 ± 0.09
MGV	4.40 ± 0.09 b	18.92 ± 0.76 a	6.76 ± 0.22 b	10.50 ± 1.07 a	0.68 ± 0.07	28.80 ± 2.24
Chemicals:						
Azatin XL	3.47 ± 0.18 a	23.39 ± 1.72	5.26 ± 0.44	20.87 ± 4.07	0.52 ± 0.07	29.47 ± 2.76
Control	3.69 ± 0.19 ab	21.97 ± 1.73	5.32 ± 0.38	19.70 ± 4.04	0.64 ± 0.09	26.89 ± 1.91
Endigo	4.08 ± 0.14 b	21.72 ± 1.47				
Entrust	4.08 ± 0.19 b	22.47 ± 1.62	5.32 ± 0.41	21.39 ± 4.35	0.56 ± 0.05	26.55 ± 1.48
Karate	3.99 ± 0.19 b	23.73 ± 1.72				
Tech. grade	3.84 ± 0.19 ab	21.28 ± 1.52	4.87 ± 0.36	19.24 ± 4.08		
Tracer	3.71 ± 0.14 ab	22.11 ± 1.85				

Mean in the same column for each fixed effect which share a lowercase letter are not significantly different (P>0.05; Tukey HSD test)

Table 4.4. Seed quantity (mean ± SE of seed weight (kg) per 2 rows and % undersize seed) of the samples taken from Center plots from different experiment locations.

	BEN HUR		DEAN LEE		CROWLEY	
	weight (kg)	% undersize	weight (kg)	% undersize	weight (kg)	% undersize
Year:						
2014	4.86 ± 0.09 b	10.39 ± 0.37 a	7.49 ± 0.10 b	3.34 ± 0.13		
2015	4.23 ± 0.07 a	23.28 ± 0.43 b	6.50 ± 0.38 a	18.81 ± 1.61	0.43 ± 0.04	36.15 ± 2.00
Variety:						
MGIII	4.45 ± 0.09	15.30 ± 0.58 a	6.62 ± 0.31	12.62 ± 1.74	0.36 ± 0.05	42.68 ± 2.92
MGV	4.66 ± 0.08	18.16 ± 0.83 b	7.37 ± 0.24	9.53 ± 1.19	0.49 ± 0.05	29.63 ± 1.70
Chemicals						
Azatin XL	4.51 ± 0.17	16.92 ± 1.42	7.19 ± 0.43	10.69 ± 2.19	0.39 ± 0.06	38.96 ± 3.80
Control	4.55 ± 0.16	17.17 ± 1.42	7.09 ± 0.38	11.92 ± 2.26	0.44 ± 0.06	34.63 ± 3.73
Endigo	4.64 ± 0.15	16.67 ± 1.40				
Entrust	4.69 ± 0.17	15.97 ± 1.27	6.92 ± 0.44	10.93 ± 2.08	0.45 ± 0.07	34.87 ± 2.95
Karate	4.80 ± 0.18	16.45 ± 1.43				
T. grade	4.58 ± 0.15	16.77 ± 1.39	6.78 ± 0.34	10.75 ± 2.03		
Tracer	4.10 ± 0.14	17.26 ± 1.36				

Mean in the same column for each fixed effect which share a lowercase letter are not significantly different (P>0.05; Tukey HSD test)

Table 4.5. Seed quality (mean ± SE of good seeds, shriveled seeds, and seed with punctured/100 seeds) of sample taken from Border plots from different experiment locations.

	BEN HUR			DEAN LEE			CROWLEY		
	good seed	shriveled	punctured	good seed	shriveled	punctured	good seed	shriveled	punctured
Year:									
2014	53.70 ± 2.00 a	8.83 ± 0.33 a	3.47 ± 0.18 a	44.30 ± 2.33 b	10.84 ± 0.42 a	3.73 ± 0.34 a	-	-	-
2015	56.53 ± 1.85 b	26.38 ± 1.65 b	11.25 ± 0.37 b	38.80 ± 3.77 a	35.28 ± 3.21 b	18.09 ± 0.95 b	9.83 ± 0.71	38.97 ± 1.23	18.11 ± 1.34
Variety:									
MGIII	38.79 ± 1.46 a	23.35 ± 1.77 b	7.36 ± 0.53	19.72 ± 1.76 a	33.47 ± 3.35 b	10.63 ± 1.28	7.83 ± 0.69	39.22 ± 1.89	13.61 ± 1.57
MGV	70.80 ± 0.84 b	11.75 ± 0.71 a	7.22 ± 0.40	63.38 ± 1.30 b	12.66 ± 0.73 a	11.20 ± 1.00	11.83 ± 1.06	38.72 ± 1.63	22.61 ± 1.59
Chemicals:									
Azatin XL	56.34 ± 3.22 b	16.91 ± 2.22 bcd	6.44 ± 0.64 ab	43.84 ± 4.52	20.44 ± 3.35	10.03 ± 1.45 a	9.25 ± 1.12	39.58 ± 2.08	16.92 ± 1.89
Control	52.90 ± 3.75 ab	19.71 ± 3.07 cd	6.71 ± 0.86 ab	43.19 ± 4.40	23.03 ± 4.09	8.69 ± 1.18 a	11.92 ± 1.21	41.17 ± 1.15	15.83 ± 2.04
Endigo	62.16 ± 2.90 c	12.52 ± 1.50 a	6.42 ± 0.86 ab	-	-	-	-	-	-
Entrust	49.78 ± 3.72 a	20.88 ± 3.21 d	9.53 ± 1.04 d	38.22 ± 4.56	23.44 ± 3.94	14.78 ± 2.15 b	8.33 ± 1.20	36.17 ± 2.75	21.58 ± 2.76
Karate	57.19 ± 3.78 bc	15.50 ± 2.29 ab	5.75 ± 0.67 a	-	-	-	-	-	-
T. grade	55.48 ± 3.93 ab	16.35 ± 2.76 abc	8.03 ± 0.87 c	40.94 ± 4.43	25.34 ± 4.25	10.16 ± 1.39 a	-	-	-
Tracer	51.81 ± 3.78 a	20.23 ± 3.23 d	8.16 ± 1.02 cd	-	-	-	-	-	-

Mean in the same column for each fixed effect which share a lowercase letter are not significantly different (P>0.05; Tukey HSD test)

Table 4.6. Seed quality (mean ± SE of good seeds, shriveled seeds, and seed with punctured/100 seeds) of sample taken from Center plots from different experiment locations.

