Spatial distribution of adult *Dectes texanus* (Leconte, Coleoptera: Cerambycidae) and its effects on Kansas soybean (*Glycine max* L.)

by

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B.S., Sul Ross State University, 2009
M.S., Sul Ross State University, 2011

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Entomology
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2019

Abstract

Dectes texanus LeConte (Coleoptera: Cerambycidae), *Dectes* stem borer, is native to North American and can be found throughout Kansas in areas with soybean (*Glycine max*, L) production fields. In Kansas, adult *D. texanus* are present in production soybean fields between mid-June and September most years*;* however, the larval stage is the most damaging stage to the plant, due to pith removal and consequent girdling of the main stem prior to overwintering. Although loss from physiological (i.e., indirect feeding of non-seed tissue) and mechanical (i.e., harvestability) mechanisms is variable, soybean growers need viable management strategies to mitigate losses caused by this annual pest. As such, there is a need to update current management recommendations for controlling *D. texanus*; however, several knowledge gaps about *D. texanus* behavior in the field exist and need to be addressed prior to successful implementation of new management strategies.

The major goal of this research was to examine and improve our understanding of the biology and behavior of *D. texanus* as well as soybean plant responses to infestation through multiple on-farm field experiments. To achieve this, we conducted three field studies. The objectives of the first study were to: 1) monitor adult *D. texanus* activity within soybean fields to determine if *D. texanus* adults and/or larvae are aggregated within the field, and if so, 2) identify when during the growing season aggregation occurs. The objective of the second study was to estimate within field dispersal capabilities of adult *D. texanus* using a protein-based, mark-capture techniques. The final objective was to investigate the utility of vegetation indices as a method to detect soybean infested with *D. texanus*.

To determine if *D. texanus* adults and or larvae aggregate and when during the growing season we conducted grid sampling throughout June-September, to monitor activity within the field. The results of this study indicate that adult aggregation occurs during July when adult presence is at its highest (mid-late July). By using protein markers, we were able to determine dispersal capabilities of adult *D. texanus*, within a given soybean field. Results showed that on average *D. texanus* traveled between 52 to 389 m. Results also found that infested soybean plants had more node and 1% larger stem diameters than non-infested plants. Multiple vegetation indices were used to examine difference in spectral response to *D. texanus* infestation. Interestingly, only the 2014 exclusion cage study showed a significant difference between *D. texanus* infested and non-infested cages for several indices, including ENDVI, ENDVI2, ENDVI3, GBNDVI, NIR Green Diff. and NIRBRVI. Given that we were able to detect changes in crop phenology through time, there is great potential in using remote sensing methods to determine optimal times to harvest soybean before *D. texanus* infestations lodge plants. Such an application would require further investigation.

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Major Professor
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Table of Contents

List of Figures

List of Tables

Acknowledgements

As we all know research would get nowhere without funding support and this case is no different. As such the Kansas Soybean Commission has been instrumental in the support and funding of this research. Additionally, thanks to the Department of Entomology at Kansas State University for logistics and financial support. I was also had the opportunity of working with some great farmers and K-State supporters, who without them none of this would have been possible, thank you.

Being in a field crop lab, the workloads can be daunting. Over the years I worked with so many wonderful students and faculty. There are so many that helped over the years and all have had a big part in assisting with experimental implementation and data collection. I would like to send a heartfelt thank you to the many people who have helped me throughout this long adventure.

I wish to acknowledge and thank my advisor, Dr. Brian McCornack for his continuous guidance, encouragement, and excitement throughout my graduate term. I would also wish to thank Drs. C. Michael Smith, James Campbell, and Douglas Goodin for being part of my advisory committee and not only providing invaluable outside perspective, but also for the dedication and interest they all showed for my project. I would further like to thank Dr. Kevin Price for being a part of my advisory committee and helping me gain my first steps down the difficult road of remote sensing.

I especially want to thank Dr. Wendy Johnson, Ximena Cibils-Stewart, Amy Raudenbush, Alysha Soper and Kent Hampton for their friendship, support, advice, assistance, and the many hours of discussions during my time with K-State. I would also like to extend a deep thank you Dr. Jeff Whitworth, Dr. Holly Davis, and JR Ewing for their assistant over the years.

Lastly, I would also like to acknowledge my family for serving as my rocks throughout this experience. I would especially like to thank my parents Marty and Debbie Harris for always being there, and my sister Micki for always “getting it”. They are everything and I would be nowhere without them, I love you all.

Dedication

For my family, who started it all.

Chapter 1 - Key factors governing colonization and dispersal of adult *Dectes texanus* (LeConte, Coleoptera: Cerambycidae) in Kansas soybean (*Glycine max* L.) production fields

Introduction

Soybean (*Glycine max* L.) is an important economic crop in North America with an estimated 11.4 million ha planted and a production value exceeding $41 billion in 2013 according to the US Department of Agriculture [USDA-NASS, 2013]. Considering the large number of production acres in the US, it is unusual that, until recently, arthropod pests have not been problematic. Consequently, the introduction and emergence of new pest complexes, such as soybean aphid (*Aphis glycines* Matsumura) , brown marmorated stink bug (*Halyomorpha halys* Stål), soybean stem borers (*Dectes texanus* LeConte), have resulted in yield loss, highlighting the need to develop sustainable and economic pest management strategies while also improving existing strategies (Ragsdale et al. 2007, 2011). Focusing on the soybean stem borer, *D. texanus* (LeConte (Coleoptera: Cerambycidae)), current management strategies include both cultural (tillage, crop rotation, trap crops) and chemical controls (insecticides) can be used to control infestations. Nevertheless, given the increased adoption of no-till cultivation practices and surge of soybean acreage in Kansas (≈1.2 to 4.0 m ha from years 2000 to 2014, respectively), increased yield losses by *D. texanus* may be expected ('USDA-NASS QuickStats Kansas Soybean Acres Planted' n.d.). This chapter will

discuss factors that have contributed to *D. texanus* becoming a notable, emerging pest species, and will emphasize the need for new and effective pest management strategies.

Geographical distribution and host range

Dectes texanus was first reported in North Carolina soybean fields in 1968 (Falter 1969). Since then, there have been reports of *D. texanus* populations throughout much of the soybean producing regions of the eastern, southern, and central US including: Missouri, Arkansas, Louisiana, Illinois, Mississippi, Texas, Nebraska, Tennessee, North Carolina, and Kansas (Laster et al. 1981; Michaud and Grant 2005, 2009; Buschman and Sloderbeck 2010; Tindall et al. 2010). In Kansas, *D. texanus* were initially detected in 1985 within five south central counties (Edwards, Barton, Kiowa, Ford, and Pawnee counties), then spreading into 41 counties by 2008 (Buschman and Sloderbeck 2010) (Fig. 1.1), and 55 counties as of 2015 (Fig. 1.2). In 38 of the 81 counties sampled in 2015, over 50% of the fields were found to have *D. texanus* larvae, adult, or both stages. These results indicate that *D. texanus* are steadily increasing in range and numbers within Kansas, strengthening the need for new and effective management strategies.

Dectes texanus, which is native to North America, can utilize (i.e. feeding (adult and larvae) and ovipositioning) several native host plants within the Asteraceae family including ragweed (*Ambrosia artemisiifolia* and *A. trifida*), native sunflower (*Helianthus annuus*), and cocklebur (*Xanthium strumarium*) (Patrick 1973a; Rogers 1985). Members of this family can be found in very diverse habitats ranging from the arctic to desert landscapes; the host plants wide distribution has been attributed to the spread of *D. texanus* (Michaud and Grant 2005; Buschman and Sloderbeck 2010). Beyond the

Asteraceae family, Michaud and Grant (2005) present several hypotheses as to why the host range for *D. texanus* expanded to include soybean. The first is host availability. Soybean may have been accepted as a host due to its increased abundance and corresponding decline in native hosts due to effective weed management strategies in soybean production systems. Second, *D. texanus* colonizing soybean are provided refuge from natural enemies like entomopathogens, predators and/or parasitoids found on native hosts. Interspecific competition is another possible explanation for a host expansion to soybean. In general, there is greater arthropod diversity observed in sunflower stalks (Michaud and Grant 2005), including those that occupy the same feeding and overwintering sites as *D. texanus* larvae. Therefore, utilizing soybean could be positively impacting *D. texanus* survival because of the reduction in resource competition. Lastly, *D. texanus* larvae develop within the stem of the soybean plant. The larvae are aggressive and cannibalistic towards each other, which results in only one larvae per plant at the end of the season. The intraspecific competition hypothesis suggests that the increase in host availability, even though larvae are cannibalistic, would provide a level of compensation for conspecific competition by providing an ample amount of available host plants and less chance larvae will interact. Through experimentation, each hypothesis may provide valuable information and a potential explanation for *D. texanus* host range expansion into soybean.

Dectes texanus life history and biology

The biology of *D. texanus* has been extensively documented. *Dectes texanus* are univoltine (Patrick 1973a), adults emerging from soybean stubble in mid-June to early

August to feed on soybean and mate, followed by females depositing new eggs into the pith of a soybean plant (Crook et al. 2004; Buschman and Sloderbeck 2010; Tindall et al. 2010). Adult *D. texanus* are relatively small, approximately 6-11 mm (Fig. 1.3) in length (Hatchett et al. 1975). Adult color ranges from dark brown to black with "banded" antennae that are as long as or longer than the body (Fig. 1.3A). Males and females can be distinguished using key morphological structures. Specifically, the last sternal segment in females are pointed and elongated (Fig. 1.4B) while the males sternal segment terminates abruptly (Fig. 1.4C). In addition, females typically have a larger abdomen and shorter antennae than males (Hatchett et al. 1973; Niide 2009). Adults mate in as little as 5 days following emergence (Patrick 1973a). Successful mating relies on documented courtship behaviors, which includes the production of a contact pheromone by the female (Crook et al. 2004). Courtship begins with antennal touching (i.e., "antennal jousting") by males to initiate copulation. Once mating commences the males will mate with multiple partners in a season while females mate with only one male (Patrick 1973a). After copulation, females enter a preoviposition period of 10-14 days before selecting a site to deposit eggs in the pith of a host plant. In order to oviposit eggs, the female will chew a hole (oviposition puncture) into the tissue of the petiole, main stem, and/or lateral branches, thrust the ovipositor into the hole, and deposit a single egg (Fig. 1.4A) (Patrick 1973a; Hatchett et al. 1975). Egg deposition highly depends on the presence of pith and whether the ovipositor of the female can reach it (Hatchett et al. 1975). While oviposition punctures may appear on host petiole, main stem, or lateral branches, eggs are mainly observed in the petioles, and not all ovipositional punctures will contain an egg (Patrick 1973a; Laster et al. 1981). Females do not differentiate between host plants already

containing eggs and will deposit eggs regardless of the number of eggs already present within the petiole or plant (Patrick 1973a; J. Michaud et al. 2007).

Newly deposited eggs (approx.1.5 to 1.9 mm in length by 0.4 mm in width) require an incubation period of 8-10 days and are yellowish-white in color before turning a dark yellow near eclosion (Patrick 1973a; Hatchett et al. 1975; Laster et al. 1981; Niide et al. 2012). Newly hatched larvae typically develop through four instars (Patrick 1973a; Niide et al. 2012). First instars will immediately begin feeding on the pith of the soybean petioles for approximately 7 days (Patrick 1973a). The second and third instars continue feeding on the pith over a 3 week period, tunneling from the petiole into the main stem creating a hole at the "entry node" (Fig. 1.4B) (Patrick 1973a; Hatchett et al. 1975; Niide et al. 2012). The third instar continues feeding well into the fall (September to October) in preparation for overwintering (Patrick 1973a; Sloderbeck and Buschman 2011). The late stage, mature larvae, range in size from 7.0 to 12.5 mm in length (Hatchett et al. 1975) (Fig. 1.3D). They are yellow in color with a brown head capsule and are cylindrical in shape. Late stage larvae are very aggressive towards conspecifics and will eliminate one another through combat or cannibalism until only one larvae remains in a plant by the end of the season (Patrick 1973a; J. Michaud et al. 2007; Niide et al. 2012). Surviving larvae tunnel to the base of the stem where they may cut or girdle the base of the mature soybean plant, which becomes the main structure used for an overwintering chamber (Fig. 1.4C). Larvae will girdle the stem 3-10 cm above the ground and block the entrance with a frass plug (Fig. 1.4D) (Patrick 1973a; Sloderbeck and Buschman 2011; Niide et al. 2012). Larvae overwinter for approximately 8-10 months, becoming active in late April before entering the pupal stage, which last for 8-10 days (Hatchett et al. 1975). Adults

will begin to emerge from the soybean stubble in mid-June and continue emerging through early August (Patrick 1973a; Niide et al. 2006, 2012; J. Michaud et al. 2007), during important developmental stages of soybean.

Management practices

Managing *D. texanus* is a challenge. The larval stages, which cause most of the damage due to early-season tunneling and late-season girdling behaviors, are found inside the developing soybean plant. Studies examining yield loss associated with the larval activity have found that developing larvae can decrease seed weight by 7-11% and that one larva can cause up to 10% yield loss per plant (Richardson 1975; Buschman et al. 2006). Even though the girdling and lodging of the mature soybean plants has been highly attributed to yield loss by farmers located in high-risk areas, the loss has not been adequately quantified. A study by Daugherty and Jackson (1969) found that fields with nearly 100% infestation resulted in a 16.8% yield loss due to lodging. In other hosts such as sunflower, *D. texanus* are reported to have no effect on seed weight, but yield losses up to 40% may result from sunflower lodging and stalk breakage (J. Michaud et al. 2007). As *D. texanus* expands its range into the soybean producing regions of the South Central US, the potential for yield loss may also increase. Management practices for yield retention, like applications of contact insecticides are inappropriate, as the larvae are well protected in the plant. As such, exploring new strategies for *D. texanus* management, outside of contact insecticides, and those that incorporate existing cultural practices may be important to the successful management of this pest in the North Central US.

Cultural practices

Several cultural practices have been proposed for managing *D. texanus* in soybean, but not all of them are practical under current agronomic practices. A common practice used by many farmers against some insect pests is crop rotation, which is used to interrupt the normal life cycle of insect pests by placing the insects in a non-host habitat on alternating years (e.g., a soybean-corn rotation). This method has been successful against pests with longer generation cycles and limited dispersal capabilities such as corn rootworm (*Diabrotica* sp.) (Levine 1996) and the Colorado potato beetle (*Leptinotarsa decemlineata*) (Wright 1984); however, the use of crop rotation as a management practice specifically for *D. texanus* has not been documented. Even so crop rotation may aide in reducing *D. texanus* infestation as mortality may occur when adults are seeking out new soybean hosts.

Another cultural practice is stem burial, which is accomplished by deep plowing, row bedding, or tilling soon after harvest. This practice has declined in popularity due to adoption of other cultural farming practices like no-till, which aims to conserve soils. Campbell and van Duyn (1977) conducted a series of experiments from 1971-1975 examining cultural and chemical controls for managing *D. texanus* in soybean. Although there were small differences between years, they did find that larval mortality was highest (20-52%) when stubble was buried 5 cm soon after harvest, with deeper burial of stems having no significant differences on larvae mortality. They also found that low-lying areas with more moisture had higher larval mortality than in any other areas in the same field. They noted that less than 36% of adults were able to dig out of the soil when buried at 5 cm and less than 15% emerged when buried at 10 cm. Effects of stem burial depth

was more evident in fields where the soil was packed or hard forming, such as clay soil, suggesting that soil type was also influencing *D. texanus* survival. Due to the increased adoption of no-till cultivation practices and rise of soybean acreage in Kansas, soybean farmers may begin to see an increase in soybean lodging by *D. texanus*.

Additional cultural practices available to farmers include trap and companion crops. Both methods can reduce *D. texanus* infestations in soybean when cultivated sunflower (*Helianthus annuus*) is planted adjacent to soybean fields (J. Michaud et al. 2007). Trap crops are plant stands, either deliberately planted or natural occurring, that serve as a preferred host, drawing the target pest away from the main crop and absorbing primary damages associated with the targeted pest (Shelton and Badenes-Perez 2006). What makes a trap crop effective is that they are more attractive to the pest than the crop and can retain the most damaging life stage of the pest at critical developmental stages of the crop. When examining trap crops, Michaud et al. (2007) used two methods employing sunflower as an ovipositional sink for *D. texanus*, which included planting sunflower in non-irrigated corners or in a six-row strip around a soybean field. Corner plantings reduced soybean infestation by 65% whereas the sunflower strips acted as a barrier for immigrating females, which reduced the number of infested soybean plants to less than 5% whereas 96% of sunflowers were infested. In using sunflower as a companion crop (two plant species planted together because they are believed to benefit one another (Parker et al. 2013)), Michaud et al. (2007) found that planting the field 50/50 (soybean and sunflower) resulted in higher *D. texanus* infestations in sunflower. In addition, Michaud et al. (2007) identified a "zone of attraction," which extended 200 m into the soybean field from the sunflower catch crop and was based on infestation patterns. The

advantage of using this method is that both hosts comprise an area large enough to produce a harvestable yield for both crops (J. Michaud et al. 2007). Conversely for trap cropping, the limiting factor for grower adoption is that the catch crop must be planted in large enough quantities to have economic value (J. Michaud et al. 2007).

Furthermore, trap and companion crops are effective because females have shown a preference for depositing eggs in sunflower over soybean (Michaud and Grant 2005; J. Michaud et al. 2007). In field and greenhouse trials, Michaud and Grant (2005) found that females had higher oviposition numbers in sunflower when encountered first in the field and lower if encountered soybean first. Lab experiments also showed that adults fed sunflower pith lived 75.6 ± 4.3 days (males) and 52.4 ± 3.7 days (females) compared to the 23.3 ± 1.4 days (males) and 23.2 ± 1.2 days (females) from soybean (Michaud and Grant 2005). Michaud and Grant (2005) were able to correlate adult feeding to ovipunctures and eggs of both plant types, suggesting that sunflower is the preferred host.

Host plant resistance

Insect resistant cultivars began to appear during the eighteenth and nineteenth centuries when the practice of applied entomology began (Smith 1989; Smith and Clement 2012). According to Smith (1989), by the mid-1970s there were over 500 arthropod resistant cultivars developed and registered in the US. Currently, there are over 25 major crops that have resistant varieties for one or more insect pests, included in this list is soybean, which has been used in the field by farmers for pest management (Wilde 2010). The increased use and importance of host plant resistance has become a foundational component of integrated pest management (IPM) programs (Dixon 1969; Panda and Khush 1995; Smith 2005a; Niide et al. 2012; Smith and Clement 2012). There

are several benefits associated with using resistant cultivars; as reviewed by Smith and Clement (2012), these include reduced or eliminated insecticide applications and residues, indirect benefit of cleaner streams and lakes from reduced insecticide use, and reduced mortality of beneficial arthropod populations in the environment. For the farmer, resistant cultivars in some cases may be a more affordable management option since arthropod control costs are included within the cost of seed (Smith 2005b; Smith and Clement 2012) and/or they reduce the need for specialized spray equipment.

There are three main categories of resistance: tolerance, antixenosis, and antibiosis. Tolerance is where the plant can withstand or recover from arthropod feeding and related damage and does not affect the growth and survival of the arthropod. Antixenosis is where morphological or chemical components in the plant adversely affect arthropod behavior, leading to delayed acceptance and possible outright rejection of a plant as a host. Lastly, antibiosis is where the plant adversely affects the life-history traits (survival, development, fecundity) of an arthropod attempting to use that plant as a host (Smith 2005c, 2005d, 2005e; Niide et al. 2012; Smith and Clement 2012). Though development is underway, there are no commercially available resistant cultivars in the US for controlling *D. texanus* in soybean (Niide et al. 2012).

In the mid-70s', Richardson (1975) and Campbell (1976) both attempted to detect resistant varieties by screening a large variety of available soybean genotypes. Using end-of-season observations of infested plants (lodged plants), they were able to identify 18 plant introductions with moderate resistance to *D. texanus*; however, they concluded that the results from these studies may misrepresent infestation pressure due to differences in plant maturity group and larval cannibalism. Since then, there has been much research

conducted on developing host plant resistant lines to be used in Kansas. Through research conducted by Kaczmarek (2003), Niide (2009; et al. 2012) and Aguirre-Rojas (2013), a plant introduction (PI) 165673 was identified as a strong candidate to use in developing host plant resistance against *D. texanus*. Niide et al. (2012) examined several soybean PIs for antixenosis (number of oviposition punctures/per plant) and antibiosis (oviposition punctures/live larvae). They found that PI165673 had the highest oviposition punctures/live larva, suggesting that antibiosis resistance could reduce the number of live *D. texanus* larvae. Further research conducted by Aguirre (2013) found that PI165673 may affect the embryos as well as delay the initial feeding by first instar larvae. The study also indicated that resistance in this PI is polygenic (controlled by more than one gene in the plant), and although beneficial, marker assisted selection would improve its resistance to attacks against *D. texanus*. Based on the study, Aguirre-Rojas (2012) suggests that additional research conducted on this, and other PIs, is needed to determine if there is delayed development to *D. texanus* or adverse effects on adults in the next season (Aguirre-Rojas 2013).

Insecticides

For *D. texanus*, there have been multiple studies examining the effectiveness of insecticides against both larvae and adults. Campbell and Duyn (1977) examined chemical control for both larvae and adult *D. texanus*. They investigated multiple insecticides and application methods including: systemic insecticides applied to furrows at time of planting (carbofuran, phorate, disulfoton; applied at 0.45 kg AI/ha), granule formulations applied over infested stubble (diazinon, chlorpyrifos, carbofuran, ethoprop, phorate, and fonos; applied at 0.45 and 0.91 kg AI/ha), stubble treated with insecticide,

and insecticide applications to adults (carbaryl and malathion (applied at rates of 0.23, 0.45, and 0.68 kg AI/ha) and methomyl and methyl parathion (applied at rates of 0.11, 0.23, and 0.45 kg AI/ha). Campbell and Duyn (1977) found that systemic insecticides were not effective in reducing the number of infested stems or girdled stems. However, they found when phorate was applied over the soybean row in mid-July there was a 50% reduction in girdled stems. The attempted to treat infested stubble with either liquid or granules over the row after harvest were found to be ineffective methods, with only the treatment of diazinon decreasing larval survival by 20%. The insecticides were further found to be ineffective at contacting larvae by penetrating stubble. In a complementary cage study targeting adults by Campbell and Duyn (1977), control was successful with all treatments (carbaryl, malathion, methomyl, and methyl parathion) killing 98-100% of the adults when applied with a 2-row back pack sprayer. Their study suggested that insecticide applications in production fields must be based on local knowledge of *D. texanus* emergence and the need for multiple well-timed applications (Campbell and van Duyn 1977).

Buschman et al. (2005, 2006, 2007a, 2007b) further conducted a series of experiments examining seed treatments, systemic, and foliar insecticide applications for use against *D. texanus* infestations. They found that the insecticide, Fipronil (Regent 4SC) (phenylpyrazole), was successful in controlling *D. texanus* using all three application methods. Foliar applications of Fipronil showed a significant increase in soybean yield (4.6 to 6.6 bu/acre) compared to the untreated control plots. Furthermore, a 7 to 11% physiological yield loss due to *D. texanus* infestations was determined by comparing yields in treatment versus control plots (Buschman et al. 2005). They also

found that there was a significant reduction (85%) in *D. texanus* infestation in plots treated with Fipronil (Buschman et al. 2005, 2006, 2007a). In a separate study, when applied as a seed treatment, Fipronil showed 100% control of *D. texanus* (Buschman et al. 2007a). When multiple varieties were compared in another study, the authors found that there were no differences in *D. texanus* control (Buschman et al. 2007b). Unlike previous studies, there was no increase in yield attributed to Fipronil, but Fipronil reduced *D. texanus* infestations between 76% and 88%, similar to findings in past studies (Buschman et al. 2007b). The studies (Buschman et al. 2005, 2006) also found that the insecticides imidacloprid (Provado 1.6 F), clothianidin (TM-44401 50 WP), acetamiprid (Intruder WSP), and thiacloprid (Calypso 4F) were also capable of reducing infestations of *D. texanus*, but not as effectively as Fipronil. Although Fipronil was successful in reducing *D. texanus* infestation under trial conditions, the insecticide is currently only registered for in-furrow use on potatoes.

More recently, the use of aerial insecticide applications for adult *D. texanus* management in soybean has been explored. A three year study was conducted to examine the impacts of aerial insecticide applications on season-long beetle populations and the resulting percentage of soybean plants infested (Sloderbeck and Buschman 2011). Study fields were sprayed with lambda cyhalothrin (Warrior™, Syngenta Crop Protection, Inc., Greensboro, NC) at an application rate of 0.028, 0.026, and 0.028 kg ai/ha in 28 1/ha of water in 2001, 2002, and 2003, respectively. All fields received two insecticide applications during adult stem borer activity (July). Aerial insecticide applications resulted in a reduction in both season-long beetle populations (74%) and percentage of plants infested (46%) in 2001, 89% and 53%, respectively in 2002, and 98% and 75%

respectively in 2003 (Sloderbeck and Buschman 2011). Based on these results and previous experiences, Buschman and Sloderbeck (2011) recommended that insecticides, specifically lambda-cyalothrin (Warrior™, Syngenta Corp.) was effective when applied during peak adult flight, followed by a second application 10 days later. These study results may be improved considering findings by Campbell and van Duyn (1977), where the local information on rate of *D. texanus* emergence should be taken into account when determining the first day for insecticide application.

Although shown to be effective, there are potential difficulties in relying solely on insecticides including resistance, upsurge of secondary pests, and unintended environmental contamination (Kogan 1998). Applying insecticides has economic impacts as well. Product price, application costs, labor, and equipment needed to apply insecticides can drive treatment decisions. In addition, the economic impacts may be compounded as insecticide control of *D. texanus* may be inconsistent due to lack of knowledge of the insect behavior within the field. This dissertation will explore possibilities for making insecticide use beneficial to farmers, either increasing its effectiveness through the addition of other management approaches, or through a better understanding of how insect biology can be used to enhance application practices.

Integrated pest management – IPM

Since the 1970s, integrated pest management (IPM) practices have gained momentum, with worldwide success managing pests in multiple cropping systems including soybean (Kogan 1998; Ragsdale et al. 2011; Flint 2012). Although its definition has evolved over the years, the driving concept behind IPM is that it incorporates different methods to manage pest organisms. The definition provided by

Kogan (1998) states that "IPM is a decision support system for the selection and use of pest control tactics, singly or harmoniously coordinated into a management strategy, based on cost/benefit analyses that take into account the interests of and impacts of producers, society, and the environment." Even though the basic definition has been modified over the years, the same underlying concept of IPM remains the same.

The major components associated with implementing and developing an IPM program include: pest identification, field monitoring and population assessment, control or action guidelines, preventative methods, treatment, and management evaluation (Flint 2012; TeyChin and CheongYew 2013). Pest identification is the first important component of an IPM program. Certain insect pest species may be morphologically similar, but biologically different, so careful identification of pests is imperative to selecting proper management practices (Flint 2012; TeyChin and CheongYew 2013). For example, *Ataxia hubbardi* (Fisher) is a Cerambycidae species that utilizes many of the same host plants as *D. texanus*. Even though they have different biology and life histories, the larvae share similar morphological characteristics, which can lead to misidentification and consequently, inappropriate management choices (Michaud and Grant 2005). After the pest has been identified, field monitoring is conducted by consistently and continuously checking the area for the presence of pests throughout the growing season. Often times, sampling can be time consuming for the farmers, especially when there are no established sampling plans (Flint 2012; Greco and Wright 2013), which is the case for *D. texanus*. By developing a sampling plan that takes into account the biology and behavior of the insect pest, famers may sample fields more accurately making better assessment of pest populations in the field. Sampling plans can also assist

in knowing when and where to treat for pests, and when control or action guidelines should be implemented. Control or action guidelines, such as economic thresholds (ET) and economic injury levels (EIL), are indicators for when management actions should occur and is based on information from the systematic monitoring of the plant crop and pest (Pedigo et al. 1986; Higley and Pedigo 1993). The EIL is the lowest population density in which economic damage could result (Pedigo et al. 1986). The ET value is set lower than the EIL and indicates when pest numbers reach a density that warrants the use of control measures in order to prevent the pest numbers from exceeding the EIL (Pedigo et al. 1986; Higley and Pedigo 1993). Studies examining *D. texanus* in soybean often find damage is inconsistent between fields and years, making it difficult to determine an EIL or ET (Buschman and Sloderbeck 2010; Sloderbeck and Buschman 2011). Sloderbeck and Buschman (2011) attempted to identify an ET for *D. texanus* by collecting 5 sets of 20 sweeps within several fields, before and after pesticides were applied. They found that there was no correlation with the number of adult *D. texanus* collected at the beginning of the season to end of season larval infestations, making them unreliable as a trigger for an ET. Further examination of the parameters affecting reliability of ET for managing *D. texanus* is needed to fill in an important knowledge gap in the soybean system.