	BEN HUR			DEAN LEE			CROWLEY		
	good seed	shriveled	punctured	good seed	shriveled	punctured	good seed	shriveled	punctured
Year:									
2014	79.50 ± 0.50 b	7.20 ± 0.28 a	3.60 ± 0.20 a	66.95 ± 0.76	11.95 ± 0.50	4.81 ± 0.28 a	-	-	-
2015	73.66 ± 0.64 a	13.31 ± 0.46 b	8.71 ± 0.29 b	66.03 ± 2.15	13.73 ± 1.39	12.38 ± 0.66 b	11.92 ± 0.68	37.72 ± 1.25	20.97 ± 0.95
Variety:									
MGIII	76.18 ± 0.70	10.78 ± 0.52 b	5.87 ± 0.37	64.84 ± 1.74	13.84 ± 1.13	8.66 ± 0.73	11.67 ± 1.08	39.61 ± 1.93	19.56 ± 1.39
MGV	77.04 ± 0.57	9.66 ± 0.44 a	6.36 ± 0.33	68.14 ± 1.44	11.84 ± 0.95	8.53 ± 0.65	12.17 ± 0.84	35.83 ± 1.52	22.39 ± 1.26
Chemicals:									
Azatin XL	79.47 ± 1.01 c	8.44 ± 0.70 a	6.13 ± 0.62	67.13 ± 2.01	11.81 ± 1.32	7.97 ± 0.87	10.17 ± 0.94	40.00 ± 2.33	21.25 ± 1.72
Control	73.34 ± 1.06 a	11.66 ± 0.97 c	6.66 ± 0.66	63.31 ± 2.71	14.06 ± 1.79	9.06 ± 0.96	14.17 ± 0.92	35.25 ± 2.33	20.83 ± 1.72
Endigo	75.35 ± 1.30 ab	11.29 ± 1.05 c	6.13 ± 0.59	-	-	-	-	-	-
Entrust	78.48 ± 1.28 c	8.55 ± 0.70 a	6.06 ± 0.85	69.75 ± 1.88	11.38 ± 1.26	8.72 ± 1.19	11.42 ± 1.37	37.92 ± 1.74	20.83 ± 1.66
Karate	76.50 ± 0.95 c	11.00 ± 0.85 bc	6.13 ± 0.51	-	-	-	-	-	-
T. grade	78.16 ± 1.30 bc	9.39 ± 0.89 ab	5.45 ± 0.69	65.78 ± 2.35	14.13 ± 1.51	8.63 ± 0.88	-	-	-
Tracer	75.09 ± 1.13 ab	11.13 ± 0.91 bc	6.25 ± 0.66	-	-	-	-	-	-

Mean in the same column for each fixed effect which share a lowercase letter are not significantly different (P>0.05; Tukey HSD test)

Discussion

From this two year study, five species of stink bugs were identified that threaten Louisiana soybean production. These five species were *P. guildinii* Say, *N. viridula* L., *C. hilaris* Say, *E. quadrator* Rolston and *E. servus* Say. This finding was similar to the pattern of stink bug composition from earlier studies (Temple et al., 2013) and justify the consistency of the redbanded stink bug and southern green stink bug as the most important species in Louisiana. During the 2015 planting season, the density of stink bugs was significantly higher than 2014. This dynamic fluctuation was apparently a result of the occurrence of several days of cold weather during the 2013 to 2014 winter season (Appendix 1). Based on two years of data, the redbanded stink bug population suffered from the cold weather with lower population densities than green and brown stink bugs (Fig. 4.1a & 4.1c). The absence of chilling days during 2014 to 2015 winter season allowed more redbanded stink bugs to survive and gained its dominance during the 2015 planting season (Fig. 4.1b & 4.1d).

In 2015, the placement of a MG III variety on the border successfully pulled stink bugs; especially when the MGIII plant was at a more preferable developmental stage. When MG V plants became more preferable, stink bugs gradually moved to the center plots (Fig. 4.2 a, b & c). Stink bug movement in plots without MG III was less dynamic as soybean growth stage at both center and border plots were at the same stage (Fig. 4.2 d, e & f). Previous studies reported that early maturing soybean varieties were highly attractive to stink bugs and attract higher populations than late maturing soybean varieties (Bundy & McPherson, 2000; McPherson et al., 1988).

Applications of selected insecticides on the border plots that were designed to attract, to repel, or to kill the stink bug did not give significant effects on stink bug populations. Nevertheless, there are several important points that can be gained from this experiment. Firstly, although statistically Entrust® (spinosad) did not perform as an effective attractant, there was a tendency for stink bug populations to be higher on plots that were sprayed with this insecticide (Fig. 3). Likewise, the application of Endigo® (λ-cyhalothrin + thiometoxam 22%) that was aimed to kill the stink bug, gave the lowest stink bug populations (Fig. 3a & 3b). Meanwhile, in field conditions, neem did not show repellent effects to stink bugs. Although it still needs to be refined with further studies, the data can be used by farmers and practitioners in pest management related to insecticide applications, particularly when it is aimed to control stink bugs or convey a potential risk of stink bug invasion.