As previously discussed, there are several types of control methods available including cultural controls (i.e. tillage, crop rotation, etc.), host plant resistance, and chemical controls that can be used in IPM programs for *D. texanus*. The type of control or combination of controls used in an IPM program typically depends on the biology of the target insect, with each method of control having their own limitations (Flint 2012). Finally, after controls have been applied, evaluations conducted during, or at the end of

the season, are used for determining if the treatments were effective and if changes and adjustments to the management plan should be made (Flint 2012). Although IPM tactics have been successfully used by farmers over the years, the adoption of IPM strategies for *D. texanus* is still limited due to significant knowledge gaps around EIL, ET, timing of infestations, and economically sound control methods. Therefore, as an alternative to whole-field IPM, site-specific management programs may be necessary for future *D. texanus* management success.

Site-specific management

Site-specific management is becoming a more widely acknowledged method for managing insect pests (Lowenberg-DeBoer and Swinton 1997; Midgarden et al. 1997; Bongiovanni and Lowenberg-DeBoer 2004). The idea behind site-specific management is that it uses different forms of information technologies in conjunction with traditional methods (sweep sampling and biology) to provide information, both spatial and temporal, on field conditions to aid in making more precise management decisions (Midgarden et al. 1997; Bongiovanni and Lowenberg-DeBoer 2004). With advances in farming technology such as software to collect and analyze site-specific data, global positioning systems (GPS), and electronic monitoring, site-specific management is becoming a more realistic management solution (Lowenberg-DeBoer and Swinton 1997). By incorporating site-specific management strategies, farmers may improve upon already implemented IPM programs while also reducing expenses and pest resistance and increase beneficial insects in the area (Weisz et al. 1996; Lowenberg-DeBoer and Swinton 1997; Midgarden et al. 1997).

Site-specific management strategies have been effective in many cropping systems in both reducing pest infestations and reducing insecticides in the environment that could result in the development of resistance (Weisz et al. 1996; Midgarden et al. 1997; Sciarretta et al. 2011). From 1992-1993, Weisz (1996) conducted a study examining whole-field IPM compared to site-specific management for the Colorado potato beetle (*Leptinotarsa decemlineata*), green peach aphid (*Myzus persicae*), and the potato leafhopper (*Empoasca fabae*) to reduce insecticide inputs in the field. In this study, all fields received the same insecticide treatments, but in the site specific IPM fields they were applied to a targeted area within the field as opposed to the whole-field IPM fields. They found that overall site-specific management was effective at reducing insecticide inputs for management against the Colorado potato beetle and green peach aphid when compared to whole-field IPM (Weisz et al. 1996). In this study, the site-specific management practices reduce insecticides applications for the Colorado potato beetle between 45-70% and ≈70% fewer application for the green peach aphid (Weisz et al. 1996). Midgarden et al. (1997) evaluated site-specific management of Colorado potato beetle to examine the development of insecticide resistance and densities of natural enemies in commercial potato fields. They used site-specific management and standard IPM (treating whole field) strategies for applying insecticides once the pest reached economic threshold (Midgarden et al. 1997). Using laboratory bioassays in pre and post-season examination of the concentration-mortality relationship as the measure of insecticide resistance of the beetle populations treated with the two management strategies, the authors found variation in amount and spatial pattern of selection pressure within both strategies. They found the standard IPM strategy showed a significant

increase in beetle populations from pre to post-season across all fields (n = 3). They also found variation within the site-specific IPM fields, with the majority of the fields showing little change in resistance from pre to post season; however, there was a greater number of parasitoids and general predators found in the areas of the site-specific IPM (Midgarden et al. 1997). The results of the study found that site-specific IPM could slow, but not completely stop, insect resistance, while conserving natural enemies in the environment (Midgarden et al. 1997).

In a production field setting, Sciarretta et al. (2008 and 2011) successfully implemented the use of site-specific IPM to treat and control egg hot spots of European grapevine moth (*Lobesia botrana*) in vineyards. In 2008, they began by investigating the spatio-temporal dynamics of *L. botrana* inside and around vineyards to evaluate the effect of the landscape elements on pest distribution (Pedigo et al. 1986; Sciarretta et al. 2008). This study showed that adult distribution in the experimental areas were aggregated and that through time, the insects dispersed shifting from the olive groves into grapevines with a large proportion of the adult populations remaining outside of the managed areas (Sciarretta et al. 2008). Using these results, they applied a pheromone trap barrier management tactic targeting the movement of male adults from the olive groves into the vineyards (Sciarretta et al. 2011). In doing so, there was a reduction in male hot spots in the olive groves, and with the deployment of additional traps the number of larval nests on vine inflorescences was significantly decreased from 24.2 nests/sample in 2005-2006 to 17.6 nests/sample in 2007-2008 (Sciarretta et al. 2011). When applying site-specific IPM control, treating only egg hot spots, farmers were able to decrease

insecticide treated areas within the vineyard, and consequently, reduced the quantity of insecticide utilized.

Even though site-specific management has been successful in several study systems, this method relies on having knowledge of the spatial distribution and dispersal behavior of the pest with the crop or cropping system, which is not well known for *D. texanus*. With the most effective management options for *D. texanus* (insecticides and tillage), also being the most damaging to the crop system and environment, alternatives to whole field applications such as site-specific management should be explored. Therefore, further studies aimed at examining the *D. texanus* biology (i.e. behavior) within the field and their environmental cues which may serve as predictors to infestation, may provide the farmers with the spatially explicit information needed to successfully implement site-specific IPM strategies for *D. texanus* management.

Insect movement

A primary component of site-specific management is understanding the dispersal capabilities and dispersion patterns of the target pest. Currently there are no formal studies examining the dispersal capabilities or dispersion of *D. texanus* in the field. There have been reports on their flight capabilities based on field observations, which are contradictory. It has been observed that when disturbed adults tend to drop to the ground instead of flying and only move as far as they need to find food, leading to the assumption that they do not undergo long distance dispersal (Hatchett et al. 1975; Michaud and Grant 2005; Michaud 2013). While other reports claim that adults are fairly strong flyers, with the ability to infest soybean fields several miles from their original

location (Buschman and Sloderbeck 2010). Additionally, dispersion patterns for *D. texanus* are relatively unknown, making management decisions, especially site-specific management, difficult for this soybean pest. Insect populations can change rapidly and it is difficult to use dispersal information for population monitoring (Osborne et al. 2001); however techniques have been developed to aide in monitoring and tracking insect dispersal in their natural habitats, which is essential to understanding insect biology, demography, and ethology (Hagler and Jackson 2001).

Monitoring pest movement can be achieved using recent advances in mark-release-recapture and mark-capture techniques (Hagler and Jackson 2001). In mark-release-recapture studies, the insects are initially collected from a laboratory colony or natural habitats, marked within the laboratory, and then released into the environment (Hagler and Jackson 2001). Mark-release-recapture studies apply direct methods that use marking techniques that are directly applied to the insect (e.g. tagging, paints, mutilation, etc.) to monitor movement of individuals (Osborne et al. 2001). Mark-capture studies generally use indirect methods, which uses marking techniques that are applied within natural habitats allowing researchers to relatively inexpensively monitor population movement patterns. Marking is normally conducted by spraying the marker in areas within the field, as in the use of protein markers, or by setting strategically located marker stations in the area of interest (Hagler and Jackson 2001; Hagler et al. 2011). Both mark-release-recapture and mark-capture methods are commonly assessed in time intervals and at different locations in and around the mark or release area to gain knowledge of insect dispersal (Osborne et al. 2001).

Monitoring insect movement using foreign markers has become an increasingly popular strategy over the past couple decades. There are many marking techniques available for mark-release-recapture and mark-capture studies, such as dye marking, pollen marking, genetic marking, tagging, mutilation, paint and ink marking, and dust marking or powders (Hagler and Jackson 2001). Paint and inks were among the first marking materials used on insects (Southwood and Henderson 2000). Typically used in mark-release-recapture studies, paints and inks allow the researcher to mark the insects for identification in any manner (location on insects, color pattern/combination, etc.) needed for the experimental design; however, the paints and inks used must be nontoxic to the insect and not alter the insect behavior. Dye marking is also useful in mark-release-recapture studies for monitoring dispersal across life stages since oil soluble dyes can accumulate in the insect's body fluids or tissues when ingested, retaining inside the insect throughout development. Of all the techniques used, dust marking remains the most common method due to low cost and capability to be used on several different insect species (Southwood and Henderson 2000). The dusts are advantageous because they are visible to the naked eye making it easy to spot in the field and the presence of the powder can be enhanced under UV light. More uncommon, but effective, methods used include mutilation and tagging. These methods are not typically chosen since they require larger bodied insects to accommodate the marker (tag or type of mutilation) and are reserved for long term mark-capture studies aimed to monitor dispersal of individuals. In addition, protein marking, as described by Hagler (1997), is a technique that has proven useful for determining insect movement and patterns in mark-release-recapture and mark-capture studies (Hagler and Jackson 2001; Jones et al. 2006). Originally conducted using

vertebrate immunoglobin proteins, protein markers have since expanded to include other proteins found in soy milk, bovine casein (milk), and egg white (Hagler 1997; Jones et al. 2006). These later protein markers are inexpensive, readily available, and can be applied to naturally occurring insect populations in large areas for monitoring insect dispersal (Hagler 1997; Jones et al. 2006). Using protein markers specifically bovine casein, (milk), and egg white can be more advantageous over other marking techniques for several reasons. First, protein markers have not been shown to have any adverse effects on the dispersal or behavior of the insects. Second, protein markers dry clear, are not visible, and are relatively inexpensive to apply and analyze. This marking method has allowed researchers to collect meaningful information on dispersal and flight capabilities for numerous insect species in many systems. For example, Hagler et al. (2011) used protein markers and fluorescent powders to quantify honey bee dispersal patterns within a commercial alfalfa seed production area in order to identify the extent of pollen-mediated gene flow. By using self-marking devices containing DayGlo™, or a combination of DayGlo™ and powdered protein markers, attached outside apiary exit, Hagler et al. (2011) were able to uniquely mark exiting bees to their apiary of origin. Bees were collected using sweep nets at given intervals and distance from each apiary. Using the markers, Hagler et al (2011) found that on average bees were recovered 800 m from their apiary of origin, allowing them to determine the apiary of origin as well as examine gene flow.

Although there has been no examination of *D. texanus* flight specifically, the flight capabilities of other Cerambycidae species has been examined. Using multiple mass mark-recapture techniques, Smith et al. (2001, 2004) showed that the mean

dispersal for male Asian longhorn beetle (*Anoplophora glabripennis)*, an invasive cerambycid beetle colonizing trees, was approximately 226 m with the dispersal potential of 1.0 to 2.4 km in a season. Gravid females had a mean dispersal of approximately 920 m with the potential to disperse 1.4-2.6 km over a single season (Smith et al. 2001, 2004). In using a marking technique they were not only able to examine the dispersal capabilities of individuals, but also the potential distance the beetle could fly in a given season. Understanding dispersal capabilities in a season would provide valuable information on potential infestation range as well as aid in identifying at risk areas for future infestation.

In regards to within field dispersion, which is unknown for *D. texanus*, many other Cerambycidae species have been found to display aggregated behavior. In Canada, two Cerambycidae species, invasive *Tetropium fuscum* and native *Tetropium cinnamopterum,* both utilize the same host volatiles and male produced pheromones for mating aggregation. Further study indicated that *T.m fuscum* had high spatial association between males and females, while *T. cinnamopterum* although still showing aggregation, had low spatial association between males and females (Rhainds et al. 2010, 2011). Other Cerambycid species, including the red milkweed beetle (*Tetraopes tetrophthalmus*) and Asian cerambycid (*Glenea cantor*), were found to display aggregated distributions throughout their life cycle (Reagel et al. 2002; Lu et al. 2011). As such, *D. texanus* may display similar behavior, which may be further examined through spatial sampling with the aid of marking techniques such as protein markers.

Of the techniques discussed (Hagler et al. 1992, 2009; Jones et al. 2006), it is important to consider the negative effects marking techniques may have on the test

subject. The main consideration when using a marking technique is that the technique must not restrict the insect movement or behavior. When using techniques that require you to add any "color" to an insect, caution should be used. Most insects have adapted their respected color scheme as camouflage from predators and by marking them with bold and/or bright colors the insect may become more susceptible to predation. Marking techniques should not inhibit normal insect dispersal or dispersion, decrease longevity, or increase mortality (Southwood and Henderson 2000). For example, a study by Dickens and Brant (2014) found that marking technique and dye color had an impact on *Aedes aegypti* survival. Fluorescent paints caused higher mortality on males versus females, while DayGlo™ had a significant reduction in overall survivorship of both males and females. Besides survivorship, cross contamination is also important to consider when handling marked individuals, as dusts and powders are easily transferred between surfaces even with minimal contact. With protein marking, a classification system using both negative and positive controls is often required for ruling out insects that had been inadvertently marked or "false-positives" (Hagler et al. 2011; Sivakoff et al. 2011). Even so, protein marking may be a useful tool to quantify the movement of *D. texanus* within soybean fields, providing information on the flight capabilities and dispersal during a growing season. Examining the dispersion of *D. texanus* within soybean fields using this technique could help identify and provide information on "where" and "when" adults are occurring in the field and ultimately provide foundational information needed for effective site-specific management strategies.

Remote sensing for detection of *D. texanus*

Apart from understanding where adults are occurring in the field, being able to identify when the soybean has become infested (early detection) with adult *D. texanus* could provide valuable information for developing site-specific management strategies. Typically, pest detection and damage is based on haphazard, field-level surveys. This may result in inaccurate sampling plans due to incorrect sampling times (e.g., day or month) and methods (e.g., sweep net versus visual examination) used. Field-level surveys can be time-consuming, laborious, and difficult for managing large-acreage farms; however, remote sensing might overcome survey limitations and provide consistent data throughout a growing season and characterize subsequent pest infestations at a field level.

Over the past several decades there has been an increased emphasis on the use of remote sensing platforms to assess agriculture areas in real-time (Hatfield and Pinter, Jr. 1993; Pinter et al. 2003; Huang et al. 2010). Techniques that are commonly used include earth observing satellites, aerial photography, and radar (Riley 1989; Huang et al. 2010). One of the more popular platforms used is satellite imagery. Specifically pictures from the Advanced Very High Resolution Radiometer (AVHRR) on the National Oceanic and Atmospheric Administration (NOAA) polar orbiting meteorological satellite have proven to be advantageous to operational crop conditions and yield assessments (Esquerdo et al. 2011). The value the AVHRR remote sensing platform brings to agriculture includes high temporal resolution (greater chance of cloud free images), ensured collection with worldwide coverage, appropriate resolution for regional scale monitoring (1 km^2 for local area coverage), low cost, real-time availability, and multi-temporal data producing 52 local area coverage (LAC) images each year (Nagol et al. 2009; Esquerdo et al. 2011).

Although the satellite can provide information at a large regional scale, the increasing popularity of SSM has decreased down to seasonal crops representing small land coverage. This makes satellite imagery less advantageous than other remote sensing platforms and the use of aerial imaging and modified cameras more prominent.

Photography is one of the oldest and well–known platforms of remote sensing (Riley 1989). Evolving from film to digital cameras, aerial photography has become a valuable tool for identifying and monitoring pest populations (Pinter et al. 2003), mainly due to its ability to generate high temporal data (measurement with respect to time) while still maintaining appropriate spatial resolution (pixel resolution) (Riley 1989). Recently, use of modified, near infrared (NIR) cameras that take pictures in the blue (400 – 500 nm), green wavelengths (500 – 600 nm), and near-infrared [NIR] band (750 – 950 nm) (Jensen 2007), has increased in use with the rise in small unmanned aircraft systems (UAS) for crop monitoring (Hatfield and Pinter, Jr. 1993; Pinter et al. 2003). As such, the NIR cameras have been used to monitor the growth and health status of many crops like corn (Wallen et al. 1976), wheat (Elliot et al. 2009) , rice (Zhao et al. 2013), and cotton (Lan et al. 2013). This is primarily because the NIR camera is easily operated, relatively inexpensive, and can be used in a range of settings and agriculture systems in conjunction with vegetation indices.

Vegetation indices and vegetation phenology metrics (VPMs)

During active photosynthesis the primary pigment chlorophyll, which is found in the chloroplast along the walls of the parenchyma cells and comprises the mesophyll layer, absorbs light in red and blue regions and reflects light in the NIR region (Gates et al. 1965). Plants that are actively photosynthesizing will absorb more of the visible light

in the red region and reflect more in the NIR; therefore, changes in the spectral properties (i.e., less photosynthetic activity) by healthy and maturing green vegetation is often correlated with plant phenology and stress (Gates et al. 1965; Jensen 2007). The relationship between photosynthesis, red and NIR wavelengths has resulted in the development of numerous vegetation indices and biomass estimating techniques that utilize multiple measurements in the visible and NIR region (Jensen 2007). Such indices include the Normalized Difference Vegetation Index (NDVI) and Green Normalized Difference Vegetation Index (GNDVI). Since the NIR camera is reading the NIR band in place of the red band, the GNDVI is often used to overcome issues of saturation observed in some vegetation types(Cicek et al. 2010). The GNDVI essentially replaces the red band with the green band from the NDVI estimator and has been considered more useful is assessing leaf chlorophyll variability when leaf area index is moderately high (Cicek et al. 2010). The use of vegetation indices to monitor and detect changes in the vegetation and is the basic theoretical idea behind the practice of remote sensing to detect pest populations (Zhao et al. 2013). Using vegetation indices to monitor insects within and around various agriculture crops have been documented in the literature with several studies successfully demonstrated the use of vegetation indices to aid in the detection of insect infestations, insect emergence, and insect habitats (Wallen et al. 1976; Wood et al. 1991; Hatfield and Pinter, Jr. 1993; Maret and Johnson 1999; Ma et al. 2005; Solberg et al. 2007). Depending upon the spectral response indicated by the vegetation indices, specific wavelengths or characteristics can be used to indicate insect damage.

Studies examining the spectral response curves of plant vegetation have been used to identify characteristic wavelengths in order to monitor insect damage. For example,

Ma et al. (2005) used NDVI and the atmospherically resistant vegetation index (ARVI) to assist in early detect of locust outbreaks in areas of East Asia. They were able to show correlations between locusts, reed biomass and LAI, and the AVRI for live instar nymphs and adult locusts (690 nm), areas of severe infestation (700 nm), as well as non-affected areas (730 nm). Lan et al. (2013) also used NDVI to examine the spectral response of spider mite (Acari: Tetranychidae) infestations in cotton to identify when miticide treatments might be needed. In this study, varying mite densities resulted in significantly different spectral signatures between treated and untreated plots (Lan et al. 2013). By identifying differences among treated cotton plants, this could result in development of SSM strategies, reducing costs and quantities of miticide in the environment. There is potential for using vegetation indices to monitor *D. texanus* infestations, but research comparing changes in reflectance patterns of infested and non-infested soybean throughout the season is needed. The values derived from vegetation indices (NDVI or GNDVI) are also used to construct the vegetation phenology metrics (VPMs), which aide in identifying changes in plant health that can be used as cues or indicators for monitoring pest populations.

Vegetation phenological metrics (VPMs), can be acquired through consultants or private companies, and can be used by a variety of stakeholders including individual farmers, government agencies, and private companies to model and predict food production in the world market and model potential agronomic issues such as drought (Jensen 2007). Used in conjunction with vegetation indices, VPMs can detect changes in plant phenology for developing efficient pest management strategies (van Leeuwen et al. 2010; Buma et al. 2013; Senf et al. 2013). In order to construct the VPM the values

derived from the vegetation index calculations are plotted against time. By plotting against time, the VPMs can then be used to compare the length and peak of different vegetation seasons within specified locations of different land cover types (van Leeuwen et al. 2010; Buma et al. 2013; Senf et al. 2013). The slopes generated in the VPMs monitor plant development and determine the length of time taken to reach maximum NDVI and full senescence. The changes in plant phenology (rate of green up and rate of senescence) seen in the VPMs can be attributed to disturbances to the vegetation canopy, which may include arthropod pests present in the field (Buma et al. 2013). Since the VPMs map the rate of green up and rate of senescence of vegetation, using vegetation index values, the length of the season and ecology of the target pest can be monitored throughout the season (Buma et al. 2013). This includes pest colonization, vegetation type, the rate of infestation, and shift to a new host (van Leeuwen et al. 2010). When inspecting insect damage, Eklundh et al. (2009) too used MODIS images and their derived VPMs to examine their use for detecting defoliation in Scots pine due to the pine sawfly (*Neodiprion sertifer*). They found that in using MODIS images, they were able to locate insect damage, but could not estimate the intensity of the insect damage (Eklundh et al. 2009).

Most pest damage detected using vegetation indices and VPMs is related to changes in plant health. However, changes in plant health can be caused by other factors (e.g. drought stress, disease, soil nutrition, etc.), apart from insect damage, which is one of the main limitations for their use in detecting insect damage (Riley 1989; Hatfield and Pinter, Jr. 1993; Pinter et al. 2003). Therefore, ground-based data and observations should be conducted to aid in determining difference between insect damage and other

forms of damage (Riley 1989). For soybeans infested with *D. texanus*, a change in reflectance values may be attributed to the infestation, which could be further verified through ground based data and observations. Furthermore, VPMs are limited by the spatial resolution of images, making it difficult to determine insect damage from other types of stresses in the plants. Such limitations have been best documented while attempting to monitor insect outbreaks in the Rocky Mountain forests using phenological and leaf area index (LAI) trends using images taken from MODIS (Buma et al. 2013). The insect damage (mainly mountain pine beetle (*Dendroctonus ponderosae*), spruce beetle (*Dendroctonus rufipennis*), and engraver beetle (*Ips spp*.)) was expected to alter observed phenological trends. However, there was no significant effect to the canopy by insects, suggesting phenological trends may be insensitive to disturbances due to pests when examined on a large scale. Fortunately, this issue may be overcome by collecting data more frequently using aerial imaging with a modified camera (for NDVI) as it allows for better spatial resolution, and high temporal data needed to track plant phenology more accurately to detect of pest populations (Buma et al. 2013). Additionally, potential disadvantages of using remote sensing to detect insect infestation lies within the methods used to acquire the images. The camera may be used in multiple methods (hand held, drone, retractable camera mount etc.) all of which allow for the high temporal and spatial data needed to monitor pests. However, each method may affect spectral readings within the same area for similar vegetation. This may occur because the images may be taken at different elevations or angles in relation to the plants, which could create inconsistency in the derived data, resulting in false representations of phenological trends and stress. Another disadvantage of the NDVI camera is that it only reads in two visible

wavelengths (blue and green) and near infrared (NIR) wavelengths. This limits the spectral view and potential characteristic wavelengths that may be needed to identify pest populations between 900-2500 nm. Fortunately, limitations of this platform may be overcome through improved data analysis and interpretation on existing methods.

Research Objectives

There is a need to update current management recommendations (tillage, crop rotation, catch crop, and insecticide application) for treating stem borer adults and larvae in IPM programs (Campbell and van Duyn 1977; Sloderbeck and Buschman 2011). Several knowledge gaps about where and when *D. texanus* are occurring in the field, both adults and larvae, exist and need to be addressed prior to implementation of new or existing management strategies. For example, understanding dispersal capability of *D. texanus* within the field and resulting dispersion patterns may provide information for implementing site-specific IPM programs. Although site-specific management will not eliminate the use of insecticides entirely, this management strategy may help conserve beneficial insects (e.g., pollinators and natural enemies) within Kansas soybean fields.

The major goal of this research was to examine and improve our understanding of the biology and behavior of *D. texanus* as well as the soybean plant responses to infestation. Concurrently, we also wanted to evaluate the potential success of site-specific pest management practices for *D. texanus* in Kansas soybean production fields. The two objectives of my research were to:

1) identify colonization patterns, and trends in populations of *D. texanus* during the soybean growing season;

2) examine host plant response as a predictor to *D. texanus* infestation by quantifying changes in soybean phenology during the growing season.

The fundamental information gained from monitoring *D. texanus* populations will lead to a better understanding of how the technology can be used to track pest species infesting soybean fields, the rate of dispersal within a given field, where pests are most likely to reside, and how to properly manage pests over time.

In chapters two and three, my objective was to understand "when" and "where" *D. texanus* were occurring within soybean fields. Specifically, my objective for chapter two was to understand *D. texanus* colonization during the growing season, while chapter three examines adult dispersal capabilities. By examining the spatial distributions and dispersal of *D. texanus* within soybean production fields, we can provide the spatio-temporal information needed for the development of site-specific management practices. Lastly, the objective of chapter four was to assess host plant response for use as predictors of *D. texanus* infestations. If a correlation can be made between *D. texanus* infestation and host plant, it may be possible to make quicker assessments of infestations for timely application of control measures. Producers may be willing to adopt improved strategies for *D. texanus* in Kansas soybean production fields if we are able to increase their effectiveness, and/or save them time and profits.

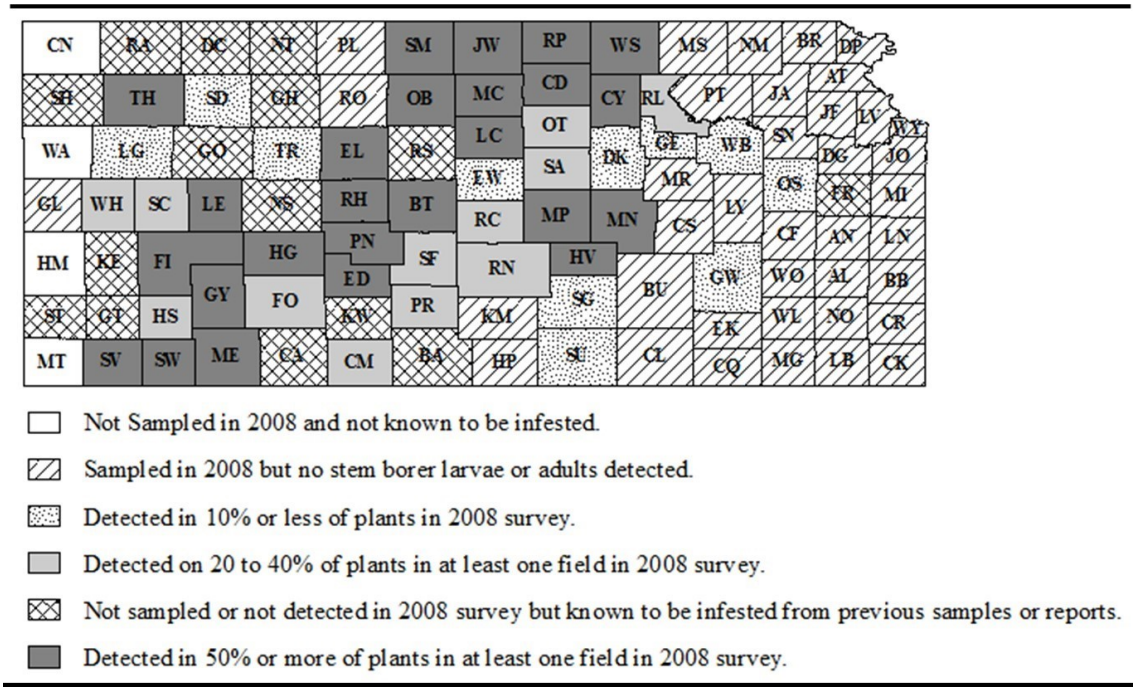

Figure 1.1. Results from a 2008 survey conducted by Buschman and Sloderbeck (2010) on the severity of *Dectes texanus* infestations by county in Kansas. The dark gray counties had high infestations (≤50% plants infested); stippled counties had significant infestations (20 to 40% of plants infested); light gray counties had low levels of *D. texanus* (>20%); black cross hatched counties were either not sampled or were not found to be infested in the 2008 survey, but are known to be infested from previous observations; black diagonally striped counties had no stem borers detected in the in the 2008 survey (and there is no history of infestations); and white counties were not sampled in 2008 and have no history of *D. texanus* infestation.