From the stand point of seed production, stink bug populations greatly affected yield and seed quality. Although yield is not influenced by a single factor, seed weight of the sample data showed a higher seed production in 2014 planting season which stink bug populations were coincidently lower (Table 3). Previous studies showed that stink bug density affects seed biomass (McPherson et al., 1979; Owens et al., 2013; Suzuki et al., 1991); even though, at a certain level, the differences in stink bug density might not give a significant effect on seed production (Corrêa-Ferreira & De Azevedo, 2002). Additionally, the reduction of seed biomass as a result of stink bug feeding activity can also be seen in the proportion of undersized seed. Generally, the higher stink bug populations and the earlier soybean pods were attacked first and at higher densities correlating with seed deterioration (McPherson et al., 1979). The data from these two years showed the proportion of undersize seed from 2015 samples was significantly higher compared to 2014 samples (Table 3). This indicates stink bugs greatly contributed to the reduction of normal size seed.

Qualitative parameters can be more reliable in evaluating the effect of stink bug feeding activity (Corrêa-Ferreira & De Azevedo, 2002). Based on the analysis of data taken from trap (border) plots, the difference in stink bug density between year and variety significantly affected seed quality. Insecticides that did not cause significant effects for quantitative parameters (Table 3) showed significant effects on qualitative parameters (Table 4). In general, seed quality was lower when it sprayed with Entrust and better when it sprayed with the synthetic insecticide. In this field study, neem did not consistently deter stink bugs nor prevent the seed from stink bug feeding activity. Perhaps rapid degradation of active compound through photolysis and hydrolysis affected neem efficacy (Isman, 1997).

Statistical analysis of qualitative parameters of the seed taken from protected (center) plots showed only the category year statistically different (Table 4.3 & 4.4). For categories 'variety' and 'insecticides', the effects appeared in Ben Hur data only. At Crowley, the explosion of stink bug populations during seed development (Fig. 4.1 & 4.2, Table 4.2) massively devastated the seed, both at quantitative and quantitative parameters (Table 4.3 & 4.4). Based on the correlation analysis, there was a connection between CID with seed quality that was represented as the percentage of good seed, the percentage of shriveled seed, and the percentage of seed with punctured (Fig. 4.4).

Based on these findings, a better arrangement of early maturing soybean as a trap crop, combined with regular monitoring of stink bug population on the trap crop, and site-specific application of effective insecticides may reduce the cost for monitoring and insecticide applications; preventing stink bugs from invading protected varieties and reducing yield losses. Future larger scale field trials would be necessary to establish the tactic before it can be officially implemented and recommended in actual soybean production.

CHAPTER 5: SUMMARY AND CONCLUSIONS

The absence of cold weather during the winter season in the southern US has enabled more diverse pest insects to survive and attack agricultural crops in Louisiana. In soybean fields, particularly in Louisiana, various pest insects can be found, these include soybean looper *Chrysodeixis includens* (Walker), velvetbean caterpillar, *Anticarsia gemmatalis* (Hübner), green cloverworm *Hypena scabra* (F.), threecornered alfalfa hopper, *Spissistilus festinus* and the stink bug complex (Hemiptera: Pentatomidae) that are considered as major soybean pests.

Among the major pests of soybean in Louisiana, stink bugs are the most economically important pest, causing severe seed damage, yield loss, and higher input costs due to increased insecticide applications. Two species, the redbanded stink bug, *Piezodorus guildinii* (Westwood) and the southern green stink bug, *Nezara viridula* (L.), are currently the most abundant species in soybeans in this area and constantly threaten soybean production. Application of insecticides is still the most common control measure applied in the field. However, these species are becoming more tolerant to insecticides with the potential for efficacy to fail.

The development of a new approach to stink bug management that focuses on minimizing insecticides will greatly benefit both farmers and the environment. For this reason, a series of experiments were conducted to evaluate the potential of applying early maturing soybean varieties, attractants, and a repellent to be implemented as a push-pull strategy to manage stink bug populations in soybean. In general, this series of experiments was aimed to evaluate the potential of spinosad as an attractant to stink bugs and neem as a repellent to stink bugs. Specifically, the experiments were designed to evaluate the olfactory response, tactile response, feeding preference, and oviposition preference of the redbanded stink bug and southern green stink bug to spinosad and neem. Finally, laboratory experiments were tested in two years of field experiments conducted to evaluate the performance of a push-pull strategy using the above components to manage stink bugs.

The olfactory response experiment showed tested stink bugs were considerably attracted to spinosad, particularly to Entrust®, and deterred by azadirachtin (Azatin XL®). This early finding showed that these natural insecticides could be used in a push and pull strategy. Further laboratory tests showed complementary outcomes that supported the hypothesis; although some inconsistency and less obvious effects were also observed. In tactile response experiments, tested stink bugs significantly avoided neem treated portions; but moved toward spinosad treated portions. A more consistent result was obtained in feeding preference experiments, where less stylet punctures were found on neem treated seeds, but higher punctures occurred on Entrust® (spinosad) treated seeds. In oviposition preference tests, stink bugs did not show a significant preference toward particular tested chemicals; indicating that both neem and spinosad may not affect oviposition behavior of stink bugs.

Diverse stink bug populations were observed during the two years of field experiments that closely correlated to weather conditions; particularly the presence of freezing days during the winter season. Redbanded stink bug suffered more from the cold weather of the 2013 to 2014 winter season; causing low population densities and less dominance than green and brown stink bugs. The absence of chilling days during 2014 to 2015 winter season allowed more redbanded stink bugs to survive winter and gained dominance during the 2015 planting season.

The placement of early maturing varieties on the border was generally able to pull stink bugs; especially when the plant was at preferable developmental stages. When the later maturing plants became more preferable, however, stink bugs gradually moved to the center plots; causing overall number of stink bugs between those plots to become less obvious. Meanwhile, the applications of selected insecticides on the border plots that were designed to attract, to repel, or to kill the stink bug did not give a significant effect. The tendency of spinosad to attract stink bug was not statistically significant. Stink bug populations greatly affected yield and seed quality, which correlated to stink bug density.