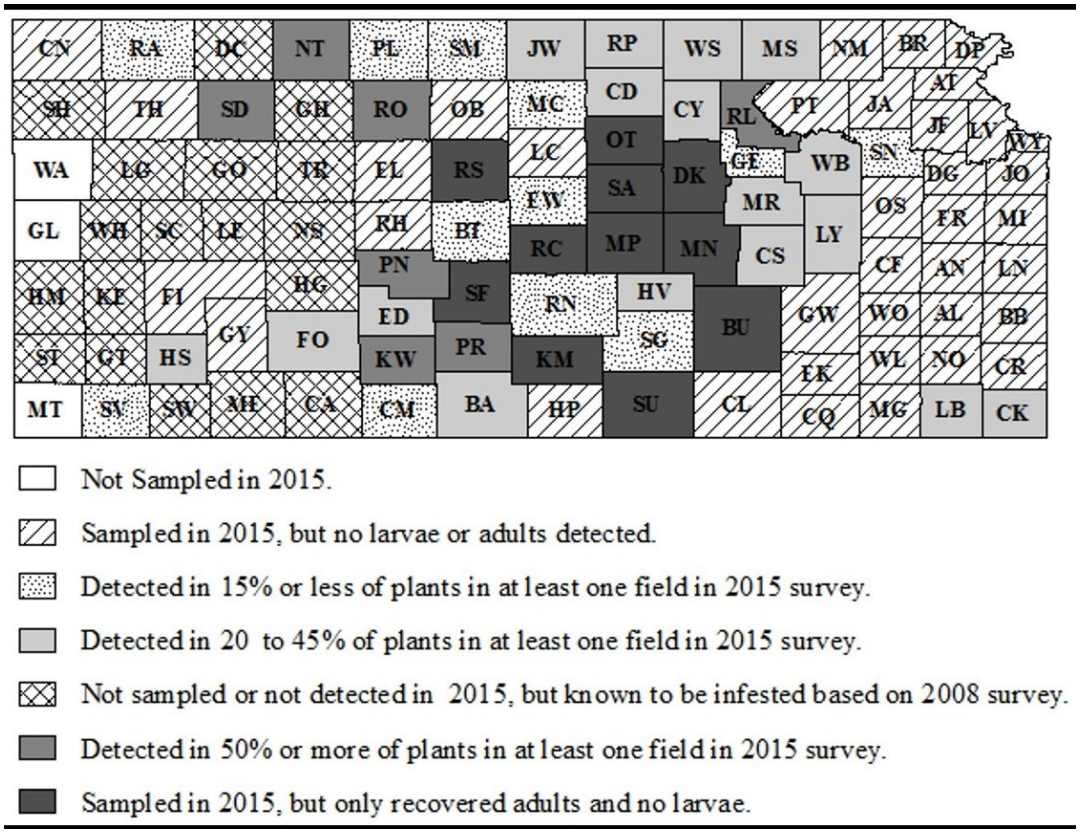

Figure 1.2. Results from a 2015 survey conducted on the severity of *Dectes texanus* infestations by county in Kansas. The medium gray counties had high infestations (≤50% plants infested); light gray counties had significant infestations (20 to 45% of plants infested); stippled counties had low levels of *D. texanus* (>15%); black cross hatched counties were either not sampled or were not found to be infested in the 2015 survey, but are known to be infested based on the 2008 survey (Buschman and Sloderbeck 2010); diagonally striped counties had no stem borers detected in the in the 2015 survey (and there is no history of infestations); dark gray counties were sampled for both adult and larvae *D. texanus*, but only adults were recovered; and white counties were not sampled in 2015 and have no history of *D. texanus* infestation.

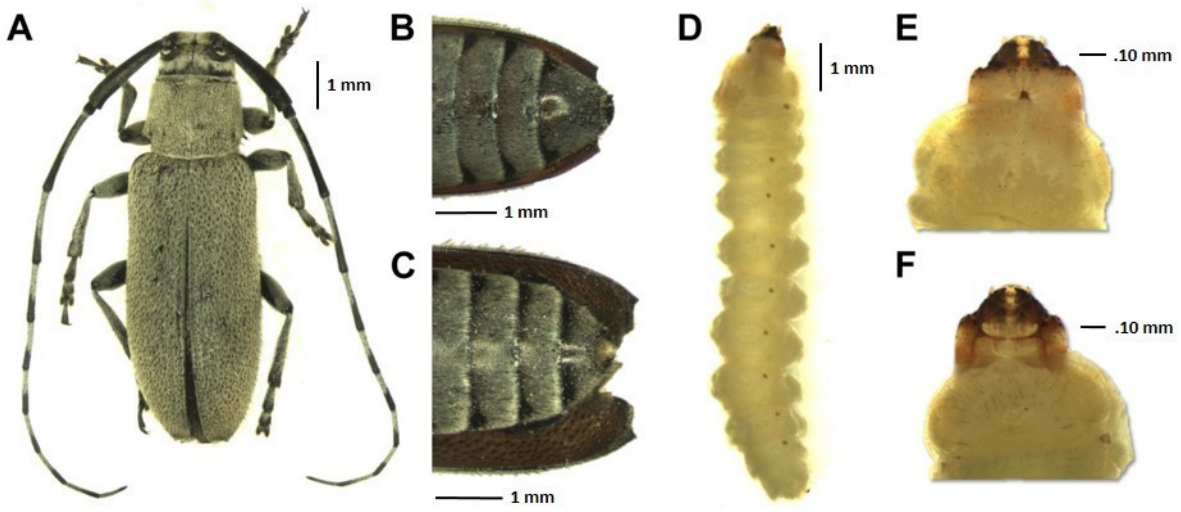

Figure 1.3. Key morphological features for *Dectes texanus* including: A) dorsal view of female adult; ventral view of a B) female adult where last sternal segment is pointed and elongated while the C) male segment terminates abruptly; lateral view of a D) late-instar stem borer; and E) ventral and F) dorsal views of a larval head capsule. Scales were adjusted to appropriately represent the actual size of *D. texanus* adults and larvae. The base of the scale was taken from the median reported size of *D. texanus* adults and larvae as documented by Hatchett et al. (1975).

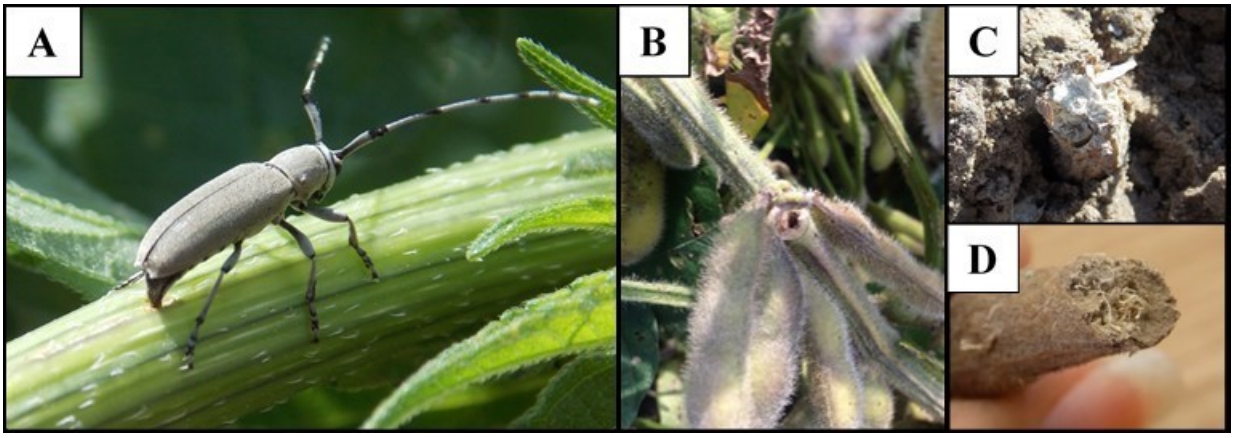

Figure 1.4. A) Female *D. texanus* ovipositing an egg into the pith of a ragweed petiole; B) entrance hole in the main soybean stem resulting from a larva feeding from the petiole into the main stem; C) girdled stem of a mature soybean plant 5 cm above the soil line; and D) a frass plug produced by a late instar in preparation of overwintering.

Chapter 2 - Within field spatiotemporal distribution of *Dectes texanus* (Coleoptera: Cerambycidae) adults and larvae in Kansas soybean (*Glycine max* L.)

Introduction

The soybean stem borer, Dectes texanus (LeConte), is native to North America and can utilize several native host plants within the Asteraceae family including ragweed (*Ambrosia artemisiifolia* and *A. trifida*), native sunflower (*Helianthus annuus*), and cocklebur (*Xanthium strumarium*) (Patrick 1973, Rogers 1985) for adult and larval feeding, as well as oviposition. The spread of *D. texanus* is likely attributed to the diversity and recent expansion to soybean (Glycine max L.), a non-native host plant (Michaud and Grant 2005, Buschman and Sloderbeck 2010) that is a major row-crop in the US. Soybean was first reported as a host for *D. texanus* during the late 1960's in North Carolina (Falter 1969). Since then, *D. texanus* has been reported in soybean-producing regions of eastern, southern, and central US (Falter 1969, Patrick 1973, Laster et al. 1981, Michaud and Grant 2005, 2009, Buschman and Sloderbeck 2010, Tindall et al. 2010). *D. texanus* were first observed in five south-central KS counties (Edwards, Barton, Kiowa, Ford, and Pawnee counties) in 1985, which later expanded to 41 counties by 2008 (Buschman and Sloderbeck 2010) (see Chapter 1, Fig. 1.1). In 2015, the number of counties reporting the presence of *D. texanus* larvae, adults, or both stages within soybean fields increased to 55 counties (see Chapter 1, Fig. 1.2). The distribution and

frequency within Kansas soybean production fields continues to rise, but the primary biological factors contributing to this expansion are largely unknown.

The lifecycle of *D. texanus* is well known (Patrick 1973; Laster 1981; Hatchet et al. 1975; Michaud and Grant 2005; Niide 2009; Buschman and Sloderbeck 2010). In Kansas, *D. texanus* are reported to emerge in late June, with adult activity peaking in early- to mid-July, then decline by late August (Sloderbeck et al. 2004, Sloderbeck and Buschman 2011). Post emergence, adults immediately begin to mate and deposit eggs into the pith of soybean petioles. Upon hatching, the early instars tunnel and feed on pith tissue and move into the main stem where larvae continue to feed until late third instar. Larvae developing in the main stem can decrease the physiological seed weight by 7-11%; one larva per plant has the potential to cause up to 10% yield loss (Richardson 1975, Buschman et al. 2005). Current physiological loss estimates are inconsistent (Buschman et al. 2005, 2007), which can be attributed to various biotic and abiotic factors governing larval growth and survival, as well as the nature of soybean to compensate under adverse conditions. Larvae disperse to the base of the soybean stem before the plant fully senesces, where a single surviving larva girdles the soybean plant approximately 5 cm above the soil line. The behavior provides the larva with a protective overwintering chamber by preventing conspecifics from reaching the base of the stem. In fields that are nearly 100% infested with larvae, girdling and subsequent lodging of mature soybean plants results in yield losses estimated up to 16.8% (Daugherty and Jackson 1969). Although loss from physiological (i.e., indirect feeding of non-seed tissue) and mechanical (i.e., harvestability) is variable, soybean growers need viable management strategies to mitigate losses caused by this annual pest.

Managing *D. texanus* is a challenge as control strategies to reduce larval densities are limited. This is primarily due to the feeding behavior of *D. texanus* larvae, which tunnel into the main soybean stem during late vegetative (V) to late reproductive (R) growth stages (Fehr et al. 1971; Sloderbeck and Buschman 2011). Current management options for Kansas growers target larval and adult growth stages and can include cultural (crop rotation, tillage, sunflower catch crops, and chemical (i.e. insecticide) control tactics. However, several practices are simply outdated or no longer effective. For example, tilling infested stubble containing overwintering pupae reduces adult emergence by ≥ 15% under certain field conditions, but this practice is not compatible with modern, no-tillage, soil conservation efforts (Campbell and van Duyn 1977, Sloderbeck and Buschman 2011). Conversely, a study found that properly timed insecticide applications can be beneficial for reducing adult *D. texanus* and consequently, reduce egg-laying and overall field infestation levels (Sloderbeck and Buschman 2011). After the three-year study, the authors concluded that aerial insecticide (lambda-cyhalothrin) applications resulted in a reduction (ranging from 0 – 89% for application one or two) in season-long beetle populations; however, the combined use of two insecticide applications showed a 74, 89, and 98% control in season-long beetle populations in 2001, 2002, and 2003, respectively. Sloderbeck and Buschman (2011) recommend that insecticides would be most effective when applied during peak adult flight followed by a second application 10 days later. However, the implementation of this strategy by growers may be limited; in addition to determining peak activity, the decision to treat an entire soybean field twice with an insecticide to control *D. texanus* also depends on the cost effectiveness of available insecticides and associated application costs (i.e., labor and equipment needs).

Site-specific management methods are effective in several agriculture systems for managing insect pests (Weisz et al. 1996, Lowenberg-DeBoer and Swinton 1997, Midgarden et al. 1997, Bongiovanni and Lowenberg-DeBoer 2004, Sciarretta et al. 2008, 2011). Site-specific management strategies use different forms of information technologies (remote sensing, global positioning systems, software, etc.) in conjunction with traditional methods (sweep sampling and arthropod biology) to provide data, both spatial and temporal, on field conditions for more precise management decisions (Lowenberg-DeBoer and Swinton 1997, Midgarden et al. 1997, Bongiovanni and Lowenberg-DeBoer 2004). Site-specific management has been effective for many cropping systems for reducing pest infestations and overall insecticides use, benefiting the environment and reducing the risk of developing insecticide resistance (Weisz et al. 1996, Midgarden et al. 1997, Sciarretta et al. 2011). For example, Weisz (1996) conducted a study from 1992-1993 examining whole-field compared to site-specific insecticide treatments for the Colorado potato beetle (*Leptinotarsa decemlineata*), green peach aphid (*Myzus persicae*), and the potato leafhopper (*Empoasca fabae*) to reduce insecticide inputs in the field. They found that site-specific treatments were more effective at reducing insecticide inputs and expenses, due to less acreage treated, for management against the Colorado potato beetle and green peach aphid when compared to a whole-field application (Weisz et al. 1996). Although the site-specific treatments had the same level of control as whole-field, they were able to apply 45-70% less insecticide for the Colorado potato beetle and ≈ 70% less insecticide for the green peach aphid in site-specific treatments (Weisz et al. 1996). In another study, Midgarden et al. (1997) evaluated site-specific management of Colorado potato beetle to examine its impact on

insecticide resistance and densities of natural enemies in commercial potato fields. They successfully demonstrated that site-specific management could slow the development of resistance to insecticides, while conserving natural enemies in the environment (Midgarden et al. 1997). In the previously described studies, the successful implementation of site-specific control tactic was dependent on characterizing the distribution and timeframe of the pest within the field, which was determined through sampling and monitoring of the pests. For successful deployment of site-specific tactics, knowledge of the spatial distribution of the pest within a cropping system is needed.

The spatial distribution of *D. texanus* within soybean fields is unknown. There are other Cerambycid species, such as *Glenea cantor* and *Tetraopes tetrophthalmus* (red milkweed beetle), that display aggregation during different stages in their lifecycle (Reagel et al. 2002, Lu et al. 2011). Lu et al. (2011) investigated host selection and colonization of *G. cantor* in kapok tree stands (*Bombax ceiba* L. = *Gossampinus malabaricus* (DC.) Merr.). They determined that females preferred to oviposit on weakened trees in areas with lower bark moisture content, resulting in aggregated egg and larval densities. Alternatively, Reagal et al. (2002) found that adult red milkweed beetles aggregated on individual stems within patches of common milkweed (*Asclepias syriaca* L.), which was dependent on host plant cues and specific male:female sex ratios. Based on these examples, it is plausible that *D. texanus* may aggregate during certain life stages. If so, information on when and where aggregation occurs in the field is needed. As previously mentioned, in Kansas *D. texanus* emerge in late June and reach peak populations in early to mid-July; although this provides a time frame when *D. texanus* can be found in the field, the distribution patterns during this time is unknown. *D texanus*

have been observed along the edges of soybean fields that neighbor previous years soybean crops and/or near 'weedy' edges (i.e., unmanaged field boarders and ditches) containing potential native hosts (Campbell 1980; Rystrom 2015). If we can determine that *D. texanus* aggregate, and when and where aggregation occurs during the growing season, then site-specific insecticide applications may be obtainable.

Our goal was to examine *D. texanus* distribution within soybean production fields. The objectives of this study were to: 1) monitor adult *D. texanus* activity within soybean fields to determine if *D. texanus* adults and/or larvae are aggregated within the field, and if so, 2) identify when aggregation occurs during the growing season. Based on the behavior of other Cerambycid species, we predicted that *D. texanus* would display aggregation during the adult life stages, consequently leading to aggregation in larvae. I hypothesized that *D. texanus* adults may aggregate on field edges, since they are not known to disperse great distances and edge effects have been observed in other studies (Buschman and Sloderbeck 2010, Campbell, 1980, Rystrom 2015).

Methods and Materials

Study sites

Spatial sampling was conducted in years 2012, 2013 and 2014, and included 8 soybean production fields in north central Kansas (Table 1). Field 1 (2012) and field 6 (2013) was the same field but sampled two years in a row; this was the only field in the study that was continuously planted to soybean, which had infested stubble already present in the field at the start of the study. Field selection was based on prior knowledge of *D. texanus* infestations and on presence of adults from pre-scouting events (sweep

sampling and stubble examination) in early June of each study year. Fields selected for sampling were separated by ≥ 5 km, with the exception of 2014; here, the fields were approximately 3 km apart from each other. Fields 3 and 4 in 2013 were dryland, whereas the remaining fields were flood irrigated as needed. All fields were planted to a 76.2 cm (30") row-spacing, except for field 4 in 2013, which was planted with a drill at a 25.4 cm (10") row-spacing. Seed variety and maturity group for the selected production fields were determined by each farmer and were not the same across fields (Table 1); therefore, each field was analyzed separately.

For each study field, a Trimble® Recon® handheld computer system (PN: 790-0025-XXQ, Trimble®, Trimble Navigation Limited: Sunnyvale, CA) connected to a Pathfinder ProXT™ (PN: 52240-20, Trimble®, GPS Pathfinder® Pro Series, Trimble Navigation Limited: Sunnyvale, CA) satellite receiver was used to trace the perimeter of each field using ArcPad® (ArcPad® V7.1.1., ESRI Inc.: Redlands, CA). Each perimeter was saved as a polygon and was downloaded into ArcMap™ (ArcGIS® V10.2, ESRI Inc.: Redlands, CA) to produce a uniformly spaced sampling grid. Grids consisted of rows of uniformly spaced sample points (25 × 25 m in 2012; and 35 × 35 m in 2013 and 2014), with the size and shape of the field determining the number of sample points (Table 2.1; Fig. 2.1A). The sample grid was then loaded onto the handheld computer system and imported into ArcPad ® V7.1.1 (ESRI Inc., Redlands, CA). The handheld GPS computer and receiver were used to navigate to each pre-determined sample point using sub-meter accuracy. Spacing between the sample points was increased in 2013 and 2014 to reduce the number of sample points within a field. This decreased the overall sampling time per field, which allowed us to include more fields in the study. Similar sampling designs have

been used in other studies to quantify the spatial distribution of other economically important pests (Boiteau 2005, Park and Tollefson 2006, Seiter et al. 2013, Reay-Jones 2014).

Sampling *D. texanus* adults and larvae

Sampling was initiated on 18 June 2012, 1 - 4 July 2013, and 24 - 26 June 2014; typically, when the soybean plants had reached V3. Sampling was postponed to this stage because sweep sampling can damage developing soybean plants. Adult *D. texanus* were sampled 1-2 times per week throughout the growing season to determine temporal and spatial activity patterns for adults in the field (Crook et al. 2004; Buschman and Sloderbeck 2010; Tindall et al. 2010); sampling ceased once adult activity decreased near zero per a set number of sweeps or were no longer found within a field. All adult *D. texanus* were collected using 38-cm sweep nets (BioQuip Products, Rancho Dominguez, CA). A total of 20 sweeps were collected in each cardinal direction (north, south, east, west) at a given sample point (80 total sweeps per sample point) within a field (Fig. 2.2B). Adult *D. texanus* collected were recorded for each sample point and then individually placed in 946 ml plastic bags (quart freezer bags, Great Value, Wal-Mart Stores, Inc., Bentonville, AR), transported back to the lab, and stored at -18°C for use in other experiments (see Chapter 3).

Dectes texanus larvae are found within the main soybean stem at the end of the growing season. To relate adult aggregation patterns with larval patterns across production fields, we collected whole plant samples prior to harvest to record presence or absence of larvae at all sample points within a field. Larval collection was conducted in fields 1-6 (2012 and 2013). Fields 7 and 8 (2014) were not sampled for larvae due to

limitations in labor and time. At each waypoint, 1-m row of whole soybean plants (including roots) were removed from the field (Fig. 3C), placed in 4.4-L paper refuse bags (Wal-Mart Stores, Inc.: Bentonville, AR), and transported back to the laboratory. Each plant collected was sliced down the middle of the main stem and examined for the presence or absence of *D. texanus* larvae and total number of larvae per plant was recorded.

Statistical analysis

Each field was analyzed separately, since soybean variety and maturity group varied between production fields. Changes in adult activity (mean number of adults collected per sample point) during the growing season for all 8 soybean fields were subject to an analysis of variance (ANOVA) with repeated measures (PROC GLM, SAS® version 9.4, SAS Institute Inc.: Cary, NC). The fixed effects were sample point, sample date, and the interaction of sample point and sample time. The sample point was analyzed as the repeated measure; autoregressive (1) (AR(1)) covariance structure was used to model the error structure of the repeated measures, which was selected based on lowest AIC value. Any significant interactions among the fixed effects were further analyzed using the adjusted Tukey Kramer method in the LS Means statement and determined significant at $\alpha = 0.05$.

To model the spatial aggregation of adults and larvae within a field, count data collected in reference to sample grids were analyzed using Spatial Analysis by Distance IndicEs (SADIE) red-blue methodology following Pilkay (2015), which was originally described by Perry et al. (1999). There were several sample dates in all three years that were excluded from analysis, because data did not meet criteria to perform aggregation or

spatial association analyses due to low counts or incomplete sampling of the field because of inclement weather (Table 2). To determine if adults and larvae aggregate in soybean, SADIE analyses provided an overall index of dispersion, I_a, where: $I_a > 1$ describes aggregated beetle counts; $I_a = 1$ equates to randomly arranged beetle counts; or $I_a < 1$, represents a uniform distribution of beetle counts across a defined space. Spatial randomness of the overall index of dispersion was rejected in this study for $P < 0.025$ (indicating aggregation) or $P > 0.975$ (indicating uniformity). To quantify the degree of clustering (aggregation) occurring, clustering indices were computed for all locations using adult and larval count data for all waypoints sampled within each field. Cluster indices provided by SADIE are indicated by positive ($\bar{v}_i$) and negative ($\bar{v}_j$) values for sampling locations with observed counts above and below the mean, respectively. These indices measure the degree to which the sampling unit contributes to a patch ($\bar{v}_i > 1.5$) or a gap ($\bar{v}_j < -1.5$) and are significant when P-values are < 0.025 or > 0.975, respectively. The mean of the two clustering indices ($\bar{v}_I$ and $\bar{v}_j$) can also be calculated and using the associated probabilities (P_i and P_j); the statistical significance of these indices can be tested against the null hypothesis of random distribution. Here, a patch is defined as areas that share similar densities and a gap is defined as areas that do not have similar densities.

To model relationships between larval and adult densities, we used linear regressions and SADIE indices of association. First, simple linear regression models were determined using the MASS package lm() function (RStudio© version 0.99.3441, The R Foundation, Vienna, Austria) to identify any relationships between adult and larval densities. Next, we calculated the index of association (X) using SADIE to determine the degree of spatial association between *D. texanus* larvae collected at the end of the 2012

and 2013 growing seasons and each sample date of adults for fields 1 through 6; recall, no larvae were collected in 2014. Positive associations between two variables indicate a patch (adults and larvae found in the same areas) or gap (adults and larvae found in different areas) for both variables where $X > 0$ and $P < 0.025$. Conversely, a negative association indicates a patch of one variable and a gap of another where $X < 0$ and $P > 0.975$ (Perry 1997, Pilkay et al. 2015). The location of patches and gaps cluster indices from respective fields were mapped using Surfer®13 (Golden Software, LLC, Surfer® V 13.1: Golden, CO), where the Kriging option was used to interpolate contours (Thomas et al. 2001, Ferguson et al. 2006). Kriging, which is a method used to estimate values for unmeasured variables at locations using the observed values at surrounding locations, allowed us to create a continuous surface where areas within the same contours were spatially associated or disassociated.

Results

Adult *D. texanus* activity

Adult *D. texanus* were recovered from all fields sampled during 2012, 2013, and 2014 (Table 2.2; Fig. 2.2). There were significant differences in the mean number of *D. texanus* collected per 80 sweeps between sample dates for all fields sampled in all years (Table 2.3). There were no consistent patterns in the timing of the peaks of activity among the years, or fields within years (Fig. 2.2). In 2012, adults were collected as early as 18 June in both fields 1 and 2, with peaks in adult activity occurring in first half of July (Fig. 2.2A, B). In 2013, adults were collected within the first week of July for all 4 fields, with levels of activity and periods of activity varying considerably among the fields

(Table 2.2; Fig. 2.2C-F). The last date *D. texanus* adults were observed in 2013 ranged from 14 August to 1 September, depending on field. In 2014, adults were first observed on 1 July, and peak periods of activity occurred throughout July and declined at beginning of August (Table 2.2; Fig. 2.2F-G). The last date *D. texanus* were collected was 13 August for fields 7 and 8.

Adult and larval aggregation

The spatial distribution of adults was random for most samples from the fields across all three years, but on 13 out of 46 sample dates (28.3%) beetles were significantly aggregated based on a significant aggregation index (I_a) from the SADIE analysis (Table 2.2). For field 1, sampled in 2012, *D. texanus* adult populations were aggregated on 29 June and 13 July, but with clustering indices significant only on 29 June (Fig. 2.3). Larvae collected at the end of the season were also aggregated and with clustering indices that were significant (Table 2.2; Fig. 2.3H). There was some overlap in distribution of the clusters between the 29 June adult sample and the larvae sample observable as a band across the field just east of the center line (Fig. 2.35). For field 2, also sampled in 2012, adults were only aggregated on 6 July, with significant clustering indices, and a significant patch on the southwest edge of the field (Fig. 2.4). Larvae in field 2 were randomly distributed (Table 2.4; Fig. 2.4). In field 3, sampled in 2013, adults were aggregated only at 8 July, with significant clustering indices, and had relatively small patches along the northwest edge of the field (Table 2.2; Fig. 2.5). All remaining sample dates for *D. texanus* adults, and the distribution of larvae, were random in distribution (Table 2.2 and 2.4; Fig. 2.5). For field 4, sampled in 2013, adults had a significant aggregation index only on 24 July, but neither the patch nor gap clustering indices were

significant (Table 2.2; Fig. 2.68). The larvae from field 4 were also aggregated, with significant cluster indices, with small patches within the field (Table 2.4; Fig. 2.6). Both larvae and adults on 24 July were primarily recovered along one edge of the field. For field 5, sampled in 2013, the aggregation and cluster indices were not significant on any adult and larvae sample dates (Table 2.2 and 2.4; Fig. 2.7). In field 6, sampled in 2013, adults were aggregated only on 12 July with significant patch and gap cluster indices (Table 2.2; Fig. 2.8), but larvae collected at the end of the season were not aggregated and had no significant patches or gaps (Table 2.4; Fig. 2.8).

Fields 7 and 8, sampled in 2014, had approximately 3 times as many sample dates as the previous 6 fields, but no larval samples were collected at the end of the season (Table 2.2). Field 7 had five out of 10 sample dates where adults were aggregated (Fig. 2.9); 8 July, 11 July, 16 July, 21 July, and 31 July. Significant patch cluster index was identified only on 16 July, but gap cluster indices were significant on multiple dates (Table 2.2). Overall, areas of the field with higher adult captures were relatively small and variable among sample dates (Fig. 2.9). In Field 8, sampled in 2014, aggregation of adults was found on 16 July and 13 August (Fig. 2.10); but only the 13 August sample date had a significant patch cluster index and no date had significant gap cluster indices (Table 2.2). In contrast to Field 7, patches with *D. texanus* adult activity appeared to build, spread, and contract from a more central location within the field and tended to be larger in coverage.

Overall, *D. texanus* were randomly distributed across most of the sample dates within all fields sampled. When there was uniformity in distribution, it was typically during the first sampling dates (1 - 17 July) or at the end of the growing season (13

August). Aggregation patterns varied within each field and year, but tended to be aggregated in late-June to mid-July timeframe. Only on two occasions did aggregation occur later in the season, which occurred at the end of July and mid-August (Fig. 2.9I; Fig. 2.10K).

Spatial associations between adults and larval counts

Linear regressions indicated that only one field and sample date had a significant relationship between adults and larvae, which was field 3 sampled for adults on 17 July ($R^2 = 0.078$, $F = 4.90_{1,58}$, $P = 0.031$) (Table 2.5). Spatial associations between adult captures and soybean infested with larvae were generally not significant (Table 2.6, Fig. 2.11 - 2.16). In field 1, the 18 June and 29 June sample dates had significant positive associations between areas of field where adults and larvae were recovered (Table 2.6; Fig. 2.11). In field 3, there was also a positive association between adult collection on 17 July and larvae (Table 2.6, Fig. 2.13). The only other significant associations were negative ones between adults collected in field 4 on 17 July and larvae (Table 2.6; Fig. 2.14), and between adults collected in field 6 on 24 July and larvae (Table 2.6; Fig. 2.16).

Additionally, as field 1 and field 6 are the same field but sampled in consecutive years we used the spatial association index to compare larvae collected in 2012 (field 1) to adults collected in 2013 (field 6). The results indicated a positive spatial association with the larvae collected from field 1 in 2012 and the adults collected from field 6 on 2 July 2013 ($X = 0.42$, $P = 0.0001$) and 12 July 2013 ($X = 0.43$, $P = 0.0003$).

Discussion

We examined the within field spatial distribution of adult and larval *D. texanus* populations for the first time in Kansas soybean fields. *Dectes texanus* adult activity peaked in late-June to mid-July with several fields having a prolonged time period of high adult activity. *Dectes texanus* adult and larval populations were sometimes aggregated at distinct times during the growing season, but there was not a consistent pattern either within fields over time or among fields. Also, while associations both positive and negative did occur between patches where adults were collected and subsequently where larvae were collected, this was also not consistent. To our knowledge this is the first study evaluating patterns of aggregation of adult and larval stages of *D. texanus* in soybean production fields.

Similar to findings in other studies (Hatchett et al. 1975, Rystrom 2015), we found that peak adult activity occurred in a majority of our sample fields from the beginning to middle of July. Contrary to Hatchett et al. (1975), where they observed two distinct peaks in adult activity with the first in early July and the second in early August; whereas, the majority of our fields had a single peak during July (ranging from 5 and 31 July, depending on field). The peak number of beetles captured, and the duration of activity, varied considerably among years and fields. Interestingly, our study also found that several fields had extended periods of high adult activity, specifically occurring from 11– 31 July. This extended peak in high activity was also similar to a study by Rystrom (2015), which found peak densities occurring specifically between 1,419 (11 July) and 2,019 (24 July) cumulative degree-days (CDD). The similarities also suggest that degree-day models could be incorporated into sampling plans for *D. texanus*, allowing farmers to

apply foliar insecticides to target adults, such as those recommended by Sloderbeck and Buschman (2011), during times of high adult activity. Further examination of *D. texanus* emergence times in Kansas soybean and the use of accumulated degree-day models requires further investigation.

Aggregation was typically observed before or after peak activity, with fields 7 and 8, the only fields to have at least one day of aggregation occur during the observed peak activity time frame. There are many Coleoptera families with species that aggregate under field conditions, including Meloidae (Snead and Alcock 1985), Carabidae (Thomas et al. 2001), Cerambycidae (Reagel et al. 2002, Rhainds et al. 2011), Chrysomelidae (Park and Tollefson 2006), and Coccinellidae (Rahman 2010). Species aggregate for several reasons, including aggregated resources, such as food and mates (Reagel et al. 2002), distribution of resources (Smith and McSorley 2000; Harmon et al. 2003) and improved defense against predators, due to increased vigilance and defense provided by the group (Sillen-Tullberg and Leimar 1988). With *D. texanus,* aggregation patterns may be due to mating behaviors since *D. texanus* do not rely on long-range sex pheromones for mate location (Crook et al. 2004); therefore, adult aggregation would likely be necessary for successful mating.

Aggregations can also occur based on where beetles enter the fields such as from adjacent areas or where they overwinter within a field. Clustering of adults along edges was similar to observations made by Campbell (1980) and Rystrom (2015). Campbell (1980) observed that higher *D. texanus* densities were located near the previous year's soybean crops and edges that were "weedy." Since larvae overwinter in soybean stubble from the previous year (Hatchett et al. 1975, Rogers 1985, Michaud and Grant 2005,

Buschman and Sloderbeck 2010), adults will seek out new hosts after emergence, which may explain why aggregation was observed along the perimeter of the field. As many farmers in the region practice crop rotation, the field containing infested stubble is often located near a new soybean field making it a convenient host. This in addition to unmanaged field borders and ditches with native host plants may also influence where aggregation is occurring (Patrick 1973, Rogers 1985). In order to implement site-specific management tactics, future studies aimed at quantifying the surrounding landscape, specifically areas with native hosts that may influence aggregation locations. This information coupled with degree day models could provide information needed to make accurate predictions as to which edges are more at risk of infestation and when it is suitable for treatment.

Contour maps produced from the SADIE analyses showed clusters of adults occurring along the edges of some fields, although this was not a consistent or predictable pattern between fields and/or years. The inconsistency in aggregation patterns may be attributed to the behavior of *D. texanus* beetles dispersing into fields. As seen with the Colorado potato beetle, there were very clear edge effects because adults walk versus fly from surrounding fields into new habitats (Boiteau 2005; Boiteau et al. 2014; Boiteau and MacKinley 2015; MacQuarrie and Boiteau 2003). The methods of dispersal, flying versus walking, for *D. texanus* is relatively unknown and the current study did not examine or compare estimates of dispersal by flight versus walking; however future studies including these factors might provide more information on *D. texanus* dispersal.

Dectes texanus larvae were not typically aggregated within the fields at the end of the season; only fields 1 and 4 showed significant larval aggregations. This is not

surprising when we consider that adults were randomly distributed across most soybean fields for a majority of the sample dates. This lack of aggregation may be attributed to the female *D. texanus* pre-ovipositional period, which can last from 7 to 14 d after mating (Hatchett et al. 1973, Patrick 1973). During this time, the female is seeking out optimal locations for ovipositing site, which may explain why larvae were not typically found to be aggregated; females are dispersing randomly across the field in search of suitable locations. The results from the current study suggest that larval distributions from the previous year affect adult distributions the following year within the same field. Recall, field 6 conditions were different from the other fields, as it was the only field that was planted to soybean the previous year (field 1, 2012). In 2013 (field 6), adults were aggregated in the central and eastern portions of the field and on the southern edge, which was similar to where the adults and larvae were aggregated in field 1 at the end of the season (2012). There was a positive spatial association between larvae collected from field 1 (2012) and the adults collected from two sample dates in field 6 on 2 and 12 July, the two sample dates prior to peak adult activity. This has potential implications for soybean management. Farmers planting consecutive soybean crops, especially those that practice no-till, may have more overwintering of larvae and increased adulty activity. This may be due so several factors. For one, as mentioned previously, tilling infested stubble containing overwintering pupae reduces adult emergence by $\geq$ 15% under certain field conditions (Campbell and van Duyn 1977, Sloderbeck and Buschman 2011); therefore chances for adult survival are likely greater. Additionally, crop rotation forces the newly emerged adult *D. texanus* to seek out new acceptable hosts. By continuously planting soybeans there may be fewer *D. texanus* dispersing away from the field resulting

in increased adult activity. There were also positive spatial associations between adult activity and larvae within the same season in fields 1 and 3. In field 1, the spatial association was observed between larvae and adults collected on 29 June, which was the sample date prior to peak adult activity. Although larvae in field 3 were not aggregated, there was a positive spatial association with adults collected on 17 July, again the sample date prior to peak adult activity. Results from both fields indicate that the adults collected on those dates were collected from the same areas as larvae at the end of the season. Because of this positive spatial association, these results suggest that a more selective placement of insecticide is possible, but only when real-time data of adult distributions is available.

We also found significant disassociations between adults and larvae in field 4 and 6. Field 4 disassociations were between adults collected on 17 July and larvae, while in field 6 the disassociations were with adults collected on 24 July and larvae. In these two instances, the adults and larvae were not collected within the same areas, meaning that it is less likely that larval distribution is attributable to adult patterns on those sample dates. Even though there were positive associations, such relationships were only observed in a few of the fields compounded with significant disassociations observed between larvae and adults could support an alternative hypothesis that *D. texanus* aggregate for mating and then disperse. This hypothesis may explain why adults and larvae were not always collected from the same areas of the field. Similar behavior of adults and larvae has been observed in other Coleoptera and Cerambycidae species (Iwabuchi 1982, Snead and Alcock 1985, Leal et al. 1994, Reagel et al. 2002, Rhainds et al. 2011, Wickham et al. 2012) and may be similar for *D. texanus*. There were a few limitations when examining

larval densities. For one, only 1 m of soybean plants were removed from each sample point, which may not have been an adequate sample size for a comparison with adult distributions. Also, *D. texanus* adults were continuously removed from the field, which could have impacted the infestation pressure within the field, and consequently larval densities and distributions.

The results of this study indicate that adult aggregation occurs during July when adult presence is at its highest (mid-late July). This information provided on peak activity time is valuable in determining effective timing of insecticide applications. The prolonged peak period is particularly beneficial when used with recommendation such as the one provided by Sloderbeck and Buschman (2011); in other words, treat adults during peak activity and then 10 days later. However, variation between years and peak activity highlights the need to incorporate degree-day models for predicting emergence and *D. texanus* adult activity in the field. This study provides support to further explore site-specific management as an option for *D. texanus*. Although populations can be aggregated during this time of peak activity, factors determine where clusters occur are still largely unknown. As this study did not address areas outside of the field, being able to examine and identify trends between aggregation locations, native hosts, and infested stubble in the surrounding landscapes would assist in forming strong predictions for *D. texanus* presence in the field. Overall, understanding dispersal capabilities of adults would add valuable information on the within field behavior of *D. texanus*, which can be used to help predict at-risk edges for implementing site-specific management strategies.

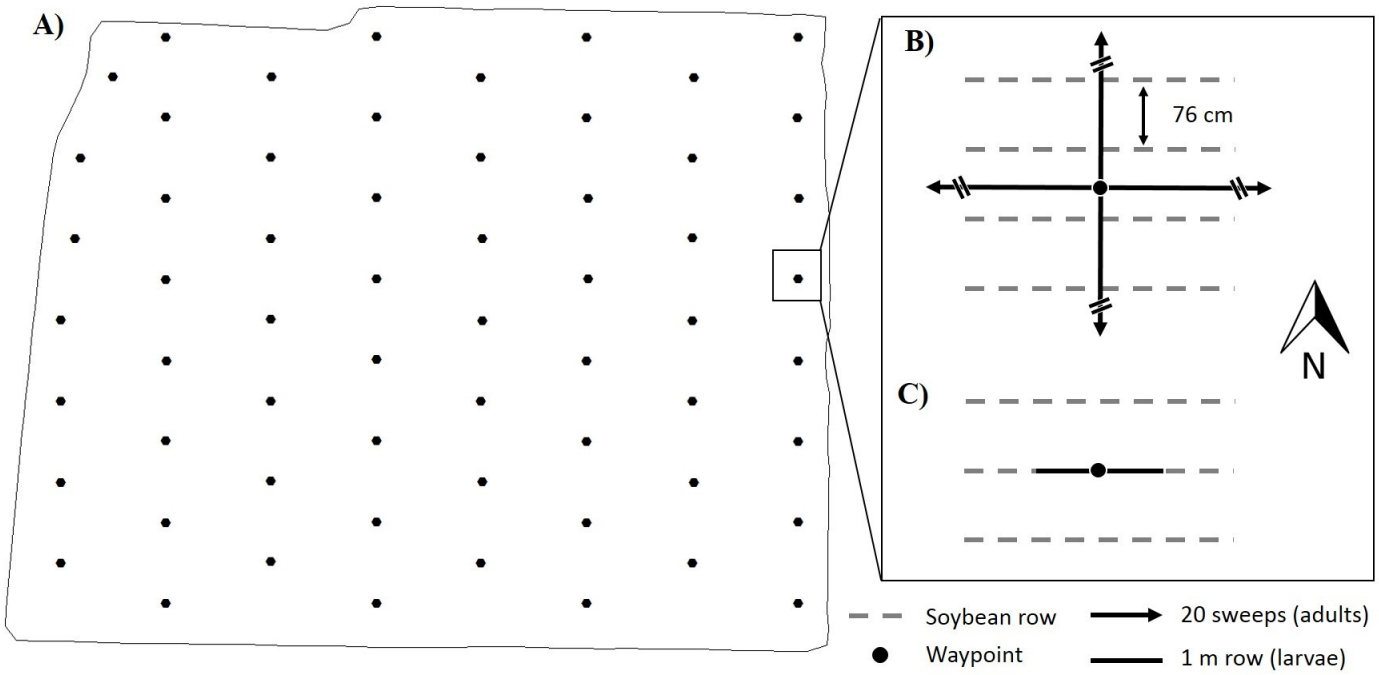

Figure 2.1. A) Uniformly spaced sampling grid used to quantify the spatial distribution of adult and larvae *D. texanus*. Each row of waypoints was offset with the adjacent row of waypoints with the size and shape of the field determining the number of waypoints; B) adult *D. texanus* sampling plant where a total of 20 sweep samples (80 total per waypoint) were taken in each cardinal direction (north, south, east, west) at each waypoint within a field; C) *D. texanus* larvae sampling plan where 1 m row of whole soybean plants (including roots) were collected from the each waypoint.

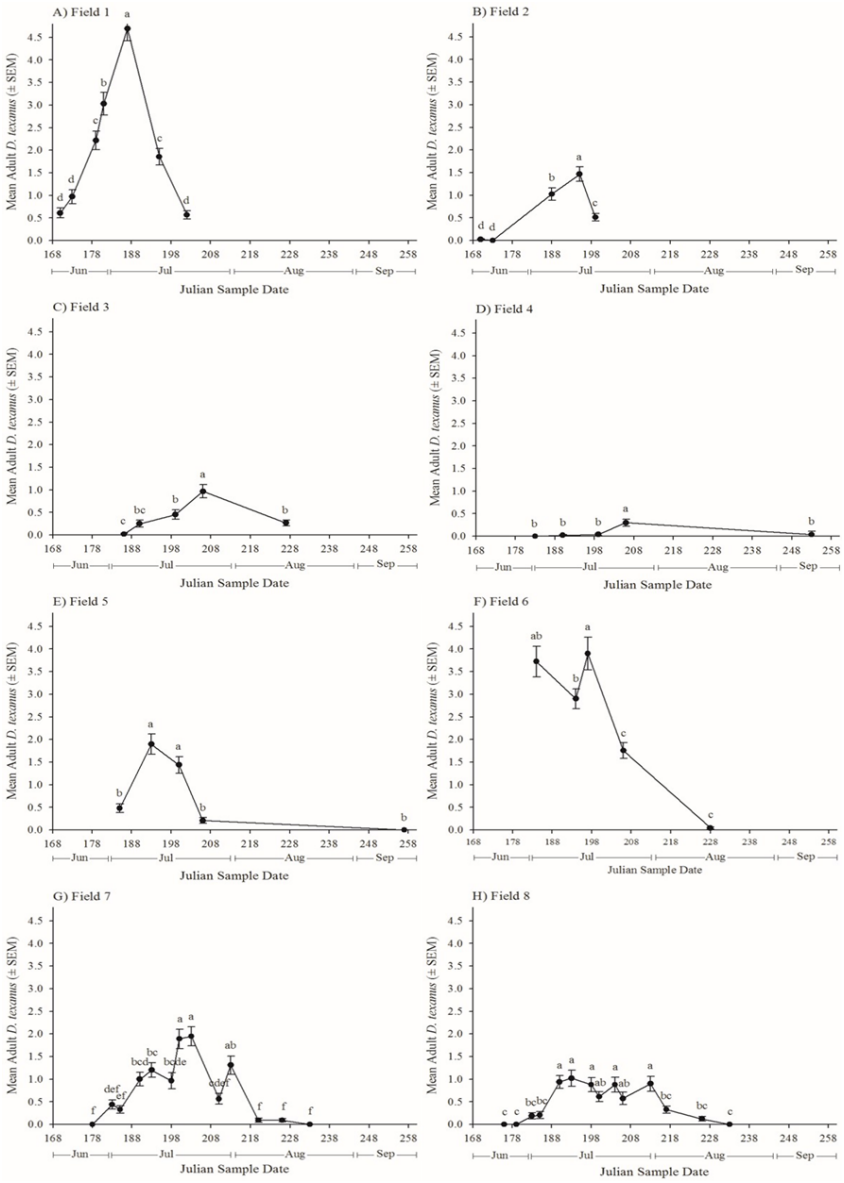

Figure 2.2**.** The mean adult *D. texanus* (+/- standard error mean (SEM)) per sample point collected on each sample date for all fields; A) field 1 2012, B) field 2 2012, C) field 3 2013, D) field 4 2013, E) field 5 2013, F) field 6 2013, G) field 7 2014, and H) field 8 2014. The x axis indicates the Julian sample date in which *D. texanus* adults were collected. Since 2012 was a leap year, the Julian days for all three years included the extra day in order to maintain consistency. The y axis indicates the mean number of adults collected per sample point on each sample date**.**

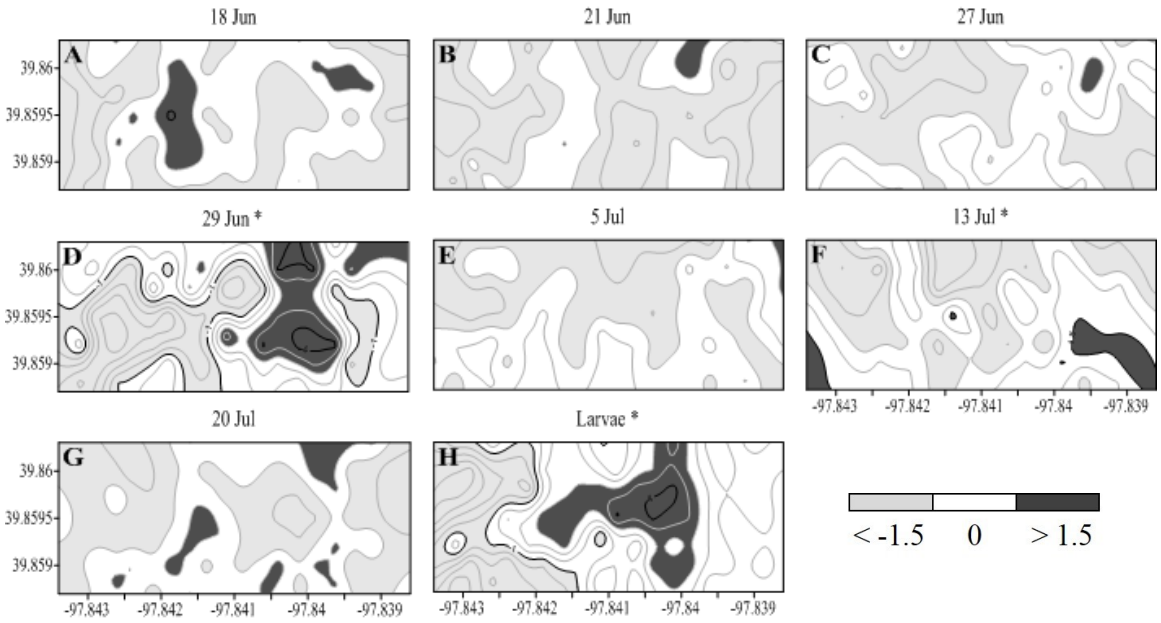

Figure 2.3. Field 1, 2012, spatial interpolation of the SADIE clustering from the local aggregation indices for all adult and larvae *D. texanus* sample dates in soybean production fields; A) 18 June, B) 21 June, C) 27 June, D) 29 June, E) 5 July, F) 13 July, G) 20 Jul, and H) Larvae. The x- and y-axed denote the longitude and latitude of the field sampled. The gray areas indicate gaps ($\bar{v}_j$ > -1.5, P < 0.975); white areas indicate values that were neither above 1.5 or below -1.5, in effect arranged at random; black areas indicate areas where patches are present ($\bar{v}_i$ > 1.5, P <0.025). Asterisks next to the date indicates significant aggregation (I_a > 1, P < 0.025).

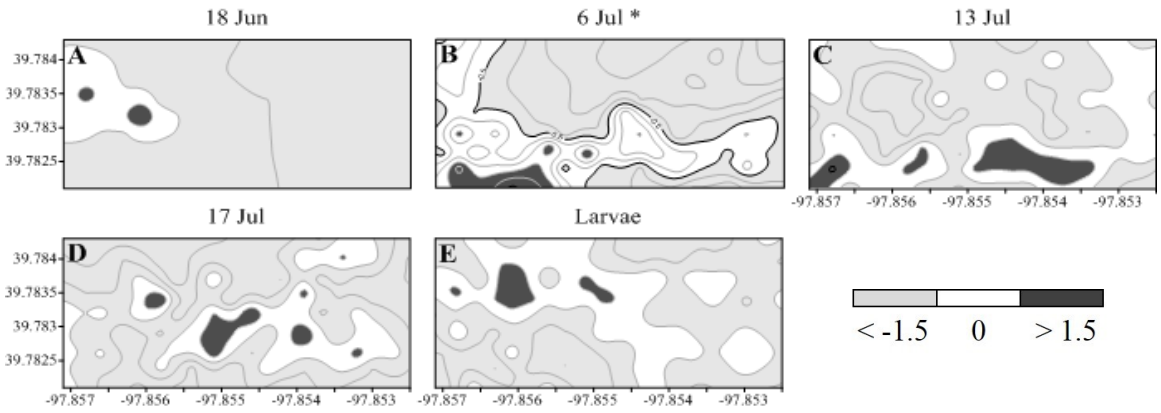

Figure 2.4. Field 2, 2012, spatial interpolation of the SADIE local aggregation indices for all adult and larvae *D. texanus* sample dates in soybean production fields; A) 18 June, B) 6 July, C) 13 July, D) 17 July, and E) 5 Larvae. The x- and y-axed denote the longitude and latitude of the field sampled. The gray areas indicate gaps ($\bar{v}_j > -1.5$, $P < 0.975$); white areas indicate values that were neither above 1.5 or below -1.5, in effect arranged at random; black areas indicate areas where patches are present ($\bar{v}_i > 1.5$, $P < 0.025$). Asterisks next to the date indicates significant aggregation ($I_a > 1$, $P < 0.025$).

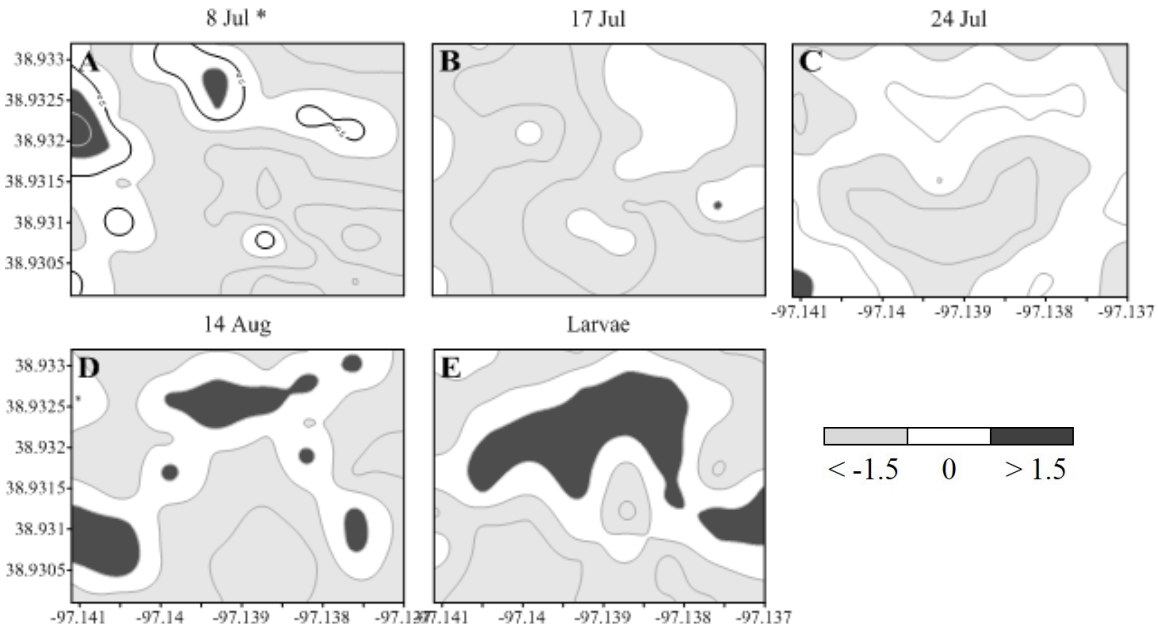

Figure 2.5. Field 3, 2013, spatial interpolation of the SADIE local aggregation indices for all adult and larvae *D. texanus* sample dates in soybean production fields; A) 8 July, B) 17 July, C) 24 July, D) 14 August, and E) Larvae. The x- and y-axed denote the longitude and latitude of the field sampled. The gray areas indicate gaps ($\bar{v}_j > -1.5$, $P < 0.975$); white areas indicate values that were neither above 1.5 or below -1.5, in effect arranged at random; black areas indicate areas where patches are present ($\bar{v}_i > 1.5$, $P < 0.025$). Asterisks next to the date indicates significant aggregation ($I_a > 1$, $P < 0.025$).