In summary, the repellency of neem and the attractant effect of spinosad to stink bugs may not strong enough as push-pull strategy components to control stink bugs in soybean fields. A better arrangement of early maturing soybean as trap crop, combined with regular monitoring of stink bug population on the trap crop, and site-specific application of effective insecticides to reduce insecticide applications, could prevent stink bugs from invading protected varieties and reduce potential yield loss due to stink bug invasion.

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APPENDIX A: CLIMATIC DATA DURING FIELD EXPERIMENT AT THREE DIFFERENT LOCATIONS

A.1. Temperature at Ben Hur Research Station during winter season 2013-2014

November 2013				**December 2013**				**January 2014**				**February 2014**			
Date	Min	Avg	**W/O**	Date	Min	Avg	**W/O**	Date	Min	Avg	**W/O**	Date	Min	Avg	**W/O**
1	53	64	1	1	35	53		1	44	51	4 1	1	46	62	
2	51	62		2	56	66		2	35	46	1 4	2	41	57	4 2 1
3	41	54		3	63	72	1	3	28	38		3	37	43	1
4	45	61		4	69	76	1	4	26	44	1	4	42	55	1 2 5 4
5	61	69		5	71	78	4	5	34	52	1 4	5	36	43	
6	60	71	4 1	6	38	56	4 1	6	**24**	**29**		6	31	37	4
7	47	57		7	34	37	1	7	**19**	**29**		7	28	41	4
8	42	54		8	38	42	1 4 5	8	**20**	**36**		8	37	50	4
9	43	58		9	40	46	1 4	9	38	53		9	42	58	1
10	54	66		10	35	43	4	10	50	59	1 2 4	10	43	50	4 1
11	49	63		11	32	42		11	41	56	4 1	11	36	40	4 1 5
12	38	55		12	35	46		12	34	52		12	34	36	4 1
13	32	45		13	34	48		13	52	60	4 1	13	29	42	1
14	33	51		14	48	58	4	14	37	52		14	32	49	
15	55	65	1	15	35	45		15	33	44		15	40	53	
16	63	73	1	16	30	46	1	16	28	45		16	37	54	
17	70	76	4 1	17	30	48	1	17	37	48		17	53	66	1
18	57	68	1 4	18	34	51	1	18	27	44		18	63	69	1 2 8
19	44	56		19	43	59		19	36	51		19	63	72	
20	39	56		20	62	71	4	20	31	51		20	63	73	4 1
21	56	64	1	21	61	71	4 1	21	33	47		21	43	55	
22	62	68	1 4	22	50	59	4	22	26	41		22	43	55	
23	46	55	1 4	23	33	42		23	32	42	3	23	55	60	4 1 2
24	38	46		24	27	41		24	**27**	**30**	**7 3 4 1**	24	55	64	1 2
25	40	44	4 1	25	29	40		25	25	40	2 1	25	55	62	1 4 8 5
26	39	44	4 1	26	32	46		26	35	51		26	37	49	4
27	31	40		27	41	48	4	27	40	51	4 2 1	27	30	44	
28	26	39	1	28	44	48	4 1	28	**24**	**32**	3	28	32	49	
29	27	45	1	29	42	53	1 2	29	**21**	**29**					
30	29	49		30	40	47		30	**17**	**34**					
				31	40	44	4	31	32	48					

Weather Occurrences Legend

1 = Fog/Mist	6 = Freezing Rain
2 = Heavy fog	7 = Snow
3 = Drizzle	8 = Haze
4 = Rain	9 = blowing snow
5 = Thunderstorm	x = tornado

A.2. Temperature at Ben Hur Research Station during winter season 2014-2015

November 2014				December 2014				January 2015				February 2015			
Date	Min	Avg	**W/O**	Date	Min	Avg	**W/O**	Date	Min	Avg	**W/O**	Date	Min	Avg	**W/O**
1	36	48		1	51	66	1 2	1	41	47	4	1	54	61	4 1
2	34	50		2	51	63	1 2 8	2	53	62	4 1	2	34	44	
3	38	56		3	47	60	1	3	58	66	1 4 5	3	31	39	
4	55	68		4	54	62	1 2	4	44	52		4	41	45	4 1
5	59	68	1	5	66	74	1 2 8	5	32	42		5	38	48	1
6	54	63	4 1 2	6	55	66	1 2 4	6	29	46		6	30	45	
7	46	56		7	43	52		7	28	42		7	33	52	
8	43	58		8	39	53	1	8	**20**	29		8	47	61	1 2
9	43	57		9	36	54		9	33	36	4 3 **7**	9	54	67	1
10	39	58	1	10	37	50	8	10	30	41		10	41	55	8
11	58	70		11	38	52		11	41	52	4 1	11	38	56	
12	42	51	4 1	12	47	57		12	47	55	1 4 2	12	39	52	
13	33	41	4	13	47	60		13	38	43		13	31	45	
14	29	40		14	43	58	1	14	36	39	1 4	14	35	55	
15	29	44		15	56	67	4	15	34	40	1 4	15	47	57	1 2 8
16	50	63	5 4 1	16	41	54		16	31	45	1	16	35	53	1 8 4
17	35	43	4 1	17	34	48		17	29	46		17	31	40	
18	29	40		18	44	55	4 1	18	38	54	1	18	28	43	
19	27	44		19	47	52	1 4	19	31	50		19	31	42	
20	37	54		20	42	47	4 1	20	39	56	8	20	34	50	4
21	43	58		21	43	52	1	21	46	59		21	59	66	4
22	50	60		22	52	65	1 2 4 8	22	44	51	4 1	22	49	63	4 1
23	63	69	4	23	49	63	1 5 4	23	38	42	4 1	23	37	43	4 1
24	46	57		24	40	47		24	33	46		24	33	40	4 1
25	36	47		25	32	45		25	39	54	1	25	36	39	1 4 5
26	31	51		26	43	53	4 1	26	45	54		26	34	44	
27	38	50		27	59	66	4 1	27	39	58		27	35	44	
28	33	48		28	51	61	1 4	28	41	56		28	33	50	
29	46	62	1	29	49	53	1	29	41	60	1 8				
30	57	69		30	M	M	1	30	38	51					
				31	33	46		31	36	49					

Weather Occurrences Legend

1 = Fog/Mist
2 = Heavy fog
3 = Drizzle
4 = Rain
5 = Thunderstorm
6 = Freezing Rain
7 = Snow
8 = Haze
9 = blowing snow
x = tornado

A.3. Temperature at Dean Lee Research Station during winter season 2013-2014

November 2013				**December 2013**				**January 2014**				**February 2014**			
Date	Min	Avg	**W/O**	Date	Min	Avg	**W/O**	Date	Min	Avg	**W/O**	Date	Min	Avg	**W/O**
1	50	64	2 1	1	36	55		1	37	50	1 4	1	44	58	1
2	45	59	1	2	53	65	8 1 2	2	**31**	45	1	2	38	55	1 4 2
3	39	53	2 1	3	59	69	1	3	**25**	36		3	37	41	1
4	41	59	1	4	69	76	1 8	4	**21**	42		4	39	46	1 4 5
5	57	68		5	45	65	1 4	5	**31**	50	4	5	33	39	1 2
6	57	67	4	6	36	41	1 4	6	**22**	28		6	30	33	4 7 3
7	39	51	1 4	7	32	34	1	7	**15**	28		7	30	36	4 1
8	36	51	1	8	32	37	1 4	8	**20**	34	4	8	35	47	1 4
9	46	56		9	36	39	1 4 3	9	42	55		9	35	51	1 4
10	46	60	1 2	10	30	40	1	10	50	59	1 2 4 5	10	33	42	4 1
11	41	58	1 2	11	28	41	1	11	37	53	1 2	11	32	33	4 1 3
12	36	50	1 2	12	29	42		12	32	52		12	M	M	
13	29	42		13	33	46	4 1	13	49	61	4 1	13	M	M	
14	24	43		14	44	53	4 2	14	32	51		14	M	M	
15	45	60	4 1 2	15	30	42	1	15	29	43		15	M	M	
16	60	70	2 1 4	16	28	46	1	16	28	48		16	M	M	
17	72	79	4 1	17	29	50	1	17	26	42		17	M	M	
18	53	66	1 4	18	32	51	1	18	25	45		18	M	M	
19	40	54		19	39	57	1	19	33	49	1	19	55	69	1 2
20	35	54	1	20	65	72	4	20	28	51		20	58	70	4
21	43	60	1 8	21	56	66	1 4 5 2	21	29	44		21	36	52	
22	57	64	1 4	22	41	52	2 1 4	22	23	41	8	22	36	54	
23	41	49	1 4	23	29	39	1	23	**25**	36	4 7 1	23	51	63	1 2
24	35	42		24	26	39	1	24	**19**	26	7	24	52	60	
25	36	39	4 1	25	25	40	1	25	**18**	38		25	51	55	5 4 1
26	39	41	4 1	26	30	45		26	34	49	1	26	32	42	1 4
27	27	38		27	35	45		27	33	45	1 2	27	26	42	
28	24	38		28	38	44	4 1	28	**22**	28	3 7 1	28	30	45	4
29	23	42		29	32	48	1	29	**18**	27					
30	26	47		30	41	46		30	**13**	32					
				31	38	43		31	41	55	1 8				

Weather Occurrences Legend

1 = Fog/Mist
2 = Heavy fog
3 = Drizzle
4 = Rain
5 = Thunderstorm
6 = Freezing Rain
7 = Snow
8 = Haze
9 = blowing snow
x = tornado

A.4. Temperature at Dean Lee Research Station during winter season 2014-2015

November 2014				**December 2014**				**January 2015**				**February 2015**			
Date	Min	Avg	**W/O**	Date	Min	Avg	**W/O**	Date	Min	Avg	**W/O**	Date	Min	Avg	**W/O**
1	32	46		1	50	65	4	1	36	39	4	1	47	62	4
2	30	48		2	43	47		2	41	47	4	2	30	40	
3	34	53		3	45	55	1	3	50	58	4 5	3	31	39	
4	49	65		4	44	61		4	35	43		4	36	45	4
5	61	68	4 1	5	59	70		5	27	38		5	33	43	
6	52	61	4	6	51	62		6	25	44		6	28	45	
7	38	51	1	7	44	50		7	25	38		7	32	52	
8	34	51		8	35	50		8	17	27		8	50	64	1
9	37	53		9	32	51		9	30	34	3 4	9	46	63	1
10	36	57	1	10	35	45		10	29	37	4	10	39	53	
11	49	64	4	11	37	47		11	37	47	4	11	32	54	
12	39	46		12	44	57		12	41	49	4	12	36	49	
13	36	42		13	40	56	1	13	33	37		13	29	45	
14	29	39		14	38	55		14	32	35	4	14	35	56	
15	29	41		15	57	68	4	15	29	40	4	15	39	49	4
16	42	49	4	16	38	50		16	26	42		16	33	51	4
17	31	41		17	32	45		17	29	46		17	27	36	
18	27	39		18	48	51	4	18	33	51	1	18	27	43	
19	24	43		19	47	50	4	19	27	48		19	28	39	
20	36	55		20	39	49		20	39	55		20	32	49	4
21	45	60	4	21	32	45		21	45	55		21	55	64	4
22	53	62	4	22	48	59		22	41	48	4	22	40	48	4
23	61	67	4	23	45	55	4 5	23	36	40	4	23	31	36	3
24	39	52		24	30	39		24	30	46	1	24	29	34	
25	32	44		25	27	44		25	30	50	1	25	35	37	4
26	32	52		26	40	51		26	37	50		26	36	44	
27	34	47		27	55	62	4	27	38	58		27	32	40	
28	29	46		28	44	50	4	28	36	55		28	31	46	
29	45	60		29	43	46		29	46	62	1				
30	51	65	1	30	M	M		30	39	49					
				31	30	41		31	34	49	4				