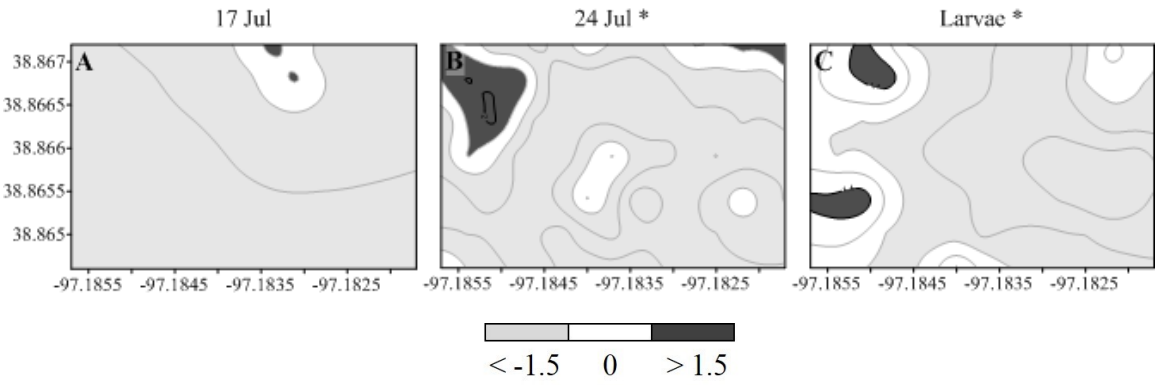

Figure 2.6. Field 4, 2013, spatial interpolation of the SADIE local aggregation indices for all adult and larvae *D. texanus* sample dates in soybean production fields; A) 17 July, B) 24 July, and C) Larvae. The x- and y-axed denote the longitude and latitude of the field sampled. The gray areas indicate gaps ($\bar{v}_j > -1.5$, $P < 0.975$); white areas indicate values that were neither above 1.5 or below -1.5, in effect arranged at random; black areas indicate areas where patches are present ($\bar{v}_i > 1.5$, $P < 0.025$). Asterisks next to the date indicates significant aggregation ($I_a > 1$, $P < 0.025$).

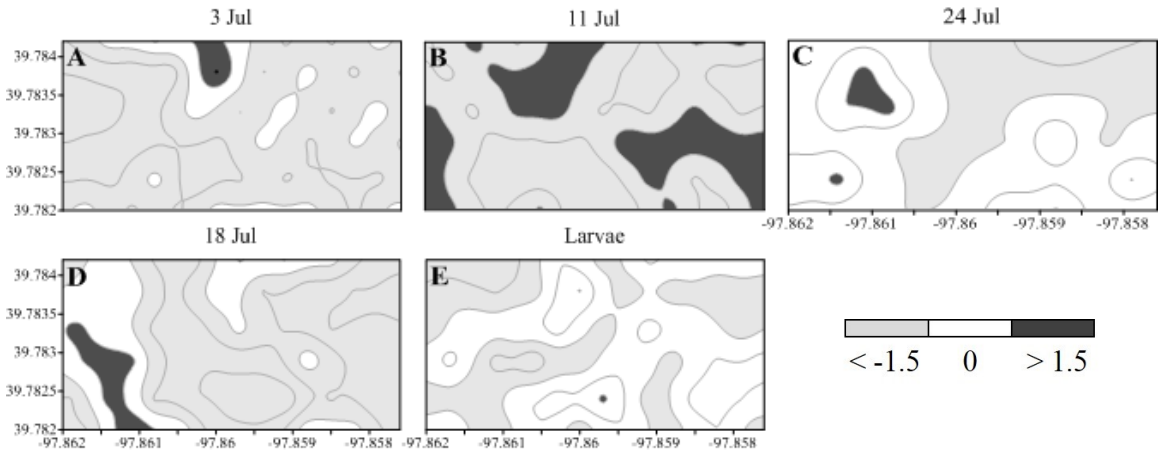

Figure 2.7. Field 5, 2013, spatial interpolation of the SADIE local aggregation indices for all adult and larvae *D. texanus* sample dates in soybean production fields; A) 3 July, B) 11 July, C) 24 July, D) 18 July, and E) Larvae. The x- and y-axed denote the longitude and latitude of the field sampled. The gray areas indicate gaps ($\bar{v}_j > -1.5$, $P < 0.975$); white areas indicate values that were neither above 1.5 or below -1.5, in effect arranged at random; black areas indicate areas where patches are present ($\bar{v}_i > 1.5$, $P < 0.025$). Asterisks next to the date indicates significant aggregation ($I_a > 1$, $P < 0.025$).

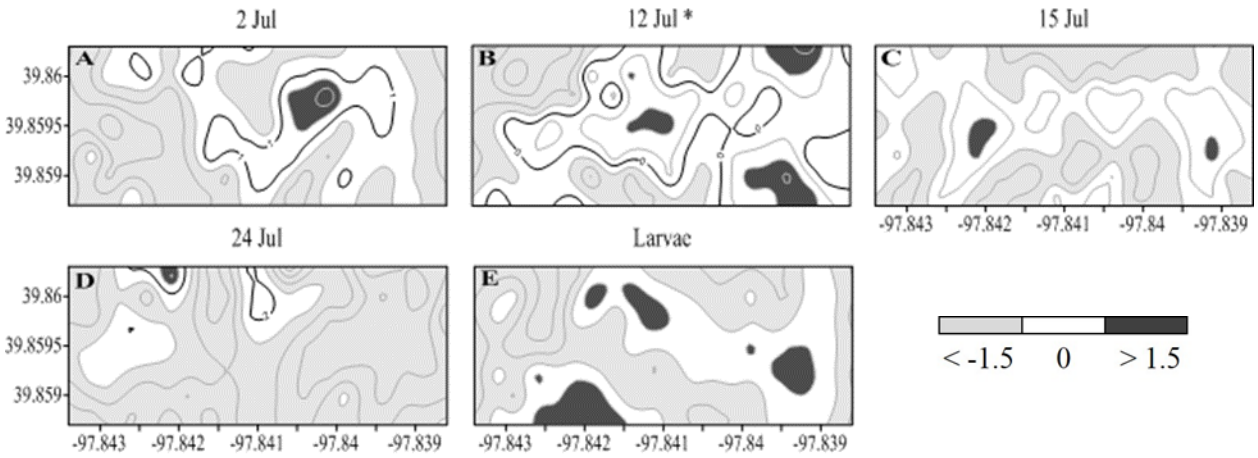

Figure 2.8. Field 6, 2013, spatial interpolation of the SADIE local aggregation indices for all adult and larvae *D. texanus* sample dates in soybean production fields; A) 2 July, B) 12 July, C) 15 July, D) 24 July, and E) Larvae. The x- and y-axed denote the longitude and latitude of the field sampled. The gray areas indicate gaps ($\bar{v}_j > -1.5$, $P < 0.975$); white areas indicate values that were neither above 1.5 or below -1.5, in effect arranged at random; black areas indicate areas where patches are present ($\bar{v}_i > 1.5$, $P < 0.025$). Asterisks next to the date indicates significant aggregation ($I_a > 1$, $P < 0.025$).

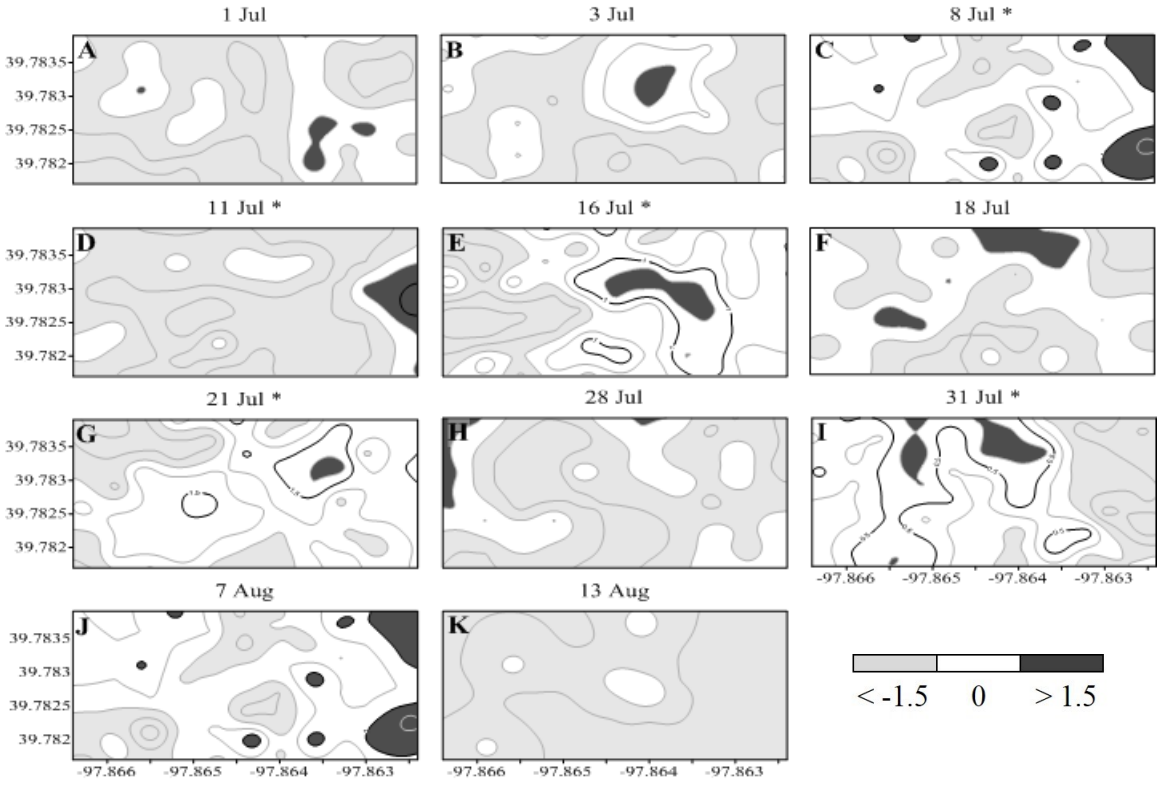

Figure 2.9. Field 7, 2014, spatial interpolation of the SADIE local aggregation indices for all adult *D. texanus* sample dates in soybean production fields; A) 1 July, B) 3 July, C) 8 July, D) 11 July, E) 16 July, F) 18 July, G) 21 July, H) 28 July, I) 31 July, J) 7 August, and K) 13 August. The x- and y-axed denote the longitude and latitude of the field sampled. The gray areas indicate gaps ($\bar{v}_j > -1.5$, $P < 0.975$); white areas indicate values that were neither above 1.5 or below -1.5, in effect arranged at random; black areas indicate areas where patches are present ($\bar{v}_i > 1.5$, $P < 0.025$). Asterisks next to the date indicates significant aggregation ($I_a > 1$, $P < 0.025$).

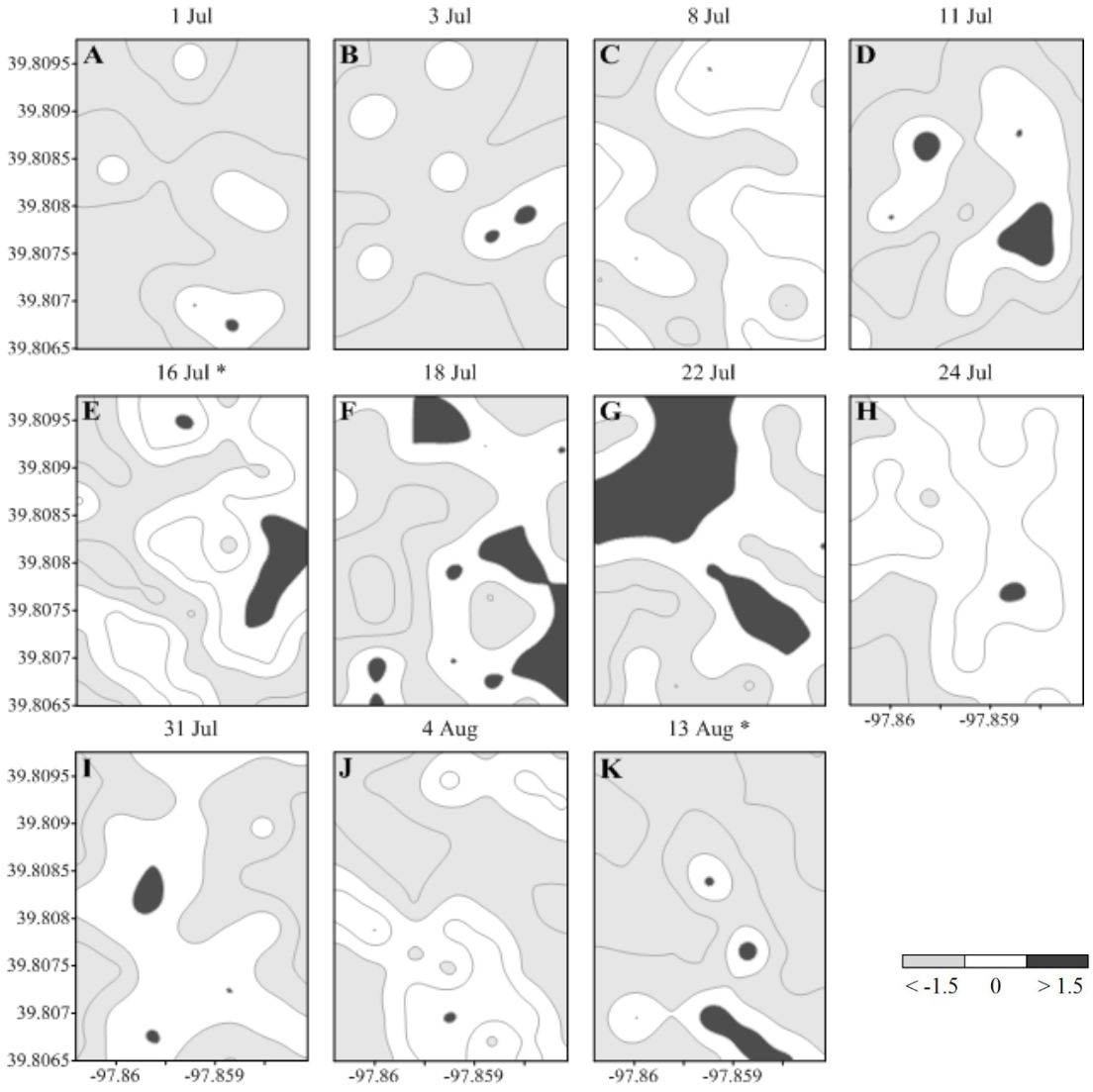

Figure 2.10. Field 8, 2014, spatial interpolation of the SADIE local aggregation indices for all adult *D. texanus* sample dates in soybean production fields; A) 1 July, B) 3 July, C) 8 July, D) 11 July, E) 16 July, F) 18 July, G) 22 July, H) 24 July, I) 31 July, J) 4 August, and K) 13 August. The x- and y-axed denote the longitude and latitude of the field sampled. The gray areas indicate gaps ($\bar{v}_j > -1.5$, $P < 0.975$); white areas indicate values that were neither above 1.5 or below -1.5, in effect arranged at random; black areas indicate areas where patches are present ($\bar{v}_i > 1.5$, $P < 0.025$). Asterisks next to the date indicates significant aggregation ($I_a > 1$, $P < 0.025$).

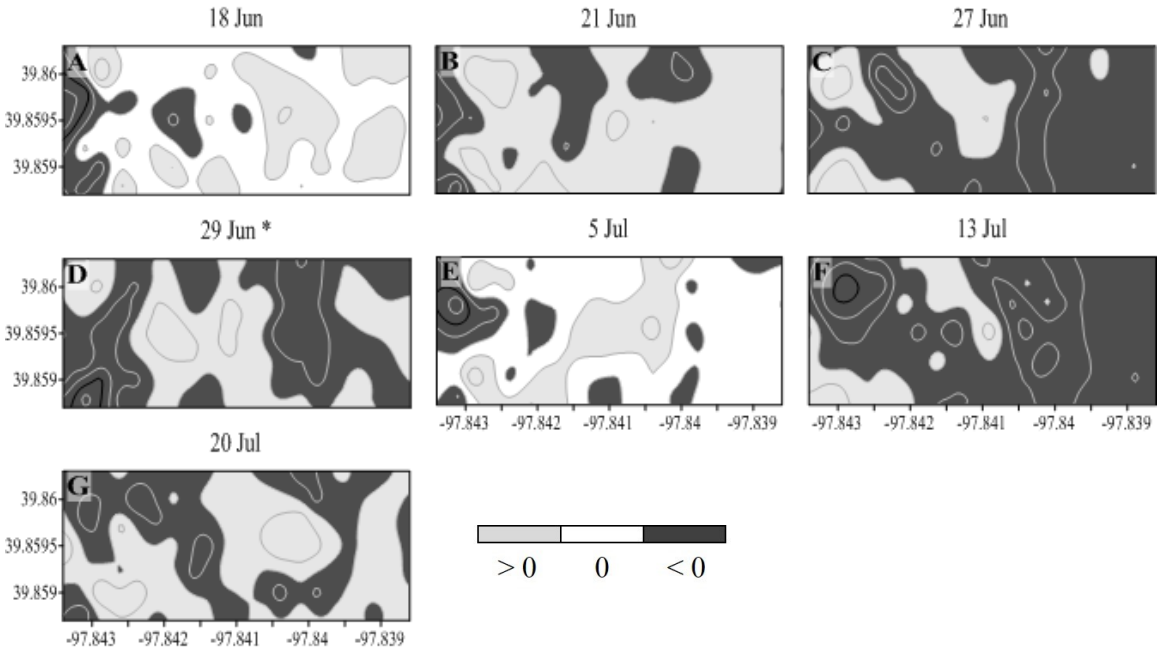

Figure 2.11. Field 1, 2012, spatial interpolation of the SADIE local spatial association for all comparisons between adult and larvae *D. texanus* sample dates in soybean production fields; A) 18 June, B) 21 June, C) 27 June, D) 29 June, E) 5 July, F) 13 July, and G) 20 Jul. The x-axis represents the longitude while the y-axis represents the latitude of the field sampled. Positive values ($X > 0$), indicates an associations between *D. texanus* adults and larvae; represented in gray. Conversely, negative values ($X < 0$) indicates disassociation; represented in black. Asterisks next to the date indicates significant positive associations ($P < 0.025$) or negative associations ($P > 0.975$).

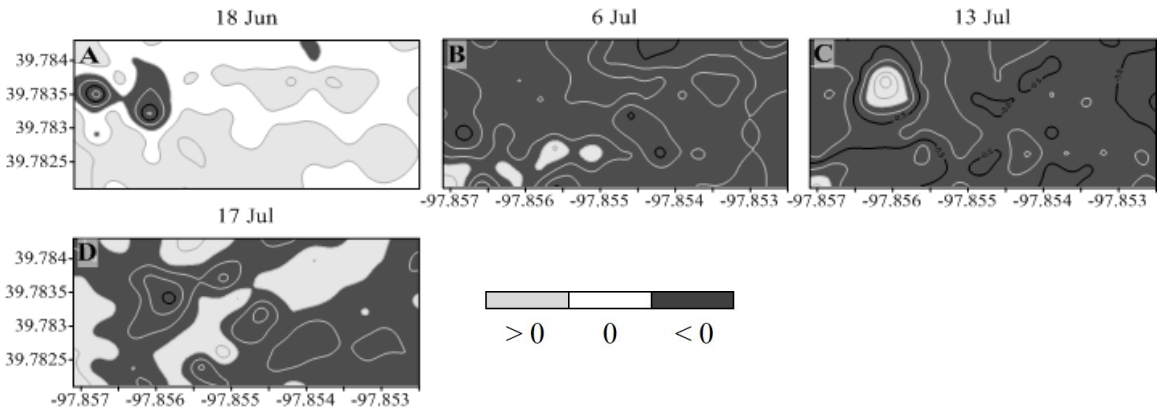

Figure 2.12. Field 2, 2012, spatial interpolation of the SADIE local spatial association for all adult and larvae *D. texanus* sample dates in soybean production fields; A) 18 June, B) 6 July, C) 13 July, and D) 17 July. The x-axis represents the longitude while the y-axis represents the latitude of the field sampled. Positive values ($X > 0$), indicates an associations between *D. texanus* adults and larvae; represented in gray. Conversely, negative values ($X < 0$) indicates disassociation; represented in black. Asterisks next to the date indicates significant positive associations ($P < 0.025$) or negative associations ($P > 0.975$).

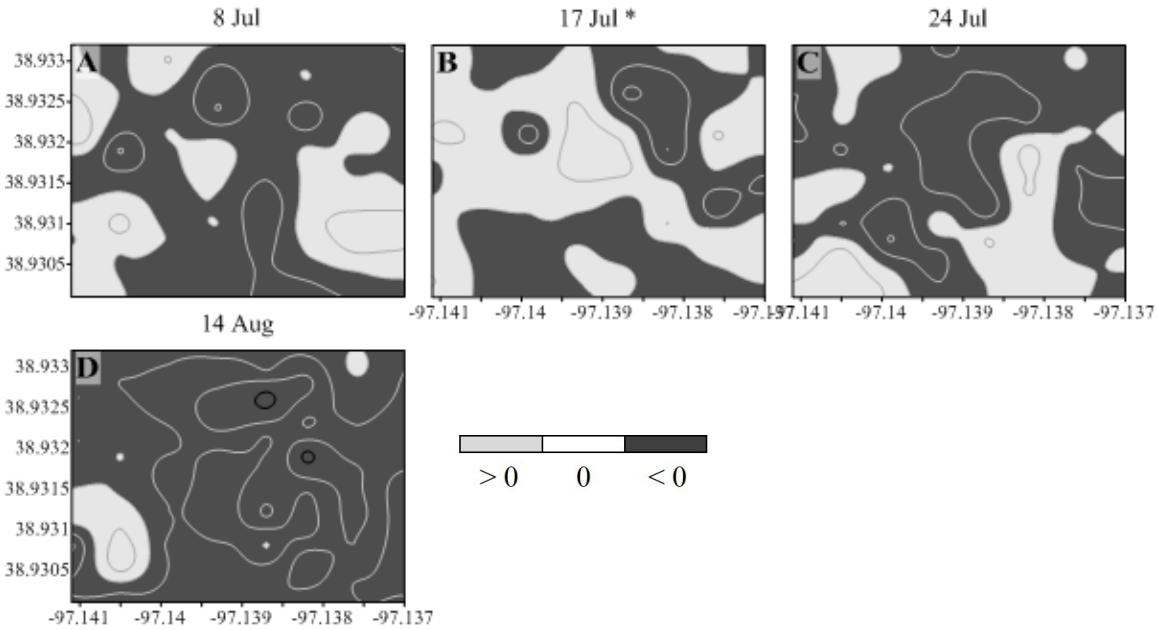

Figure 2.13. Field 3, 2013, spatial interpolation of the SADIE local spatial association for all adult and larvae *D. texanus* sample dates in soybean production fields; A) 8 July, B) 17 July, C) 24 July, and D) 14 August. The x-axis represents the longitude while the y-axis represents the latitude of the field sampled. Positive values ($X > 0$), indicates an associations between *D. texanus* adults and larvae; represented in gray. Conversely, negative values ($X < 0$) indicates disassociation; represented in black. Asterisks next to the date indicates significant positive associations ($P < 0.025$) or negative associations ($P > 0.975$).

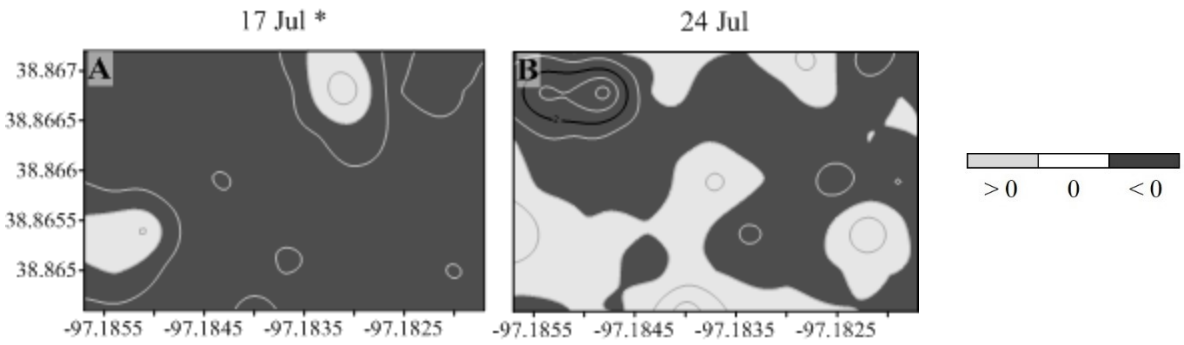

Figure 2.14. Field 4, 2013, spatial interpolation of the SADIE local spatial association for all adult and larvae *D. texanus* sample dates in soybean production fields; A) 17 July and B) 24 July. The x-axis represents the longitude while the y-axis represents the latitude of the field sampled. Positive values ($X > 0$), indicates an associations between *D. texanus* adults and larvae; represented in gray. Conversely, negative values ($X < 0$) indicates disassociation; represented in black. Asterisks next to the date indicates significant positive associations ($P < 0.025$) or negative associations ($P > 0.975$).

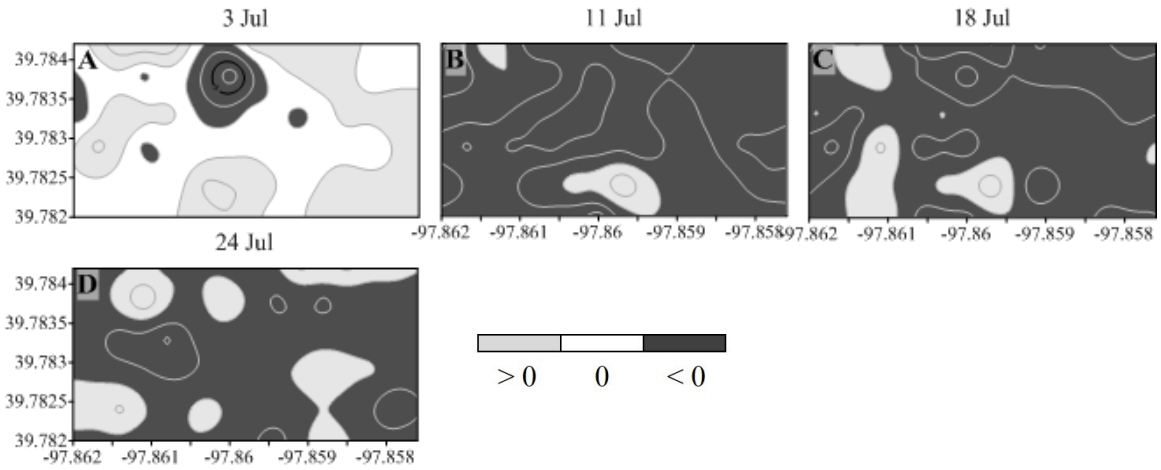

Figure 2.15. Field 5, 2013, spatial interpolation of the SADIE local spatial association for all adult and larvae *D. texanus* sample dates in soybean production fields; A) 3 July, B) 11 July, C) 18 July, and D) 24 July. The x-axis represents the longitude while the y-axis represents the latitude of the field sampled. Positive values ($X > 0$), indicates an associations between *D. texanus* adults and larvae; represented in gray. Conversely, negative values ($X < 0$) indicates disassociation; represented in black. Asterisks next to the date indicates significant positive associations ($P < 0.025$) or negative associations ($P > 0.975$).