Weather Occurrences Legend

1 = Fog/Mist
2 = Heavy fog
3 = Drizzle
4 = Rain
5 = Thunderstorm
6 = Freezing Rain
7 = Snow
8 = Haze
9 = blowing snow
x = tornado

A.5. Temperature at Crowley – Lafayette Research Station during winter season 2013-2014

November 2014				**December 2014**				**January 2015**				**February 2015**			
Date	Min	Avg	**W/O**	Date	Min	Avg	**W/O**	Date	Min	Avg	**W/O**	Date	Min	Avg	**W/O**
1	55	66	1	1	36	53	1	1	43	50	1 4	1	49	61	
2	52	62		2	56	66	1	2	37	47	1 2	2	43	58	4 1 2
3	45	56		3	61	70	1 2	3	30	39		3	38	43	
4	48	62	1	4	69	74	1	4	27	45		4	43	57	1 2 5 4
5	62	70	1	5	71	76	1	5	32	52		5	36	44	
6	65	74		6	39	56	1 4 2	6	27	31		6	30	37	3 7
7	49	59		7	34	37	1	7	20	29		7	29	38	4
8	42	54		8	35	40	3 1 4	8	22	36		8	37	48	
9	45	59		9	39	44	1	9	41	55		9	45	58	1 8
10	53	65		10	35	42		10	52	61	1 2 4 5	10	39	50	1 4
11	49	62		11	32	41		11	54	63	4	11	33	37	4 3 7 1
12	40	57		12	35	45		12	36	52		12	33	35	3 1
13	33	45		13	34	50		13	36	53	4 1	13	30	43	1
14	33	50		14	48	57	4 1	14	36	52		14	36	52	1
15	54	66	4	15	35	44	2	15	M	M		15	36	52	
16	67	73	1 2	16	31	46	1 2	16	30	46		16	41	55	1
17	72	77	4 1	17	31	49	2 7 1	17	34	46		17	56	66	1
18	M	M	2 1 4	18	36	52	1 2	18	30	44		18	58	66	1 2
19	48	58		19	43	59	1	19	40	53	1 2	19	64	71	1 2
20	41	58		20	66	72		20	35	53	1 2	20	64	70	4 1 5
21	56	66	1 2 7 8	21	64	70	1 4	21	38	49	1 2 4	21	46	56	
22	65	69	1 2 8 4	22	47	57	4 1	22	44	50		22	61	65	
23	46	56	1 4	23	33	42		23	30	44	4	23	58	66	4 1 2 5
24	39	47		24	29	41	1	24	25	28	3 1 7 4	24	56	63	1
25	39	43	4 1	25	29	40		25	24	39		25	56	62	1 2 4
26	40	43	4 1	26	34	47		26	38	51	1	26	39	48	4 1
27	30	39		27	44	50		27	37	49	1 2	27	32	44	
28	27	38		28	44	46	4 1	28	25	31	3	28	36	49	
29	28	45	1	29	35	49	1 2	29	23	30					
30	30	49	1	30	42	48		30	19	35					
				31	40	43	4	31	40	53					

Weather Occurrences Legend

1 = Fog/Mist	6 = Freezing Rain
2 = Heavy fog	7 = Snow
3 = Drizzle	8 = Haze
4 = Rain	9 = blowing snow
5 = Thunderstorm	x = tornado

A.6. Temperature at Crowley – Lafayette Research Station during winter season 2014-2015

November 2014				**December 2014**				**January 2015**				**February 2015**			
Date	Min	Avg	**W/O**	Date	Min	Avg	**W/O**	Date	Min	Avg	**W/O**	Date	Min	Avg	**W/O**
1	39	50		1	52	66	1 2	1	43	50	1	1	55	63	4 1
2	36	52		2	49	58	4 1	2	56	62	1 2	2	34	44	
3	42	58	1	3	45	60		3	59	66	1 5 4	3	33	40	
4	58	69		4	56	65	1 2 8	4	44	52		4	43	47	4 1
5	65	71	4 1	5	64	72	1 2	5	34	42		5	39	48	
6	56	64	4	6	58	70	2	6	31	48		6	33	47	
7	47	55		7	47	54		7	30	43		7	37	53	1
8	46	59		8	42	54		8	23	30		8	51	63	1 2
9	46	58	1	9	41	56	1 8	9	35	37	3 4	9	57	67	1
10	42	59	1 2	10	40	52		10	33	42		10	43	55	8
11	56	68		11	43	55		11	44	52	4 1 8 2	11	41	58	
12	43	50	4 1	12	45	58	8	12	47	54	1 2	12	42	53	
13	35	42	4	13	49	62	1 8	13	38	43		13	33	46	
14	30	40		14	47	60	1 2	14	36	38		14	39	55	1
15	32	43		15	60	69		15	36	45	4 1	15	52	59	1 2 8
16	49	62	4 1 5	16	45	56		16	31	45		16	37	55	4 1
17	38	44	4 1	17	39	50		17	34	48	1 8 2	17	31	39	3
18	33	42	2	18	48	58		18	46	59	8 1	18	30	44	
19	27	43	1	19	49	54	1 4	19	32	51		19	33	44	
20	42	57	1	20	43	47	1	20	41	57	1	20	40	54	
21	53	63		21	42	49	1	21	47	59	1 2	21	63	67	4
22	55	63	4	22	50	63	1 2	22	45	53	4 1	22	45	59	1 4
23	62	68	4 1	23	49	61	1 2 5 4	23	39	42	1 4	23	33	39	4 1 3
24	49	59	1	24	39	46		24	35	47		24	31	37	3 1
25	37	47	1	25	35	47		25	41	54	1 2	25	38	40	1 4
26	36	53	1 8	26	48	55	1 4	26	44	53		26	36	43	
27	40	51		27	60	67	1 4	27	46	61		27	35	44	
28	37	51	None	28	49	58	4	28	45	58		28	33	50	
29	52	64		29	48	51	1 8	29	48	61	1				
30	56	67	8	30	M	M	1 8	30	42	52					
				31	35	46		31	41	53					