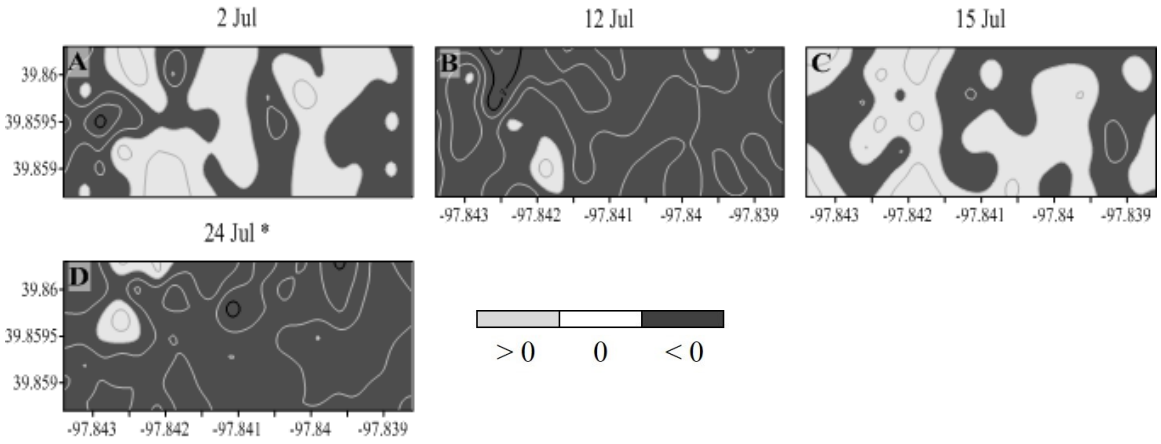

Figure 2.16. Field 6, 2013, spatial interpolation of the SADIE local spatial association for all adult and larvae *D. texanus* sample dates in soybean production fields; A) 2 July, B) 12 July, C) 15 July, and D) 24 July. The x-axis represents the longitude while the y-axis represents the latitude of the field sampled. Positive values ($X > 0$), indicates an associations between *D. texanus* adults and larvae; represented in gray. Conversely, negative values ($X < 0$) indicates disassociation; represented in black. Asterisks next to the date indicates significant positive associations ($P < 0.025$) or negative associations ($P > 0.975$).

Table 2.1. Location and seed information of the soybean production fields spatial sampled during 2012, 2013, and 2014.

Year	Field	County	Longitude	Latitude	ha[a]	No. of Waypoints[b]	Seed Company/Brand	Variety	Relative Maturity
2012	1*	Republic	-97.841373	39.859500	8	69	Syngenta	S36-B6	3.6
	2	Republic	-97.854640	39.783184	11	90	Kruger	K2-3701	3.6
2013	3	Dickinson	-97.138745	38.930330	15	58	Pioneer	94Y23	42
	4	Dickinson	-97.183109	38.865889	12	49	Ohlde	421	4.2
	5	Republic	-97.857947	39.783313	11	47	Syngenta	S36-B6	3.6
	6*	Republic	-97.840875	39.859501	8	69	Syngenta	S39-U2	3.9
2014	7	Republic	-97.864401	39.782906	11	55	--[c]	--	--
	8	Republic	-97.859210	39.806500	10	49	Syngenta	S38-W4	3.8

*: Fields that were sample in consecutive years from each other.

[a]: Field size measured in hectares, rounded to the nearest hundreths.

[b]: The number of sample points located within each field.

[c]: Information on the variety of soybean used was not collected.

Table 2.2. The sample dates for each field by year, including the total number of adults collected per sample occasion, the total number of larvae collected pre-harvest, and the vegetation (V) and reproductive (R) stages at each sample date.

Year	Field No.	Sample Date	Total No. of collected adults	V	R	I_a	P_a	$\bar{v}_i$	*Pvi*	*vj*	*Pvj*
2012	1	18-Jun	42	--[a]	--	1.0	0.413	0.961	0.453	-1.020	0.355
		21-Jun	67	--	--	1.0	0.329	0.996	0.401	-1.083	0.283
		27-Jun	152	--	--	1.0	0.386	0.996	0.383	-1.032	0.322
		29-Jun	205	--	--	2.3	0.001	2.195	0.001	-2.372	0.000
		5-Jul	323	--	--	1.0	0.316	1.051	0.298	-1.007	0.370
		13-Jul	128	7	2	1.5	0.040	1.410	0.050	-1.482	0.038
		20-Jul	38	11	3	1.0	0.469	0.923	0.548	-0.988	0.402
	2	18-Jun	2	--	--	1.3	0.106	1.420	0.008	-1.295	0.117
		21-Jun †	0	--	--	--	--	--	--	--	--
		6-Jul	92	8	1	2.3	0.000	2.141	0.0002	-2.106	0.000
		13-Jul	131	8	2	1.1	0.221	0.991	0.413	-1.071	0.269
		17-Jul	45	8	3	1.2	0.167		0.089	-1.149	0.181
2013	3	4-Jul †	1	5	0	--	--	--	--	--	--
		8-Jul	15	6	0	1.5	0.013	1.543	0.009	-1.473	0.017
		17-Jul	27	8	2	0.9	0.682	0.932	0.615	-0.900	0.699
		24-Jul	58	9	2	1.0	0.407	0.954	0.561	-0.988	0.467
		14-Aug	16	13	3	1.0	0.440	1.027	0.357	-0.978	0.476
	4	1-Jul †	0	3	0	--	--	--	--	--	--
		8-Jul †	1	4	0	--	--	--	--	--	--
		17-Jul	2	5	1	1.1	0.209	1.178	0.162	-1.118	0.239
		24-Jul	15	8	1	1.3	0.040	1.267	0.069	-1.340	0.044
		9-Sep †	1	16	6	--	--	--	--	--	--
	5	3-Jul	23	4	0	0.9	0.667	0.952	0.517	-0.918	0.616
		11-Jul	91	6	1	0.8	0.902	0.795	0.960	-0.822	0.881
		18-Jul	69	6	2	1.2	0.119	1.253	0.084	-1.144	0.175
		24-Jul	10	6	1	1.1	0.294	1.073	0.279	-1.043	0.330
		13-Sep	0	13	6	--	--	--	--	--	--

	6	2-Jul		257	5	0	1.3	0.098	1.298	0.096	-1.300	0.095
		12-Jul		200	8	1	1.6	0.019	1.584	0.026	-1.661	0.018
		15-Jul	†	268	9	1	1.2	0.149	1.173	0.175	-1.179	0.184
		24-Jul		116	7	2	1.3	0.124	1.312	0.082	-1.234	0.127
		15-Aug		3	11	4	--	--	--	--	--	--
2014	7	26-Jun	†	0	3	0	--	--	--	--	--	--
		1-Jul		25	5	1	1.1	0.260	0.997	0.413	-1.078	0.265
		3-Jul		19	5	1	1.0	0.402	0.970	0.465	-1.003	0.390
		8-Jul		55	7	1	1.6	0.016	0.608	0.038	-1.550	0.021
		11-Jul		66	6	2	1.4	0.046	1.153	0.188	-1.124	0.221
		16-Jul		53	7	2	1.6	0.007	1.462	0.019	-1.645	0.008
		18-Jul		104	8	2	1.0	0.478	0.899	0.697	-0.939	0.573
		21-Jul		107	9	3	1.4	0.047	1.273	0.073	-1.291	0.073
		28-Jul		31	8	3	1.0	0.337	0.971	0.468	-1.031	0.342
		31-Jul		71	10	4	1.6	0.015	1.348	0.041	-1.541	0.014
		7-Aug	†	5	12	5	--	--	--	--	--	--
		13-Aug		5	11	5	0.9	0.760	0.900	0.673	-0.857	0.771
		20-Aug	†	0	14	6	--	--	--	--	--	--
	8	24-Jun	†	0	6	0	--	--	--	--	--	--
		27-Jun	†	0	6	1	--	--	--	--	--	--
		1-Jul		9	6	1	0.9	0.597	1.014	0.390	-0.922	0.623
		3-Jul		10	7	1	0.9	0.715	0.975	0.488	-0.891	0.710
		8-Jul		46	9	2	1.0	0.360	0.965	0.491	-1.027	0.355
		11-Jul		50	7	2	1.1	0.234	1.094	0.239	-1.030	0.355
		16-Jul		43	7	2	1.3	0.041	1.219	0.084	-1.329	0.046
		18-Jul		29	10	2	1.1	0.190	1.072	0.252	-1.133	0.178
		22-Jul		43	11	3	1.0	0.429	0.951	0.560	-1.028	0.359
		24-Jul		28	11	3	1.0	0.520	0.930	0.620	-0.968	0.495
		31-Jul		44	13	4	1.0	0.558	0.914	0.687	-0.944	0.567
		4-Aug		16	13	5	1.1	0.215	1.185	0.120	-1.086	0.252
		13-Aug		6	14	5	1.4	0.033	1.466	0.015	-1.360	0.040
		20-Aug	†	0	15	6	--	--	--	--	--	--

a: indicates tha data was not recorded on that day

†: indicates sample dates that were excluded from SADIE analysis

∗: Indicates spatial randomness of the overall index of dispersion at $P_a < 0.025$ (indicating aggregation) or $P_a > 0.975$ (indicating uniformity).

Table 2.3. Results of generalized linear model repeated measures analysis of variance (PROC GLM) comparing the total number of adult *D. texanus* collected during each sample date within the years 2012, 2013, and 2014.

Year	Field No.	*F*	*df*	*P*
2012	1	64.72	6	<0.0001*
	2	42.31	4	<0.0001*
2013	3	16.46	4	<0.0001*
	4	10.79	4	<0.0001*
	5	36.61	4	<0.0001*
	6	43.64	4	<0.0001*
2014	7	25.16	13	<0.0001*
	8	25.16	12	<0.0001*

*: indicates signficance at $\alpha = 0.05$

Table 2.4. Results of the SADIE analyses overall index of dispersion conducted on the *D. texanus* larvae collected from 2012 and 2013.

Year	Field	*D. texanus* Larvae Collected	*Ia*	*Pa*	$\bar{v}_i$	*Pvi*	*vj*	*Pvj*
2012	1∗	529	1.9	0.004	1.857	0.007	-2.143	0.002
	2	336	0.9	0.597	0.952	0.494	-0.929	0.563
2013	3	165	1.0	0.321	1.038	0.340	-1.000	0.432
	4∗	8	1.4	0.021	1.468	0.019	-1.453	0.020
	5	366	1.0	0.446	0.997	0.403	-0.994	0.412
	6	755	1.2	0.185	1.042	0.308	-1.099	0.241

∗: Indicates spatial randomness of the overall index of dispersion at $P < 0.025$ (indicating aggregation) or $P > 0.975$ (indicating uniformity).

Table 2.5. The 2012 and 2013 results of the linear regression models used for identifying relationships between adult and larvae *D. texanus*.

Year	Field No.	Sample Date	R^2	*F*	*df*	*P*
2012	1	18-Jun	0.033	2.32	1, 67	0.133
		21-Jun	0.147	11.57	1, 67	0.001
		27-Jun	0.001	0.09	1, 67	0.768
		29-Jun	0.039	2.71	1, 67	0.104
		5-Jul	0.001	0.08	1, 67	0.781
		13-Jul	0.043	3.01	1, 67	0.087
		20-Jul	0.004	0.27	1, 67	0.602
	2	18-Jun	0.019	1.73	1, 88	0.192
		6-Jul	0.005	0.49	1, 88	0.488
		13-Jul*	0.000	0.02	1, 88	0.879
		17-Jul	0.034	3.07	1, 88	0.083
2013	3	8-Jul*	0.000	0.03	1, 58	0.868
		17-Jul	0.078	4.90	1, 58	0.031
		24-Jul	0.051	3.13	1, 58	0.082
		14-Aug	0.031	1.88	1, 58	0.175
	4	17-Jul	0.008	0.38	1, 48	0.538
		24-Jul	0.026	1.30	1, 48	0.261
	5	3-Jul	0.029	1.36	1, 46	0.249
		11-Jul	0.065	3.22	1, 46	0.079
		18-Jul	0.011	0.52	1, 46	0.473
		24-Jul*	0.001	0.03	1, 46	0.859
	6	2-Jul	0.046	3.23	1, 67	0.077
		12-Jul	0.010	0.65	1, 67	0.424
		15-Jul	0.037	2.60	1, 67	0.112
		24-Jul	0.018	1.20	1, 67	0.278

∗: Indicates significant relationships between larvae and adults collected on the repsected date at $\alpha = 0.05$.

Table 2.6. Results from SADIE spatial association index for 2012 and 2013 comparing larval collected at the end of the season to adult sample collection dates.

Year	Field	Sample Date	*X*	*Xp*
2012	1	18-Jun	0.382	0.001
		21-Jun	0.196	0.051
		27-Jun	-0.091	0.774
		29-Jun*	0.267	0.017
		5-Jul	-0.012	0.532
		13-Jul	0.015	0.456
		20-Jul	-0.018	0.562
	2	18-Jun	0.084	0.261
		6-Jul	-0.069	0.731
		13-Jul	-0.176	0.946
		17-Jul	0.008	0.480
2013	3	8-Jul	-0.008	0.527
		17-Jul∗	0.400	0.003
		24-Jul	0.129	0.221
		14-Aug	0.263	0.041
	4	17-Jul∗	-0.375	0.979
		24-Jul	0.160	0.143
	5	3-Jul	0.145	0.164
		11-Jul	0.140	0.189
		18-Jul	-0.173	0.875
		24-Jul	-0.052	0.629
	6	2-Jul	0.075	0.264
		12-Jul	0.229	0.040
		15-Jul	0.120	0.175
		24-Jul∗	-0.245	0.979

∗: Positive associations between *D. texanus* adults and larvae were found significant at $X > 0$ and $P < 0.025$. Conversely, a negative association were determined significant at $X < 0$ and $P > 0.975$.

Chapter 3 - Estimating dispersal of *Dectes texanus* LeConte (Coleoptera: Cerambycidae) in soybean (*Glycine max*, L.) using mark-capture techniques

Introduction

Dectes texanus LeConte (Coleoptera: Cerambycidae), *Dectes* stem borer, is native to North American and can be found throughout Kansas soybean (*Glycine max*, L) production fields (Michaud and Grant 2005; Buschman and Sloderbeck 2010). *Dectes texanus* was first observed across five south central Kansas counties (Edwards, Barton, Kiowa, Ford, and Pawnee counties) in 1985, then spread to 41 counties by 2008 (Buschman and Sloderbeck 2010) (see Chapter 1, Fig. 1.1). By 2015, number of counties with *D. texanus* increased to 55 (see Chapter 1, Fig. 1.2). This spread of *D. texanus* may be attributed to the diversity and availability of hosts and non-native hosts, which are found across most of Kansas and include several native plants in the Asteraceae family (Patrick 1973, Rogers 1985): ragweed, both annual and giant ragweed (*Ambrosia artemisiifolia* (Linnaeus) and *A. trifida* (Linnaeus), respectively), native sunflower (*Helianthus annuus* (Linnaeus)), and cocklebur (*Xanthium strumarium* (Linnaeus)). More importantly, this spread is likely due to the steady rise in soybean acres planted and harvested across Kansas (Michaud and Grant 2005, Buschman and Sloderbeck 2010).

In Kansas, adult *D. texanus* are present in production soybean fields between mid-June and September most years. Soon after emergence, adults will mate and females deposit eggs into the tissue of the petioles (Patrick 1973). The larval stage is the most

damaging stage to the plant, due to pith removal and consequent girdling of the main stem prior to overwintering. Upon hatching, early instars will tunnel and feed on the pith of the petiole and move into the main stem continuing feeding throughout later instars. Developing larvae in the main stem can decrease soybean seed weight by 7-11% (Richardson 1975, Buschman et al. 2005). However, results documenting physiological yield losses are inconsistent (Buschman et al. 2005, 2007), which is likely due to varying infestation severity between production years. Toward the end of the growing season, larvae move towards the base of the stem and girdle or cut the soybean plant approximately 5 cm above the soil line to prepare an overwintering chamber, which also prevents conspecifics from reaching the base of the stem. In fields where nearly 100% of the plants are infested with larvae, girdling and lodging of the mature soybean plants can result in yield losses of up to 16.8% (Daugherty and Jackson 1969). Yield losses are quite variable across production fields, but this is not attributed to the larval stage as larvae do not move from plant to plant. The adult stage accounts for all of the spatial variability across soybean fields yet dispersal capabilities of this stage are not well understood.

In general, many insects use dispersal to adapt to changes in the environment, but more specifically insects move through space to find mates, colonize new habitats, secure food resources, or to avoid overcrowding (Ranius 2006). Effective dispersal behaviors allow a species to adapt to changes in the environment, which can include sudden loss of habitat or fragmentation (Hanski et al. 1994, Thomas 2000, Ranius 2006). Several cerambycid species are known to disperse long distances to find suitable hosts. For example, Asian longhorn beetles, *Anoplophora glabripennis* (Motschulsky), are capable of dispersing 1.0 to 2.6 km in order to find preferred host trees for ovipositioning in

landscapes that are have more heterogeneity (Smith et al. 2001, 2004). In addition, *Phoracantha semipunctata,* can disperse > 5 km when suitable host plants are limited within unsuitable habitats (Drinkwater 1975, Hanks et al. 1998). Togashi (1990) conducted a three-year study using mark-recapture techniques to model the distances flown by *Monochamus alternatus* (Hope), which ranged from 7.1 to 37.8 m per week in search of oviposition sites in Japanese black pine, *Pinus thunbergii* Parl. Similarly to these species, *D. texanus* may be dispersing in search of suitable host; however the short and long-range dispersal capabilities are not known for *D. texanus*.

Currently there are no formal studies examining the dispersal capabilities of *D. texanus* in the field. There are several field-based observations about adult dispersal that are contradictory (Hatchett et al. 1975, Michaud and Grant 2005, Buschman and Sloderbeck 2010, Michaud 2013). For example, it's been observed that adults tend to drop to the ground instead of flying when disturbed and only move as far as needed to find food sources (Hatchett et al. 1975, Michaud and Grant 2005, Michaud 2013). Other reports claim that adults are fairly strong flyers with an ability to infest soybean fields several miles from source locations (Buschman and Sloderbeck 2010). In order to gain better understanding of *D. texanus* dispersal, different methods of population monitoring should be explored. Over the years, there have been several techniques developed to aide in monitoring and tracking insect dispersal in their natural habitats, which is essential to understanding insect biology, demography, and ethology (Hagler and Jackson 2001).

Monitoring pest movement is achievable using simple yet effective mark-capture techniques (Hagler and Jackson 2001). Mark-capture studies generally use indirect methods like mass marking techniques, which are applied within the environment and

allow researchers to monitor populations without disrupting natural insect behaviors. Mass marking is normally conducted by spraying a marker in defined areas within a study area or by setting strategically located marker stations in an area of interest (Hagler and Jackson 2001, Hagler et al. 2011). Mark-capture methods are commonly assessed over time and space to quantify factors affecting insect dispersal (Osborne et al. 2001).

Monitoring insect movement using foreign markers has become an effective strategy over the past couple decades to monitor pest movement. Proteins, dye, pollen, paint and ink, and dust marking or powders have been successfully deployed to track insect movement in various systems (Hagler and Jackson 2001). Paint and inks were among the first marking materials used on insects (Southwood and Henderson 2000). Paints and inks allow the researcher to mark the insects for identification in any manner (location on insects, color pattern/combination, etc.) needed for the experimental design; however, the paints and inks used must be nontoxic to the insect or not alter the insect behavior. Dye marking is also useful in studies for monitoring dispersal across life stages since oil soluble dyes can accumulate in the insect's body fluids or tissues when ingested, retaining inside the insect throughout development. Of all the techniques used, dust marking remains the most common method due to low cost and capability to be used on several different insect species (Southwood and Henderson 2000). Dusts are highly visible to the naked eye, making it easy to spot in the field; and the detection of the powder can also be enhanced under ultraviolet light. Protein marking, as described by Hagler (1997), has been widely used to track insect movement and patterns in mark-capture studies (Hagler and Jackson 2001, Jones et al. 2006). Originally conducted using vertebrate immunoglobin proteins, protein markers have since expanded to include other

proteins found in soy trypsin inhibitor (soymilk), casein (bovine milk), and egg albumin (chicken egg whites) (Hagler 1997, Jones et al. 2006). These later protein markers are inexpensive, readily available, and can be applied to naturally occurring insect populations in large areas for monitoring insect dispersal (Hagler 1997, Jones et al. 2006).

Protein marking, in particular, has become a common marking technique for estimating insect dispersal in natural habitats (described by Hagler 1992). Protein markers are often applied directly in a field to naturally occurring insect populations using various application methods (Hagler and Jackson 2001; Jones et al. 2006). Samples are collected through time or distances, depending on experimental design, and examined for presence of the proteins using enzyme linked-immunosorbent assays (ELISAs) (Hagler and Jackson 2001; Jones et al. 2006). For example, Swezey et al. (2013) used protein markers to monitor dispersal, distribution, and movement of *Lygus* spp. in trap-cropped organic strawberries. They were able to successfully mark *Lygus* spp. and found that nymphs were able to disperse into neighboring alfalfa, 62 m away in as little as 24 h, which provided new insight into their dispersal capabilities. Hagler et al. (2011) also used protein markers in addition to fluorescent powders to quantify honey bee dispersal patterns within a commercial alfalfa seed production area to identify the extent of pollen-mediated gene flow. On average, marked bees were recovered 800 m from the original apiary, which allowed the researchers to determine the apiary of origin. To our knowledge, such methods examining dispersal of pests in soybean, particularly *D. texanus*, have not been studied. Effectiveness of such techniques to estimate the dispersal of *D. texanus* within soybean fields is not known.

Therefore, the objective of this study was to estimate within field dispersal capabilities of adult *D. texanus* using a protein-based, mark-capture technique developed by Jones et al. (2006). Positive identification of markers in lab assays were used to indicate point of origin based on the marker identified and distance traveled, which was determined by measuring the distance between sample location and spray zone for adult *D. texanus*. *Dectes texanus* specifically are described as strong, but reluctant fliers, often dropping to the ground when disturbed (Michaud and Grant 2005, Michaud et al. 2007, Buschman and Sloderbeck 2010). We hypothesized that once in the field, adults would not disperse across the field, but rather displaying trivial flight and remaining close to field edges. Additionally, the methods used for applying the protein markers have not been used for pest monitoring in soybean to our knowledge. In a companion study, we examined the duration of the protein markers within the canopy and whether the methodology used in our study would be an effective protein-marker application for soybean in general. Based on previous research by Hagler and Jones (2010), we hypothesized that protein markers would remain on the mid-to-lower canopies longer than the upper portion of the canopy. We further predicted that there would be minimal drift across soybean rows with the rows directly under the spray boom receiving the most coverage and a decrease in foliar coverage the further a row was from the spray zone.

Material and Methods

Study sites

A protein marking and spatial sampling study was conducted in 2012, 2013, and 2014. Eight commercial soybean fields of varying sizes were selected (Table 3.1): six

were located near Scandia, Kansas (39.796829°N, -97.783995°W) and the remaining two fields were located near Abilene, Kansas (N 38.923902°N, -97.224139°W). Fields were selected in early June based on preliminary sampling for adults as well as evidence of larval-infested stubble in nearby soybean fields (AH unpublished data); larval-infested stubble was only found in field 6 in 2013. Other considerations for selecting suitable fields for this study included proximity to native hosts including giant ragweed and wild sunflower as well as the proximity to other fields used in this experiment. Fields were ≥ 5 km apart from one another within each year of the study, with the exception of 2014 when fields were ≈ 3 km apart. Proper spacing of field sites helped prevent protein-marked individuals from moving between marked fields. Selected fields ranged in size from 8-14 ha. Seed variety varied from field to field (Table 3.1) based on farmer preferences and agronomic growing requirements; therefore, each field was analyzed separately. All fields were planted with a 76.2 cm (30") row-spacing with the exception of field 4, which was planted with a seed drill with a 25.4 cm (10") row-spacing. Field management varied between study sites. Fields 3 and 4 were rain-fed systems and remaining fields were flood-irrigated throughout the growing season and watering frequency was determined by each respective landowner. Information on wind speed and wind direction was obtained from nearby weather stations located near fields (Courtland, KS (KKSCOURT2) and Abilene, KS (KKSABILE11); www.wunderground.com).

Once field sites were selected, a Trimble® Recon® handheld computer system (PN: 790-0025-XXQ, Trimble®, Trimble Navigation Limited, Sunnyvale, CA) connected to a Pathfinder ProXT™ (PN: 52240-20, Trimble®, GPS Pathfinder® Pro Series, Trimble Navigation Limited, Sunnyvale, CA) satellite receiver was used to trace the perimeter of

each field using ArcPad® (ArcPad® V7.1.1., ESRI Inc., Redlands, CA). Each perimeter was saved as a polygon and was downloaded into ArcMap™ (ArcGIS® V10.2, ESRI Inc., Redlands, CA) to produce a uniformly spaced sampling grid. Similar sampling designs are described in other studies quantifying spatial distributions of other economically important pests (Boiteau 2005, Park and Tollefson 2006, Seiter et al. 2013, Reay-Jones 2014) (Fig. 3.1A). In 2012, the distance between waypoints was 25 m in all cardinal directions. Distances between waypoints was increased in 2013 and 2014 to 50 m to reduce the number of waypoints, which decreased overall sampling time and allowed more fields to be included in the study. Sampling grids were uploaded to the handheld navigation system described above and was used to navigate (sub-meter accuracy) to each waypoint within a field during sampling events.

Application of protein markers

Protein markers were applied along transects using predetermined waypoints as a guide on two opposing edges within each field. This resulted in three distinct zones within a field site: two spray zones (egg albumin and bovine casein, hereafter referred to as egg white and milk) and a protein-free zone, which was unsprayed soybean between the spray zones (Fig. 4.3). This design allowed us to estimate dispersal distances for *D. texanus* within a field by marking adults already present in the field at the time of application or adults entering marked areas and then dispersing from field edges. Within each field, the egg white zone received 57 L of 10% egg albumin and water solution (Great Value 100% Liquid Egg White, Wal-Mart Stores, Inc., Bentonville, AR). This protein marker is considered more durable than bovine casein (Jones et al. 2006, Hagler and Jones 2010) and is more likely to be detected on *D. texanus* individuals collected

between protein applications. Since adults emerge from stubble of the previous soybean crops, the egg white spray zone was selected based on proximity to previous years infested soybean and/or alternative host plants. Once the egg white spray zone was selected, the opposite edge was also identified as a spray edge to assess movement across the field. The opposite edge was then sprayed with a total of 57 L of milk, which was not diluted with water (skim milk, Great Value Fat Free 0% Milk, Wal-Mart Stores, Inc., Bentonville, AR; skim milk, Kansas State University: Call Hall Dairy, Manhattan, KS). The opposite edge was selected as a second spray zone using milk because it would reduce the potential of contamination between spray zones during application while also allowing us to look at two different potential areas of entry by *D. texanus* adults.

The protein markers were applied using a CO_2 backpack sprayer attached to a 3-m boom, which directly covered 5 rows of soybean. Separate booms were used for each protein to reduce cross-contamination between spray zones. Since both protein markers were applied on the same day, disposable coverall suits (Polpropylene coverall, SKU# CO35, Cordova Safety Products, Memphis, TN) and disposable boots covers (DuPont™ Tyvek® boot covers, No. 19-813-211, Fisher Scientific, Waltham, MA) were worn during marker application. Between the fields and spray zones, suits were changed, and all spray equipment was thoroughly cleaned to reduce cross contamination. Protein markers are reported to remain in the environment up to 14 d before decreasing in reactivity to enzyme linked immunosorbent assays (Hagler and Jones 2010). For this study, protein markers were reapplied to the fields every 10-12 d, unless there was inclement weather (i.e., rain) in which case protein spray zones were retreated sooner to ensure markers were constantly present in the field during peak adult activity. The length

of each spray zone extended the full length of a given field used in the study (Table 3.1). Protein applications ceased after sweep sampling resulted in the recovery of 50 or fewer *D. texanus* adults in marked fields post protein applications.

Sampling for *Dectes texanus* adults

Spatial sampling for adults was conducted 1 to 2 d after the protein markers were applied to field edges and all fields were sampled 1-2 times per week throughout the remainder of the growing seasons. Adult *D. texanus* were sampled at each waypoint using 38-cm sweep nets (BioQuip Products, Rancho Dominguez, CA). Sweep nets were changed between the protein marked and non-marked areas to reduce cross-contamination within and between fields. A total of 20 sweeps were collected in each cardinal direction (north, south, east, west) at a given waypoint (80 total sweeps per waypoint) within a soybean field (Fig. 4.1B). All field sites were sampled prior to applying protein markers to determine presence of adults in a field. Count data on adult *D. texanus* per 20 sweeps was collected and recorded per waypoint. Live adults were individually placed in plastic, 946-ml storage bags (Great Value, Wal-Mart Stores, Inc., Bentonville, AR) to reduce cross-contamination of protein-marked individuals contacting unmarked individuals from the same waypoint. All samples were placed immediately in a cooler to slow down protein degradation, transported to the lab, and stored at -18°C until analyzed for presence of protein markers. Specimens used in this study were deposited as voucher number 244 in the Kansas State University Museum of Entomological and Prairie Arthropod Research.

Sampling for protein marker drift

Single leaf samples were taken in conjunction with *D. texanus* sweeps in field 7 and Field 8 during 2014. Recall, the 3-m spray boom covered 5 rows of soybean and 3 additional rows on either side of the spray-area was examined for protein; therefore, 11 rows were evaluated for presence or absence of select proteins. We collected leaflets from existing waypoints within the spray zones; a total of 10 waypoints (5 each within milk and egg white spray zones) in Field 7, and five waypoints (2 within milk and 3 within egg white spray zones) were examined in Field 8. To quantify the vertical distribution of protein markers within the soybean canopy, three single leaflet samples within the canopy (top and middle of canopy and lowest leaf on the plant) were collected from one plant within each of the 11 rows. All leaf samples were stored in a cooler at the time of collection and transported to the lab, where they were stored at -18°C until assayed for presence of the specific proteins.

Enzyme linked immunosorbent assay preparation

Dectes texanus. Presence of the proteins was determined using enzyme linked-immunosorbent assays (ELISA) as described by Hagler and Jackson (2001) and Jones et al. (2006). Samples were prepped by removing adult *D. texanus* from storage bags and placing each *D. texanus* into individual 1.5-ml microcentrifuge tubes (Fisherbrand ™, Fisher Scientific, Waltham, MA); samples were then returned to -18°C until assayed. Samples were removed from the freezer and 1000 µl of tris-buffered saline (TBS, ph 8.0; Sigma-Aldrich, T-1503, St. Louis, MO) was added to each sample tube and incubated for 60 min at 23°C. To examine individual movement within the field, all *D. texanus* samples were assayed for the presence of both markers, casein in bovine milk (milk) and egg

albumin in chicken egg white (egg white), following methods described by Hagler et al. (2014). In addition, adult *D. texanus* beetles were collected in the Ashland Bottoms Research Farm near Manhattan, KS (Riley, Co.) and used as negative controls for all ELISA assays. Protein markers were not applied in this area; therefore, *D. texanus* collected would not have come into contact with either protein. *Dectes texanus* negative controls were processed similar to the protein-marked samples and were checked for the presence of casein and egg albumin proteins. Post assay samples were stored at 4°C until the completion of the sample set. All samples were then placed in long-term storage at -18°C to prevent protein degradation in case future assays were needed.

Soybean leaf disk. Once in the lab, leaf discs samples were taken from each leaf collected at all waypoints (as previously described) and prepared for ELISA assays following Hagler and Jones (2010). For each leaf, we selected a 6-mm diameter sub-sample using a disposable soda straw (OurFamily Flexible Straws, Nash Finch Company, Minneapolis, MN); the cutting edge was cut off after each use to prevent cross contamination between samples. The sub-sample was then placed into a 1.5-ml microcentrifuge tube. An aliquot of 1000 µl of TBS was added to each sample tube and incubated for 60 min at 23°C. Negative controls used for determining positively marked samples were collected from greenhouse grown soybean plants, which were not exposed to the protein markers used in our study. All soybean leaf negative samples were collected and stored the same way as previously described for soybean leaf disk samples from our experiments. The leaf disk samples were assayed for one protein or the other (egg white or milk), depending on where a leaf was collected following the methods described by Hagler et al. (2014). Once the soybean leaf disc samples were assayed, they

were stored at 4°C until the completion of the sample set. After analysis, all samples were placed in long-term storage at -18°C to prevent protein degradation in the event that future assays from the samples were needed.

Protein specific ELISAs

For determining presence of protein markers, both the *D. texanus* adults and soybean leaf disc samples were assayed for the specific proteins of casein in bovine milk and egg albumin in chicken egg white using the ELISA procedures described below.

Anti-casein ELISA for milk. The assay began with a 100 µl aliquot of each sample, pipetted into individual wells of an uncoated Nunc™ 96-well ELISA assay plate (#12-565-136, Thermo Scientific, Waltham, MA). Plates were incubated for 60 min at 4°C, cell contents were then emptied, and plates were washed 5 times with a 300 µl of phosphate buffer saline-tween 20 (PBST, 0.5% tween); tween is a cleaning solution used to aide in the washing process. Each well was then coated with 100 µl of hydrogen peroxide (Vi-Jon, St. Louis, MO) and incubated for 30 min at 23°C to reduce potential reactions to plant material and other unknown substances found within the sample (Hagler et al. 2015). The plates were washed two more times with PBST. Next, 300 µl of a blocker (25% egg white dilution in Deionized H_2O or DH_2O) was added to each well and stored at 4°C for 30 min; plates were then washed three more times with PBST. The primary antibody used for the milk assays was anti-bovine casein polyclonal (#ABIN1118451, antibodies-online.com, Agro-Bio, Belgium) (1:10000 diluted in 50/50 mix of PBS-BSA (1%) (1.0% BSA, w/v, pH 7.4, #P3688; Sigma-Aldrich, St. Louis, MO) & soy milk), which was added at 50 µl/well and allowed to incubate for 60 min at 23°C; samples were then washed 5 times with PBST. Then 50 µl of goat anti-rabbit IgG,

conjugated to horseradish peroxidase (#A6154, Sigma-Aldrich, St. Louis, MO) (1:000 diluted in PBS-BSA-Silwet (PBS-BSA (1%) and Silwet L-77 (1300 µl/L; #VIS-01, Lehle Seed, Round Rock, TX)) was added to each well and incubated for 60 min at 23°C. The plate was then emptied and washed five times with PBST. The substrate used for the assays was TMB substrate (#TMBW-1000-01, Microwell One Component Peroxidase Substrate, BioFX Laboratory Inc, Owings Mills, MD). For substrate, 50 µl/well was added to the plates and then allowed to soak for 10 minutes at 23°C, and then read immediately at an absorbance of 650 nm using a microplate spectrophotometer (#1021000, BioTek® EON™, BioTek® Instruments, Inc., Winooski, VT).

Anti-egg albumin ELISA for egg white. A 100 µl aliquot from the sample was pipetted into individual wells of an uncoated Falcon™ 96-well ELISA assay plate (Corning Life Sciences, DL, Manassas, VA). All plates were incubated for 60 min at 27°C. Plates were emptied after the incubation time and were washed 5 times with PBST (described above). Each well was then coated with 100 µl of hydrogen peroxide (Vi-Jon, St. Louis, MO) and incubated for 30 min at 27°C. Plates were washed twice more with PBST. Next, 300 µl of the blocker solution, phosphate buffer saline-bovine serum albumin (PBS-BSA, ph 7.4; #S3688, Sigma-Aldrich Co, St. Louis, MO) was added to each well and then incubated for 30 min at 27°C. Plates were then washed two times with PBST. The primary antibody used in the egg white assays was anti-chicken egg albumin (Ovalbumin) (#C6534, Sigma-Aldrich, St. Louis, MO) (1:8000 dilution in PBS-BSA (1%)-Silwet L-77), added to each plate at 50 µl/well and incubated for 60 min at 27°C. Plates were washed 5 times with PBST. The secondary antibody is the same as described in the above method; 50 µl of goat anti-rabbit IgG, conjugated to horseradish peroxidase

(1:000 diluted in PBS-BSA-Silwet) and then incubated for 60 min 27°C. The plate was emptied and washed five times with PBST. The TMB substrate used in the previously described assay was also used in this assay and was added at 50 µl/well. The substrate was allowed to soak for 10 minutes at 23°C, and then read immediately at an absorbance of 650 nm using the microplate spectrophotometer described above.

Data analysis

Protein marked samples. The standard normal variate transformation threshold criteria was used to identify positively marked samples if the ELISA optical density (OD) value exceeded the negative control mean readings by six standard deviations (Hagler 1997, Sivakoff et al. 2011). This conservative approach of limiting positives to six standard deviations reduced the number of false positives reported (Swezey et al. 2013). The proportion of positively marked samples from the total number of collected samples is reported, which is consistent with other studies incorporating this technique (Hagler et al. 2011, Sivakoff et al. 2012, Swezey et al. 2013). Additionally, simple linear regression models were determined using the MASS package lm() function (RStudio© version 0.99.3441, The R Foundation; Vienna, Austria) to identify any relationships between the proportion of positively marked samples and the mean adult *D. texanus* collected per waypoint.

The number of positively marked *D. texanus* adults collected within each of the spray zones were analyzed using a repeated measure generalized estimating equations (GEE) approach to Poisson regression (PROC GENMOD, SAS® version 9.4, SAS Institute Inc., Cary, NC). The GEE model was selected because it is an extension of generalized linear models (GLM) and can be used to analyze a broad range of data

discrepancies including missing observations and time–dependent explanatory variables (Stokes et al. 2012). The Poisson distribution was selected due to the count nature of the response measured (i.e., number of *D. texanus* captured). The waypoint was analyzed as the repeated measure and the exchangeable correlation structure was used for the error structure of the repeated measure, which was selected based on having the lowest QIC values. Under the GEE model, standard assumptions of normality and heterogeneity are not required because of the assumption that waypoints are correlated and not independent from each other. The fixed effects were spray zone, marker status, and sample date. Spray zones included two areas marked with protein, either egg white and milk, and a "protein-free zone", which was soybean between the spray zones where no direct protein applications were made. Marking status was defined as marked individuals that were collected and included: egg white, milk, and protein-free (samples not containing egg white or milk). Samples containing both proteins (n = 5) were excluded from analysis due to the low number of positives collected. The marking status was treated as a fixed effect to compare the number of positively marked samples, regardless of spray zone. Significant interactions of fixed effects were further analyzed using pair-wise comparisons, which were determined significant at $\alpha = 0.05$.

The soybean leaf disc sub-samples collected from fields 7 and 8 were also analyzed using the same methods described above. Soybean leaf discs were analyzed using a repeated measure generalized estimating equations (GEE) model in SAS (PROC GENMOD). The sample date was analyzed as the repeated measure and the exchangeable correlation structure was used for the error structure of the repeated measure, which was selected based on having the lowest QIC values. To examine the

longevity of the protein in the soybean as well as drift from the spray zone, we focused on samples collected after the second application (17 July) for both Field 7 and 8. The main effects examined in this analysis included protein (egg white and milk), row (row numbers 1-11), canopy location (top, middle, and bottom), and time (number of days after protein application) and were determined significant at $\alpha = 0.05$. Significant interactions were further analyzed using pair-wise comparisons, which were determined significant at $\alpha = 0.05$.

Adult Dectes texanus dispersal distances. The minimum and maximum dispersal distance was estimated for each positively marked *D. texanus* adult collected outside of the spray zones using ArcMap™ (ESRI, ArcGIS V. 10.2, Redlands, CA). The waypoints located within the spray zones were identified as a "point of origin" to approximate the distance traveled. Minimum distance traveled was then measured perpendicular to the spray zone into the field to where the adult was collected. Maximum distance traveled was measured from where the protein positive adult was collected in the field, to the furthest waypoint located in the spray zone of the identified protein. *Dectes texanus* testing positive for a protein marker found within the spray zone with the same marker were excluded from the measurements, as it is not possible to determine a point of origin within a spray zone. The distances traveled by each marked adult were estimated within each field and were averaged within a field as well as across the three years to approximate the within-field dispersal of adult *D. texanus*.

Results

Marked *Dectes texanus*

Of the total *D. texanus* adults collected (n = 2,742) across the eight fields and three years sampled, 132 individuals were positively marked based on ELISA OD values (Table 3.2). Of those positively marked with proteins, 63% (n = 83) were positive for egg white while 37% (n = 49) were positive for milk. Of the positively marked samples, 0.15% (n = 4) was positively marked with both egg white and milk proteins (1 adult in Field 7 and 3 adults in Field 8). We found that of the 83 samples positive for egg white, 81% (n = 67) of adults were recovered from within the egg white spray zone (Table 3.2). A total of 18% (n = 15) of adults positively marked with egg white were recovered from the middle of the field or protein-free zone, while 1% (n = 1) was found within the milk spray zone, which is on the opposite side of the field. The majority (67%; n = 33) of individuals positively marked with milk were recovered from within the milk spray zone, while 22% (n = 11) were recovered from within the middle of the field. Lastly, 10% (n = 5) of the milk-marked *D. texanus* adults were recovered from within the area sprayed with the 10% egg white solution. For both proteins, the greatest numbers of recovered individuals were collected from the respective spray zones. Positively marked milk samples were recovered 10, 5, and 14 d after protein applications after the last application for years 2012, 2013, and 2014, respectively. Samples positive for egg white were recovered 10, 8, and 22 d after the last protein application for years 2012, 2013, and 2014, respectively.

There were no significant differences between the total number of egg white and milk marked adults collected within fields 2, 6, 7, and 8 (Ps > 0.05). There was a

significant difference in field 1 ($X^2(1) = 3.8$, $P = 0.05$), with 3.18-fold more positively marked egg white *D. texanus* than milk. There were significantly more non-marked samples than marked samples within fields 1 (20 fold more than marked samples), 6 (300 fold more than marked samples), 7 (10 fold more than marked samples), and 8 (10 fold more than marked samples) (Ps < 0.05) (Table 3.3). There were no significant differences in the number of positively marked samples recovered from within the different spray zones for fields 1, 2, 6, and 7 (Table 3.3). There was a significant difference in the spray zones in field 8 ($X^2(2) = 10.43$, $P = 0.0054$) (Table 3.3). Pair-wise comparison indicated that there was a significant difference between the numbers of positively marked samples recovered between spray zones in field 2 ($X^2(1) = 4.11$, $P = 0.04$), with more being collected in the egg white spray zone than milk. There were no significant differences between the numbers of positively marked samples recovered within the respective spray zones for all other fields (Ps > 0.05), but there were differences between the numbers recovered from the non-sprayed area and the milk and egg white spray zones, respectively. Overall, there were more positive samples collected in the egg spray zone than protein-free zone, but more positives were collected from the protein-free zone than milk spray zone. Additionally, there was a significant difference in the number of positively marked samples recovered through time for fields 1 (Fig 3.3A), 2 (Fig. 3.3B), 6 (Fig. 3.3C), 7 (Fig. 3.3D), and 8 (Table 3.3; Fig 3.3E); the number of positively recovered samples declined through time after each application (Table 3.3). Fields 3, 4, and 5 were not included in analysis because there was either no positively marked *D. texanus* recovered or the number of positively marked *D. texanus* were too low for analysis. The regression analysis indicated that there was a relationship between the

proportion of positively marked samples and the mean number of *D. texanus* collected per waypoint in fields 1 ($F = 7.2$; $df = 1, 4$; $P = 0.05$; $R^2 = 0.55$), and 7 ($F = 4.7$; $df = 1, 9$; $P = 0.05$; $R^2 = 0.27$), but no relationship was found in the remainder of the fields sampled (Table 3.4).

Adult *Dectes texanus* dispersal

Approximate distance dispersed was measured using positively marked *D. texanus* collected from Fields 1, 2, 6, 7, and 8 (Table 3.5). Positively marked *D. texanus* collected within a respected spray zone, egg white or milk, were excluded from these measurements and included 69 and 33 individuals from the egg white and milk spray zones, respectively. The mean estimated minimum and maximum distance dispersed by adult *D. texanus* (marked with at least one of the two proteins; n = 23) was 98 m (± 15 m) and 275 m ± 14 m, respectively. The estimated mean minimum dispersal distances exhibited by adult *D. texanus* ranged from 52 to 217 m. The estimated mean maximum dispersal distances exhibited by adult *D. texanus* ranged from 65 to 389 m.

Leaves

Within the spray zones for field 7 there were a total of 2,166 leaf discs assayed. Of that, there were a total of 172 (8%) discs positively marked with milk and 147 (7%) positively marked with egg white (Table 3.6) for soybean leaves collected and processed after the protein application on 2 July. In field 8 there was a total of 1,152 leaves sampled with 70 (6%) discs positively marked with milk and 53 (5%) positively marked with egg white.

Analysis conducted on leaves collected after the second protein application (17 July) indicated that there was no difference between the number of positively marked egg

white and milk samples recovered for field 7 ($X^2(1) = 1.66$, $P = 0.20$) (Figure 3.4 Table 3.7). There was a difference in the number of positively marked egg white and milk samples in field 8 ($X^2(1) = 16.57$, $P < 0.0001$); there were 1.3-fold more positive milk marked samples recovered than egg white (Figure 3.4 Table 3.7). When examining drift across rows, row numbers 4-8 were located directly under the 3-m spray boom (Figure 3.5). There was a significant difference between the rows in both Fields 7 ($X^2(10) = 111.58$, $P < 0.0001$) and 8 ($X^2(10) = 40.24$, $P < 0.0001$) (Figure 3.5). Within the milk spray zone for field 7, rows 5 had the highest proportion of marked leaves for the top and middle of the canopy, which was 37% (Figure 3.5; Table 3.8). For the bottom of the canopy, rows 6 and 7 had the highest proportion of marked leaves, 41 and 43% (Figure 3.5; Table 3.8). The egg white spray zone had the highest proportion of marked leaves recovered from row 6 for the top, middle, and bottom of the canopy, which was 31, 38, and 37% of the discs, respectively (Table 3.8). In field 8, the milk spray zone had the highest proportion of marked leaves, 31%, collected from the bottom of the canopy (Table 3.9). In the egg white spray zone the middle of the canopy had the highest proportion of marked leaves, 21% (Table 3.9). The rows with the highest proportion of marked leaves for both milk and egg white spray zones were row 6 at 48 and 45%, respectively (Table 3.9). In both fields 7 and 8, the proportion of positively marked leaves decreased as they reached the outer rows with rows 1, 2, 10, and 11 having the least amount of positively marked leaves, which was < 21% in milk spray zones and < 16% positively marked found in the egg white spray zone (Figure 3.5; Table 3.6). There was no significant differences between the positively marked samples recovered from within the canopy for field 7 ($X^2(2) = 1.07$, $P = 0.59$) or field 8 ($X^2(2) = 2.98$, $P = 0.22$).

Across both fields the top of the canopy had the lowest numbers of positively marked samples when compared to the middle and bottom of the canopy.

There was a change in the number of positively marked samples through time in field 7 ($X^2(5) = 169.64$, $P < 0.0001$) and 8 ($X^2(6) = 70.16$, $P < 0.0001$) (Figure 3.5; Table 3.7); both proteins decreased in the number of positives recovered as we neared the end of the study. For field 7, there was no significant difference between sample time 11 and 27 ($X^2(1) = 3.60$, $P = 0.06$) or samples times 14 and 21 ($X^2(1) = 0.10$, $P = 0.8$) (Figure 3.4). In field 8 there was no significant differences between sample 4 and 11 ($X^2(1) = 0.47$, $P = 0.5$), 4 and 14 ($X^2(1) = 0.35$, $P = 0.6$) and 11 and 14 ($X^2(1) = 0.15$, $P = 0.2$) (Figure 3.4). When examining the number of positively marked samples within the canopy there were no differences between the canopy levels for fields 7 and 8 ($X^2(2) = 1.07$, $P = 0.59$; $X^2(2) = 2.98$, $P = 0.22$) (Figure 3.5); number of marked leaflets decreased equally in the canopy through time. Positively marked milk samples were recovered almost 4 wk after proteins were applied (Table 3.7; Table 3.8). The egg white spray zone from field 7 only had positively marked samples on the top and bottom of the canopy 1 and 4 d after spray application, after they were only recovered from the middle of the canopy (Table 3.8). For field 8, we saw more variation in recovered samples from the different areas of the canopy (Table 3.8). In the milk spray zones there were no protein marked samples collected on 4 Aug (17 d after application), but they were recovered from the middle of the canopy on 13 Aug (4.5%) and the top of the canopy on 20 Aug (4.5%) (Table 3.9). In the egg white spray zone the last positive sample was recovered on Aug 13 (3%) from the bottom of the canopy (Table 3.8), which was 27 d from the last protein application to either field.

Discussion

In this study, we used a protein-based, mark-capture technique to quantify the within field movement and approximate dispersal capabilities of adult *D. texanus* during the soybean season. The readily available protein markers, egg white and milk, were effective at measuring *D. texanus* dispersal. We found that *D. texanus* travel throughout the field during the season, with some individuals dispersing the full length of the field. Furthermore, based on the leaf disc analysis, we found that the methodology used in this study was appropriate for use in soybean. Additionally, we found minimal drift and no obvious cross-contamination between spray zones. Due to our findings in regard to the protein application methods, these methods may be useful for marking other insects in soybean.

In using the protein markers, we were able to effectively mark adult *D. texanus* in soybean allowing us to monitor their dispersal within the field. This is the first study to our knowledge that quantifies dispersal distances for this pest in soybean. Although dispersal measurements were limited to the size of the field, our study found that *D. texanus*, on average, traveled between 52 to 389 m. This information is beneficial for future identification of fields that may be "at risk" of becoming infested; however future studies focusing on dispersal across the landscape and factors that may be driving and influencing that dispersal will be key in making management decisions.

Number of marked *D. texanus* individuals was comparable to previously published research using protein-based markers. In our study, marking of *D. texanus* relied on adults coming into contact with the proteins by walking on the leaf surfaces or being directly sprayed with the protein marker during application. When comparing the

spray zone and concentration of the protein markers applied in the current study to two other large-scale mark-capture studies (Jones et al 2006; Sweazy et al. 2013), the spray zone within these two studies were 0.4 ha area (Jones et al. 2006) and 0.21 ha (Sweazy et al. 2013); both were larger spray zones than in the current study, which ranged from 0.08 – 0.12 ha. Jones et al. (2006) applied 552 L of the proteins (either egg white, milk, and soy milk) to 0.4 ha plots within an apple tree orchard. They were able to collect a total of 87 *Cydia pomonella* L. using sticky cards with 46.5% positively marked with at least one of three markers (egg white, milk, and soy milk). The spray zones were 5 and 3 times larger than the smallest and largest spray zones in the current study, respectively, with 9 times more protein applied. Similarly, Sweazy et al. (2013) conducted a two-year study collecting *L. hesperus* (Knight) nymphs and adults from alfalfa and strawberries. They applied protein markers at 7.6 L/ha to 0.21 ha plots. They had 30.1% and 28.9% of the total number of collected nymphs and adults, respectively, positively marked with egg white; however, they had only 8.3% and 17.8% of the total number of collected nymphs and adults, respectively, positively marked in the second year of their study. The spray zones were 3 and 2 times larger than our smallest (0.08 ha) and largest (0.12 ha) spray zones, respectively; however, we applied 7 times more protein than in Sweazy et al. (2013) study. Therefore, our study applied a comparable amount of marker within designated zones, but additional abiotic factors may have impacted protein marker longevity on soybean leaf surfaces.

Abiotic factors such as rain have been shown to reduce the effectiveness of the milk and egg white protein markers (Jones et al., 2006). Jones et al. (2006) found that the egg albumin is water-soluble and under a light rain may redistribute on the leaf but wash

off with heavy or continuous rainfall. In addition, they found that milk markers were more likely to be rain-fast, thus lasting longer in the field. There were differences in number of marked individuals recovered between years, which were likely due to differences in precipitation and other abiotic factors. For example, there was few to no positively marked *D. texanus* collected (0.5% total) in 2013 from fields 3-6, even though numerous adults (n = 1173) were collected within these fields (Table 3.2). Conversely, protein marked individuals in 2012 and 2014 were approximately 5% and 9%, respectively. Interestingly the cumulative rainfall during the timeframe of applications ranged as well with 10.62 cm, 9.4 cm, and 3.78 cm in 2012, 2013, and 2014, respectively. The low level of precipitation in 2014 was advantageous for protein marking and resulted in the highest percent of positively marked individuals. Although the amount of rainfall in 2012 would suggest there would be less positively marked individuals recovered, there was also 1-2 more protein marker applications applied than in 2013 and 2014. In this case the additional protein applications helped overcome the amount pf precipitation and allowed us to collect more positively marked individuals to examine dispersal. In general, number of marked individuals can be quite variable, despite protein concentration and size of spray zones. Future studies need to address issues around protein solubility, which may affect protein effectiveness within the soybean canopy and influence the number of positively marked *D. texanus* within a field.

Dectes texanus adults are reported to rest in the upper one-third of the canopy (Campbell 1980). Protein markers were predominantly found and/or remained longer in the lower portions of the canopy based on results from the leaflet disc bioassay study. Both fields 7 and 8 had more milk positives leaves collected from the bottom of the

canopy (26% and 31% of the total leaves collected for fields 7 and 8, respectively). Similarly, both fields also had more egg white positive leaves recovered from the middle of the canopy at, 23 % and 21% of the total leaves collected for fields 7 and 8, respectively (Table 3.9). This may be explained by the architect of the soybean canopy. As the soybean plant grows, the petioles of the middle canopy will grow up and over the shorter petioles of the newer trifoliates at the top of the plant. Since the older, predominate foliage is from the middle nodes, this would help in explaining why most of the protein markers were detected in the mid to lower parts of the canopy longer; more protein is coming into contact with the leaves of the mid canopy and ultimately adult *D. texanus*.

We were able to evaluate the longevity within the soybean canopy and drift of protein markers away from spray zones. On a field basis the highest percent of positively marked samples were recovered on the collection day following the protein application (Fig. 3.5). For example, in field 7, 23% of the *D. texanus* adults collected the day after a protein application were positively marked with at least one of the two proteins. Overall there were more leaves positively marked with milk (24% compared to 20% in field 8) in field 7, while field 8 had more egg white (28% and 17%) positive samples. Consistent with other studies conducted by Jones et al. (2006) and Hagler and Jones (2010), proteins were retained within the canopy for 34 and 27 d after bovine casein was applied and 27 and 20 d after egg albumin, for fields 7 and 8, respectively. We did observe a sharp decline in the number of positively marked leaves with egg white in both fields 7 and 8, 4 d after the protein marker was applied. In field 7 the number of positive samples collected declined from 31% to < 1%, while in field 8 the number of positive samples decreased

from 30% to < 1%. There is no obvious explanation for why this occurred in the egg white spray zones and not in the milk spray zones. As previously discussed, abiotic factors, such as precipitation and humidity likely have a greater effect on the egg white marker more than the milk marker. The study also found that the 5 rows directly under the spray boom had more positively marked leaf samples than the other rows sampled, while outer rows had the fewest marked samples in both fields. This indicates that there was minimal drifting of protein markers when using our application method, and cross contamination unlikely. Therefore, with appropriately spaced out application timings and distances between marking locations this method could be successfully used for future monitoring of *D. texanus* as well as other species in experiments where multiple markers are used in the same vicinities.

This study found that protein markers can be effective for investigating the dispersal capabilities of *D. texanus* and supports the hypothesis that *D. texanus* of moving throughout a soybean field. More importantly, through this study we gained knowledge on previously unknown dispersal distance capabilities of *D. texanus* within the field. Furthermore, the methods used in this study may also prove beneficial in monitoring dispersal of other arthropod species found within soybean (i.e. bean leaf beetles, stink bugs, etc.). Future studies that incorporate more detailed environmental conditions (i.e. precipitation, wind, temperature, humidity) for specific fields samples in examining dispersal would be beneficial and possibly explain the variability seen between fields and years.