Weather Occurrences Legend

1 = Fog/Mist	6 = Freezing Rain
2 = Heavy fog	7 = Snow
3 = Drizzle	8 = Haze
4 = Rain	9 = blowing snow
5 = Thunderstorm	x = tornado

A.7. Average temperature and precipitation during winter months at three experiment locations

Baton Rouge:

Temperature Data	Nov13	Dec13	Jan14	Feb14	Nov14	Dec14	Jan15	Feb15
AVERAGE MONTHLY	57.0	52.0	44.5	52.8	54.6	56.4	49.0	49.8
MIN 32 OR BELOW	5.0	6.0	16.0	6.0	5.0	1.0	8.0	6.0
HEATING DEGREE DAYS:								
TOTAL THIS MONTH	268	437	630	352	326	263	493	424
COOLING DEGREE DAYS:								
TOTAL THIS MONTH	36	44	0	20	19	14	1	3
Precipitation Data								
TOTAL FOR THE MONTH	1.71	3.72	2.09	7.19	3.50	5.68	6.37	3.38
GREATEST 24HR TOTAL	1.02	2.05	0.46	2.13	2.64	2.41	3.52	0.93
NUMBER OF DAYS WITH:								
- 0.01 INCH OR MORE PRECIP	7	8	9	13	5	9	8	7
- 0.10 INCH OR MORE PRECIP	3	4	8	8	3	5	4	6
- 0.50 INCH OR MORE PRECIP	1	3	0	5	1	3	2	4
- 1.00 INCH OR MORE PRECIP	1	1	0	3	1	3	2	0

Alexandria:

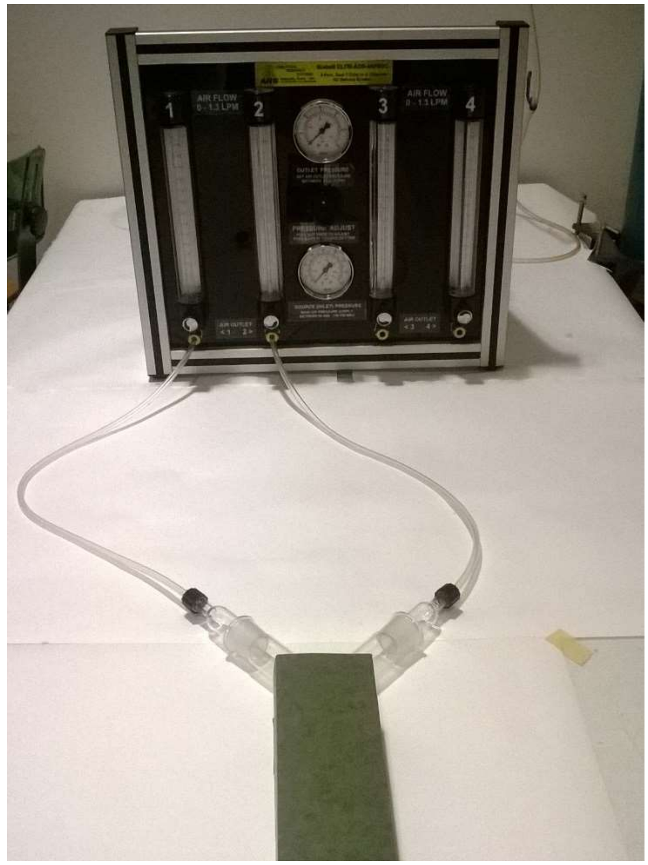
Temperature Data	Nov13	Dec13	Jan14	Feb14	Nov14	Dec14	Jan15	Feb15
AVERAGE MONTHLY	53.8	49.1	43.0	49.0	52.0	52.2	45.5	47.1
MIN 32 OR BELOW	6.0	14.0	22.0	6.0	10.0	6.0	13.0	14.0
HEATING DEGREE DAYS:								
TOTAL THIS MONTH	351	510	672	341	388	382	598	494
COOLING DEGREE DAYS:								
TOTAL THIS MONTH	25	23	0	9	5	8	0	0
Precipitation Data								
TOTAL FOR THE MONTH	4.28	2.37	2.61	6.36	2.91	3.30	5.67	1.45
GREATEST 24HR TOTAL	2.89	0.79	0.92	1.87	2.01	0.69	2.09	0.66
NUMBER OF DAYS WITH:								
- 0.01 INCH OR MORE PRECIP	13	9	9	11	7	10	11	8
- 0.10 INCH OR MORE PRECIP	4	6	4	7	2	7	7	4
- 0.50 INCH OR MORE PRECIP	3	2	2	5	2	4	3	1
- 1.00 INCH OR MORE PRECIP	1	0	0	3	1	0	3	0

Lafayette:

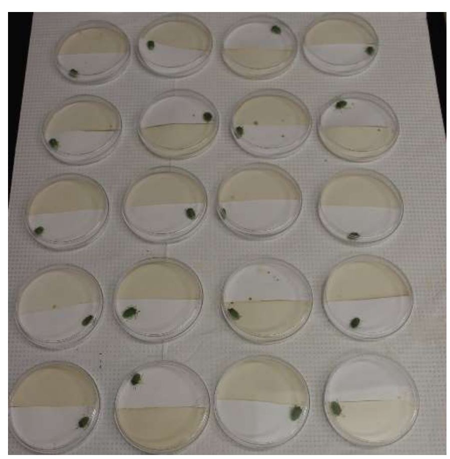
Temperature Data	Nov13	Dec13	Jan14	Feb14	Nov14	Dec14	Jan15	Feb15
AVERAGE MONTHLY	57.2	51.6	45.2	53.0	55.5	57.0	50.0	50.2
MIN 32 OR BELOW	4.0	5.0	14.0	4.0	3.0	0.0	5.0	3.0
HEATING DEGREE DAYS:								
TOTAL THIS MONTH	258	447	584	340	296	250	459	411
COOLING DEGREE DAYS:								
TOTAL THIS MONTH	41	38	0	14	18	19	1	4
Precipitation Data								
TOTAL FOR THE MONTH	1.49	2.79	1.63	5.63	2.65	3.50	4.61	2.48
GREATEST 24HR TOTAL	0.66	1.09	0.34	1.44	1.02	2.05	2.22	0.78
NUMBER OF DAYS WITH:								
- 0.01 INCH OR MORE PRECIP	6	8	9	13	7	6	9	8
- 0.10 INCH OR MORE PRECIP	3	4	6	7	6	4	4	4
- 0.50 INCH OR MORE PRECIP	2	3	0	6	2	2	2	3
- 1.00 INCH OR MORE PRECIP	0	1	0	2	1	1	2	0

APPENDIX B: EXPERIMENT DOCUMENTATION

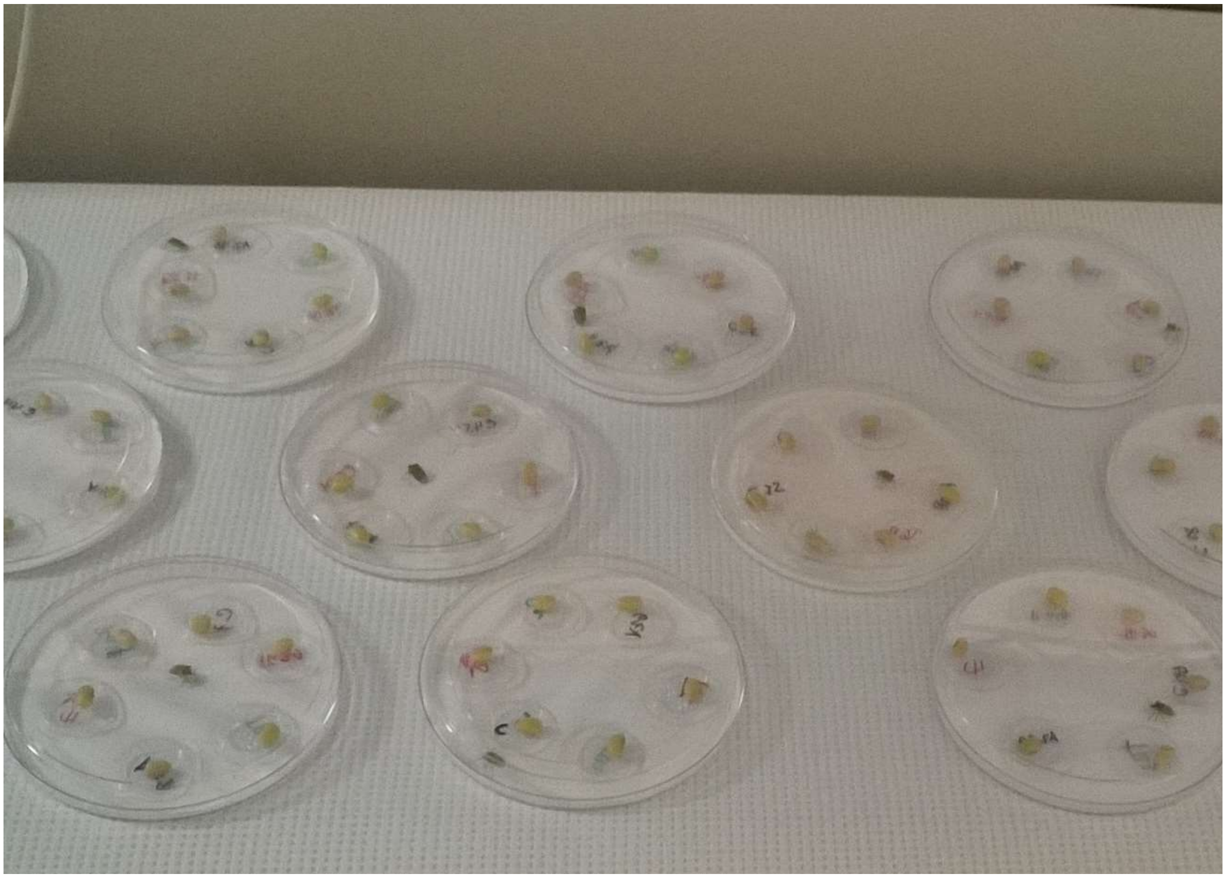
B.1. The set up of Y-tube for olfactory response experiment



B.2. Tactile Response arenas



B.3. Feeding preference arenas



B.4. Oviposition Preference arenas

B.5. The field experiment plots at early stage of soybean growth

B.6. The field of experiment plots at late stage of soybean growth

B.7. The appearance of samples plants and pods, showing the effect of stink bug.

B.8. The appearance of seeds taken from two different sample plots, showing the difference in damage severity.

B.9. The appearance of sample seeds after soaking in water, showing different symptoms of damage

VITA

Kukuh Hernowo, was born in Wonosobo, Indonesia to parents, Soeharsono (father) and Sri Umiyati (mother). He attended the University of Jenderal Soedirman in Purwokerto, Indonesia for his Bachelor degree in Agriculture and graduated as Master of Philosophy from the University of Queenslan, St. Lucia, Australia. In 2011, he enrolled at Louisiana State University, Baton Rouge, Louisiana to pursue a PhD in Entomology under the guidance of Dr. Jeffrey A. Davis in the Soybean Entomology Laboratory. He is married to Wahyu Eka Hartanti and has two children, Tiara Cahyadien Syifa and Rifky Pasha Wisnuwardhana. Kukuh Hernowo is currently a doctoral candidate in the Department of Entomology at Louisiana State University.