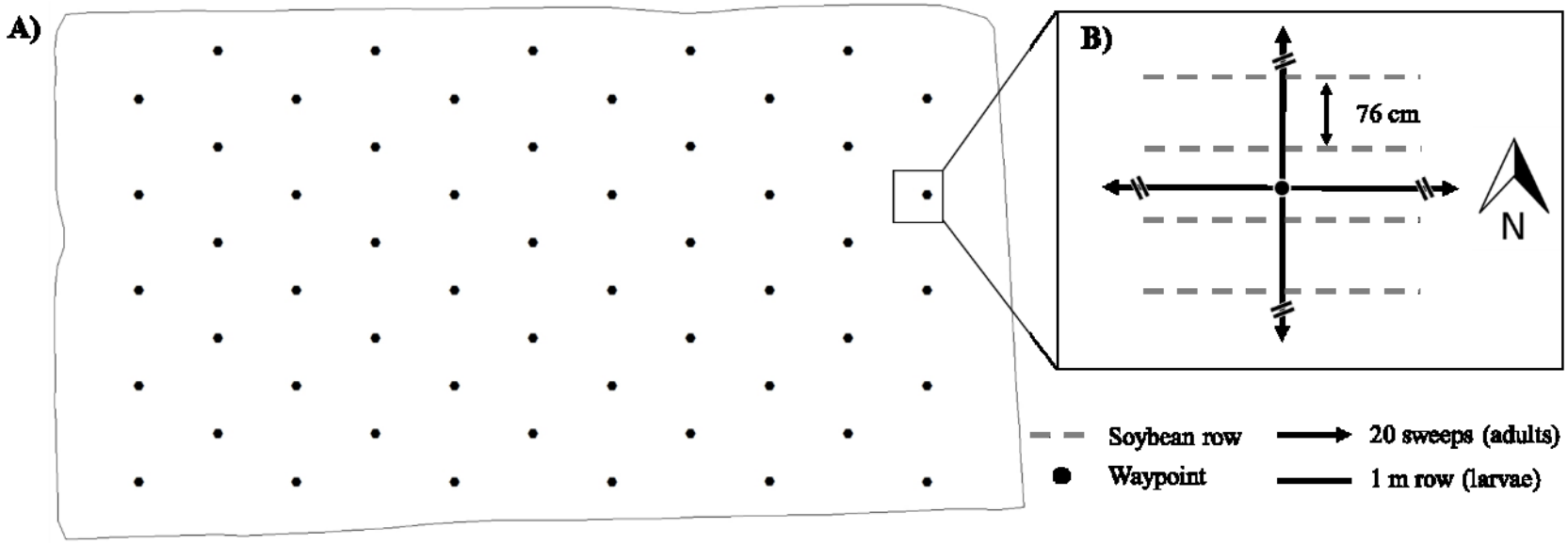

Figure 3.1. Diagram depicting the sweep net sampling scheme used to collect adult *D. texanus* from each sample point within the soybean fields. A) example grid sampling plan. B) sampling scheme for adult *D. texanus*. The gray dash lines represent the soybean rows (30 in. row spacing), while the black arrows represent the cardinal directions (North, South, East and West) in which samples were taken from the waypoint. Sweep samples (n = 20) were taken in each cardinal direction resulting in a total of n = 80. The distance between waypoints was 25 × 25 m in 2012 and 50 × 50 m for 2013 and 2014.

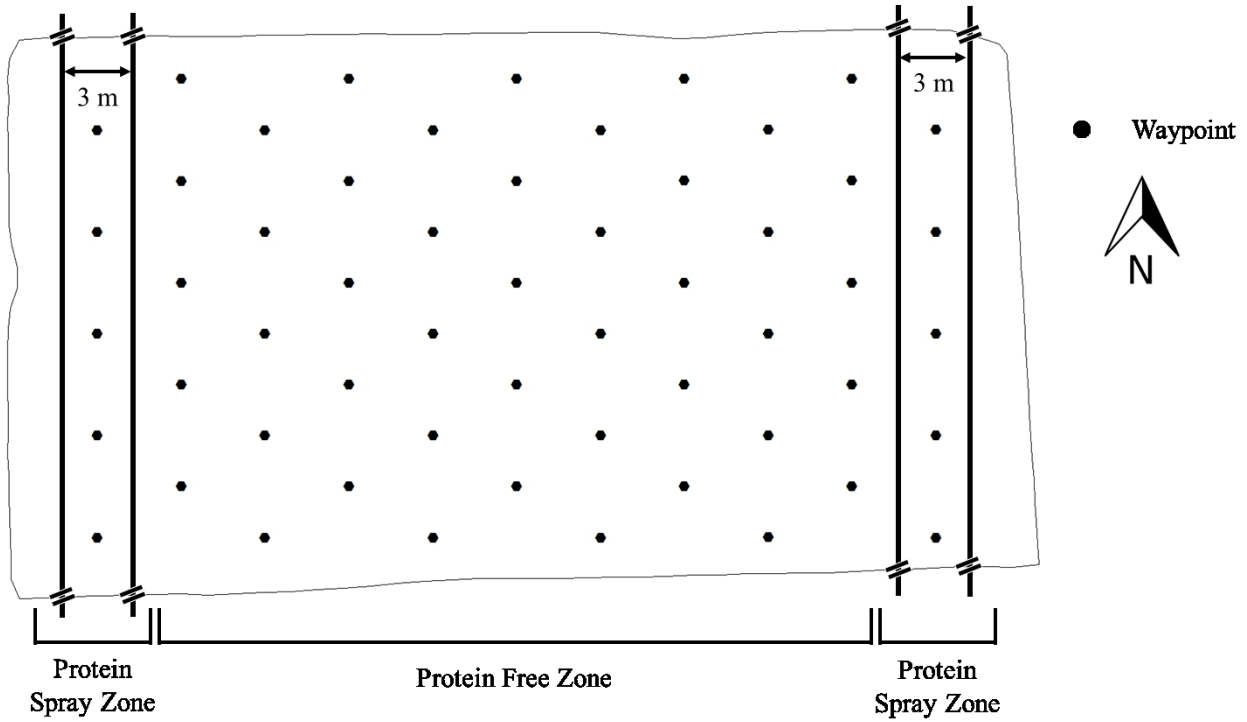

Figure 3.2. Example protein marker application scheme used for Fields 1-8 in 2012, 2013, and 2014. The protein markers were applied along the sample points (from the grid sampling plan) on two opposing edges within each field, resulting in three distinct areas in the field: two spray zones (egg albumin (egg white) and bovine casein (milk)) and a protein-free zone, which was soybean between the spray areas where no direct protein applications were made.

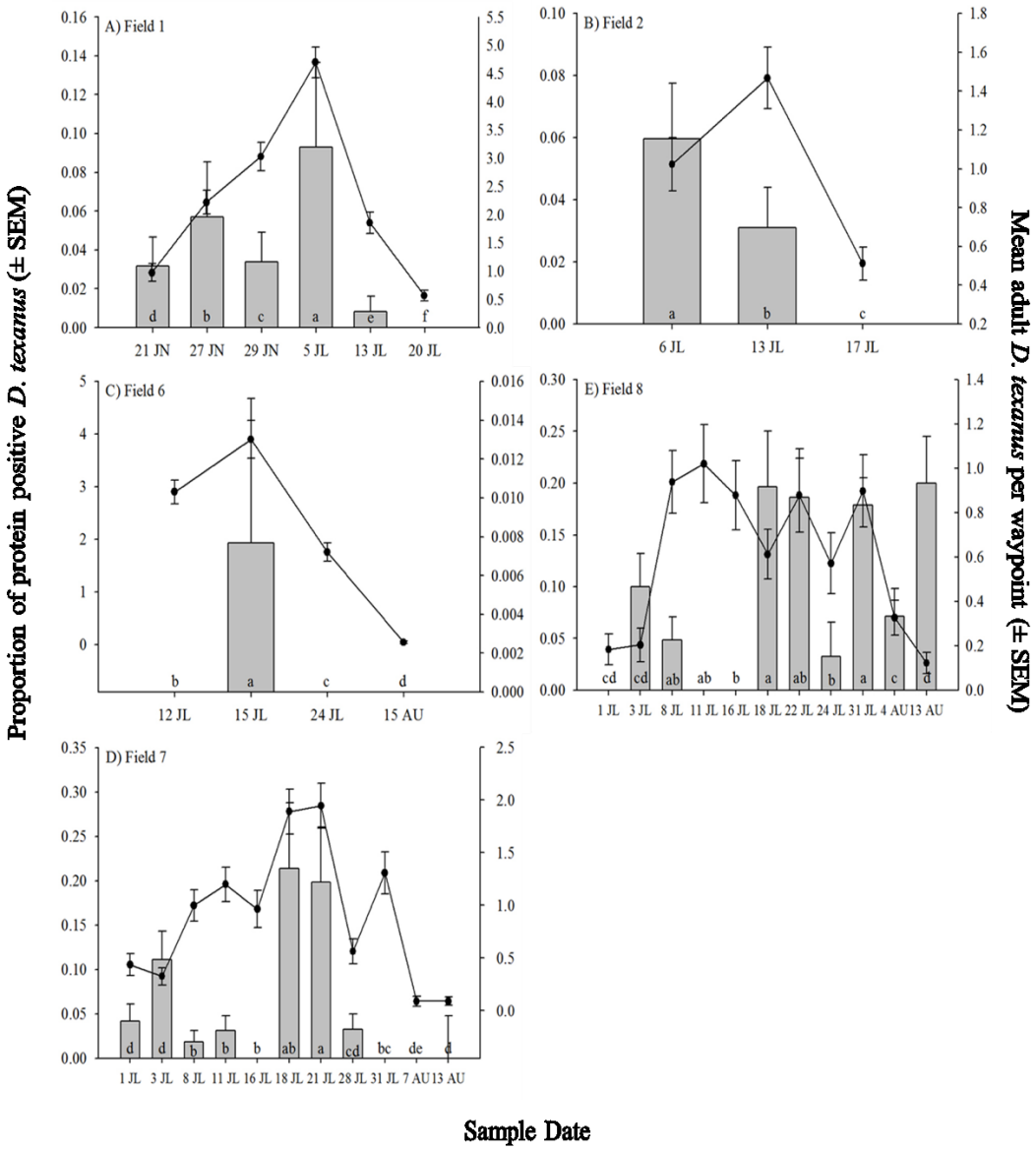

Figure 3.3. Proportion of protein positive marked *D. texanus* adults recovered through time and mean adult *D. texanus* collected per waypoint for fields 1 (A), 2 (B), 6 (C), 7 (D), and 8 (E). The X-axis represents the sample dates through the season with JN = June, JL = August and AU = August. The Y-axis represent the proportion of protein positive *D. texanus* collected (gray bar) and the mean adult *D. texanus* collected per waypoint (solid line). The letters represent significant differences between the proportion of protein positive samples collected between dates.

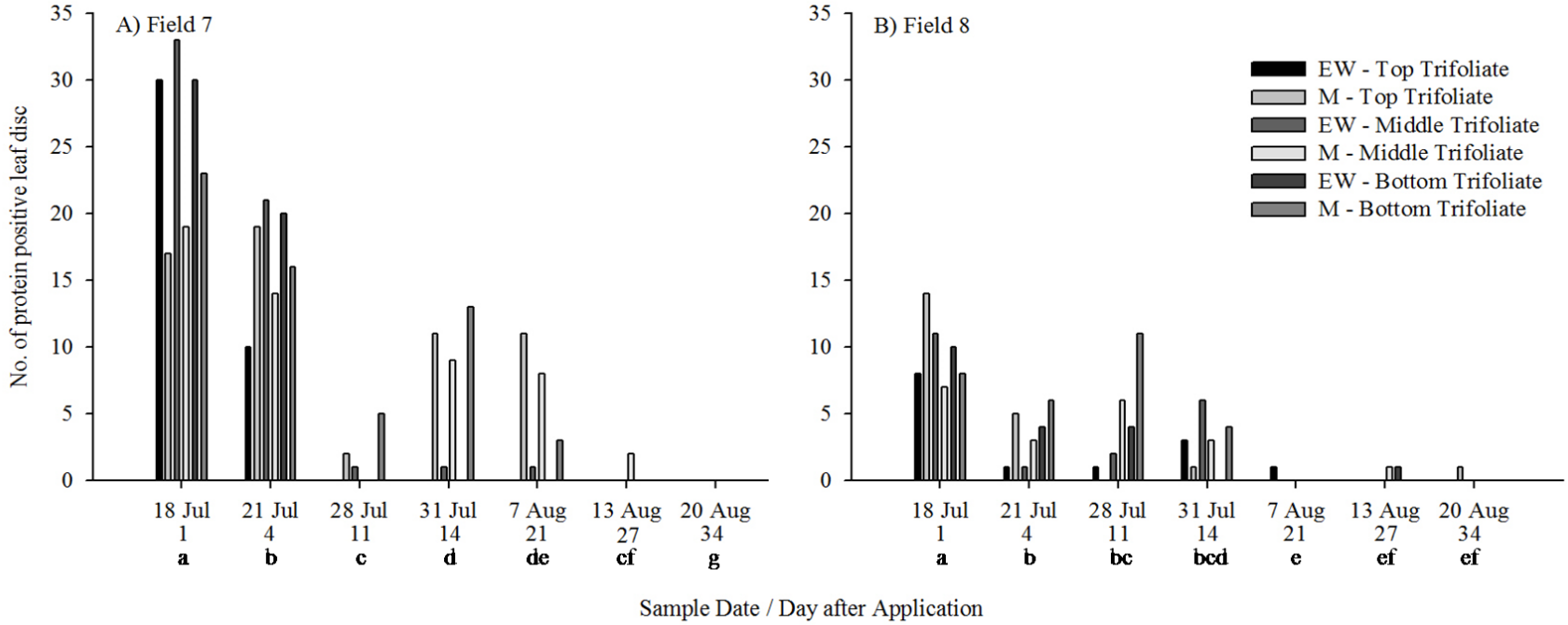

Figure 3.4. The total number of positively marked leaf samples through time, in each trifoliate section, after the second protein application (17 Jul) in: A) Field 7 and B) Field 8. The figure shows the positively marked egg white (EW) and milk (M) for the: top, middle and bottom trifoliates.

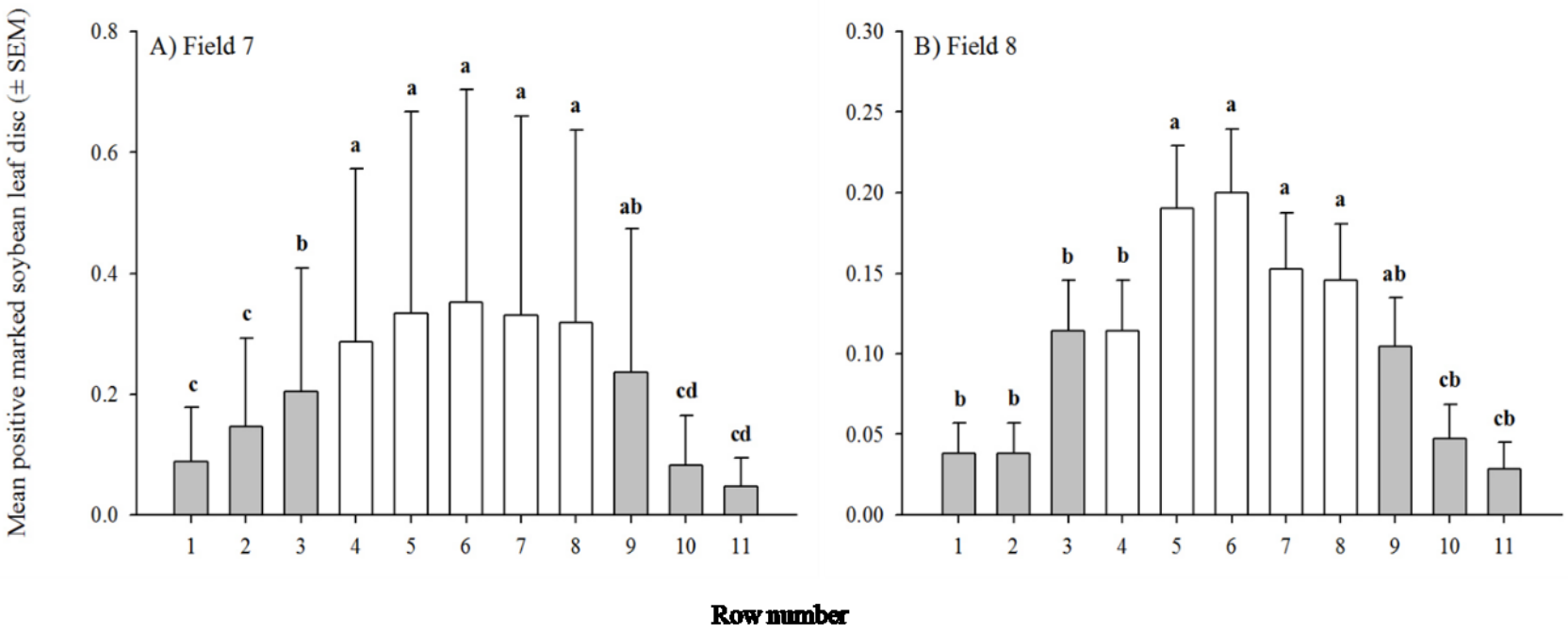

Figure 3.5. Comparison between the proportion of positively marked leaf disc samples collected from field 7 (A) and Field 8 (B) (± standard error mean) across the 11 rows and sample dates; significant differences are indicated above. The white bars indicate rows directly located under the spray boom and the gray bars indicate the rows beyond the boom used to examine drift.

Table 3.1. Field information for the soybean production fields sampled during 2012, 2013, and 2014 for the protein marking studies.

Year	Field	County	Longitude	Latitude	ha	No. of sample points	Date of Protien Application	spray area size (m^2)	spray rate (L/m)	Seed Company/ Brand	Variety	Relative Maturity
2012	1	Republic	-97.841373	39.859500	8	69	19-Jun 25-Jun 3-Jul	1272	1 L/28 m	Syngenta	S36-B6	3.6
	2	Republic	-97.854640	39.783184	11	90	19-Jun 25-Jun 3-Jul 10-Jul	1227	1 L/27 m	Kruger	K2-3701	3.6
2013	3	Dickinson	-97.138745	38.930330	15	58	16-Jul	1170	1 L/26 m	Pioneer	94Y23	4.2
	4	Dickinson	-97.183109	38.865889	12	49	16-Jul	1149	1 L/25 m	Ohlde	421	4.2
	5	Republic	-97.857947	39.783313	11	47	10-Jul 22-Jul	1170	1 L/26 m	Syngenta	S36-B6	3.6
	6	Republic	-97.840875	39.859501	8	69	10-Jul 22-Jul	1272	1 L/28 m	Syngenta	S39-U2	3.9
2014	7	Republic	-97.864401	39.782906	11	55	30-Jun 17-Jul	840	1 L/18 m	--	--	--
	8	Republic	-97.859210	39.806500	10	49	30-Jun 17-Jul	1182	1 L/26 m	Syngenta	S38-W4	3.8

Table 3.2. The total number of positively marked *D. texanus* adults recovered from all fields sampled in 2012, 2013, and 2014.

Year	Field	Total No. Collected[a]	Protein Marker	Location[b]	No. of Positive[c]	Proportion positive[d]
2012	F1	869	Egg White[e]	non-marked area	2	0.002
				EW marked area only	32	0.037
				M marked area only	1	0.001
			Bovine Milk[f]	non-marked area	3	0.003
				M marked area only	7	0.008
				EW marked area only	1	0.001
2012	F2	257	Egg White	non-marked area	1	0.004
				EW marked area only	4	0.016
				M marked area only	0	0.000
			Bovine Milk	non-marked area	0	0.000
				M marked area only	4	0.016
				EW marked area only	0	0.000
2013	F3	96	Egg White	non-marked area	0	0.000
				EW marked area only	1	0.010
				M marked area only	0	0.000
			Bovine Milk	non-marked area	0	0.000
				M marked area only	0	0.000
				EW marked area only	0	0.000
2013	F4	18	Egg White	non-marked area	0	0.000
				EW marked area only	0	0.000
				M marked area only	0	0.000
			Bovine Milk	non-marked area	0	0.000
				M marked area only	0	0.000
				EW marked area only	0	0.000
2013	F5	166	Egg White	non-marked area	0	0.000
				EW marked area only	1	0.006
				M marked area only	0	0.000
			Bovine Milk	non-marked area	0	0.000
				M marked area only	0	0.000
				EW marked area only	0	0.000

2013	F6	580	Egg White	non-marked area	1	0.002
				EW marked area only	0	0.000
				M marked area only	0	0.000
			Bovine Milk	non-marked area	1	0.002
				M marked area only	0	0.000
				EW marked area only	0	0.000
2014	F7	499	Egg White	non-marked area	5	0.010
				EW marked area only	20	0.040
				M marked area only	0	0.000
			Bovine Milk	non-marked area	3	0.006
				M marked area only	15	0.030
				EW marked area only	1	0.002
2014	F8	340	Egg White	non-marked area	6	0.018
				EW marked area only	11	0.032
				M marked area only	0	0.000
			Bovine Milk	non-marked area	4	0.012
				M marked area only	7	0.021
				EW marked area only	3	0.009

[a] : Total number of adult *D. texanus* collected within each field, respectively.

[b] : Location in the field where marked individuals were recovered.

[c] : Total number of postiviely marked samples per zone

[d] : Proportion of the samples testing positive for the protein that is targeted

[e] : Egg white protein, which will be depicted as EW under location

[f] : Bovine casein protein, which will be depicted as M under location

Table 3.3. Results of the generalized estimate equations conducted on the number of positively marked *D. texanus* samples recovered within Fields 1, 2, 6, 7, and 8. Fields 3, 4, and 5 were excluded from analysis due to the low or no numbers of positively marked samples recovered.

Year	Field	Variable	*df*	X^2	*P*
2012	1	Zone	2	1.83	0.4009
		Marker	2	89.1	<0.0001∗
		Sample Date	5	60.19	<0.0001∗
	2	Zone	2	5.08	0.0787
		Marker	2	74.68	<0.0001∗
		Sample Date	2	24.29	<0.0001∗
2013	6	Zone	2	4.01	0.1346
		Marker	2	93.96	<0.0001∗
		Sample Date	3	29.94	<0.0001∗
2014	7	Zone	2	3.75	0.153
		Marker	2	59.11	<0.0001∗
		Sample Date	10	49.21	<0.0001∗
	8	Zone	2	10.43	0.0054∗
		Marker	2	52.9	<0.0001∗
		Sample Date	10	37.14	<0.0001∗

∗: Indicates significant differences at $\alpha = 0.05$

Table 3.4. Results of the regression analysis examining the relationship between the proportion of positively marked samples and the mean number of *D. texanus* collected per waypoint in fields 1, 2, 6, 7 and 8. Field 3-5 were excluded from analysis because of the low or no positively marked adults collected.

Year	Field	*F*	*df*	*P*	R^2
2012	1	7.18	1,4	0.05*	0.55
	2	0.28	1,1	0.69	-0.56
2013	6	1.84	1,2	0.31	0.22
2014	7	4.74	1,9	0.05*	0.27
	8	0.002	1,10	0.96	-0.11

*: Indicates significant differences at $\alpha = 0.05$

Table 3.5. The calculated minimum and maximum distance traveled for adults positively marked with egg white and milk. Measurements were taken on adults found outside of the spray zones.

Field	Protein Marker	No. of Adult Dispersal Measured	Min. Mean Distance Flown	± SEM	Max Mean Distance Flown	± SEM	Protein Spray Edge
1	Milk	3	90	30.8	65	24.2	North
	Egg White	2	102	39.8	279	0.0	South
2	Milk	--[a]	--	--	--	--	South
	Egg White	1	211	--	373	--	North
6	Milk	1	55	--	304	--	South
	Egg White	1	55	--	389	--	North
7	Milk	3	217	43.3	270	37.8	West
	Egg White	5	52	8.1	212	12.1	East
8	Milk	4	114	20.9	114	36.9	West
	Egg White	3	76	43.7	268	32.2	East

a: (--) indicates no samples recovered to measure distance flown.

Table 3.6. The number of positively marked leaf samples collected from the top (new growth), middle (middle nodes) and bottom (bottom most node) of the canopy based on the optical density (OD) values derived from the Enzyme Linked Immunosorbent Assay (ELISA) analysis.

Field	Protien Marker	Node Location of Leaf	Total Collected[a]	Total Marked	Proportion Marked
7	Milk	T	371	60	0.16
		M	371	52	0.14
		B	369	60	0.16
	Egg White	T	351	40	0.11
		M	352	57	0.16
		B	352	50	0.14
8	Milk	T	153	21	0.14
		M	154	20	0.13
		B	154	29	0.19
	Egg White	T	230	14	0.06
		M	230	20	0.09
		B	231	19	0.08

a: Total number of collected leaf discs across all rows
b: "T" inicates top or new growth
c: "M" indicates nodes from the middle of the canopy
d: "B" indicates nodes from the bottom of the canopy

Table 3.7. The total number of collected samples from the egg white and milk spray zones in field 7 for each date samples were collected from the field. The information includes the number of positively marked samples recovered from the top, middle, and bottom of the canopy.

		Protein Marker					
		Milk			Egg White		
Date	Canopy Location of Leaf	Total Collected	Total Marked	Proportion Marked	Total Collected	Total Marked	Proportion Marked
1-Jul	T	55	27	0.491	55	27	0.491
	M	55	24	0.436	54	31	0.574
	B	55	24	0.436	55	22	0.400
3-Jul	T	55	36	0.655	55	37	0.673
	M	55	44	0.800	54	37	0.685
	B	54	40	0.741	55	37	0.673
8-Jul	T	55	5	0.091	55	0	0.000
	M	55	19	0.345	55	6	0.109
	B	55	30	0.545	55	2	0.036
11-Jul	T	55	1	0.018	55	0	0.000
	M	55	1	0.018	55	0	0.000
	B	55	0	0.000	55	0	0.000
18-Jul	T	55	17	0.309	55	30	0.545
	M	55	19	0.345	55	33	0.600
	B	54	23	0.426	55	30	0.545
21-Jul	T	55	19	0.345	55	10	0.182
	M	55	14	0.255	55	21	0.382
	B	54	16	0.296	55	20	0.364
28-Jul	T	55	2	0.036	54	0	0.000
	M	55	0	0.000	55	1	0.018
	B	55	5	0.091	55	0	0.000
31-Jul	T	52	11	0.212	55	0	0.000
	M	52	9	0.173	55	1	0.018
	B	52	13	0.250	55	0	0.000

7-Aug	T	44	11	0.250	22	0	0.000
	M	44	8	0.182	22	1	0.045
	B	44	3	0.068	22	0	0.000
13-Aug	T	55	0	0.000	55	0	0.000
	M	55	2	0.036	55	0	0.000
	B	55	0	0.000	55	0	0.000
20-Aug	T	55	0	0.000	55	0	0.000
	M	55	0	0.000	55	0	0.000
	B	55	0	0.000	55	0	0.000

Table 3.8. The total number of collected samples from the egg white and milk spray zones in field 8 for each date samples were collected from the field. The information includes the number of positively marked samples recovered from the top, middle, and bottom of the canopy.

		Protein Marker					
		Milk			Egg White		
Date	Canopy Location of Leaf	Total Collected	Total Marked	Proportion Marked	Total Collected	Total Marked	Proportion Marked
1-Jul	T	22	12	0.545	33	15	0.455
	M	22	15	0.682	33	20	0.606
	B	22	9	0.409	33	15	0.455
3-Jul	T	22	13	0.591	32	15	0.469
	M	22	16	0.727	33	19	0.576
	B	22	17	0.773	33	18	0.545
8-Jul	T	21	10	0.476	33	2	0.061
	M	22	12	0.545	33	13	0.394
	B	21	13	0.619	33	13	0.394
11-Jul	T	22	1	0.045	33	0	0.000
	M	22	9	0.409	33	3	0.091
	B	22	7	0.318	33	2	0.061
18-Jul	T	22	14	0.636	33	8	0.242
	M	22	7	0.318	33	11	0.333
	B	22	8	0.364	33	10	0.303
22-Jul	T	21	5	0.238	33	1	0.030
	M	22	3	0.136	31	1	0.032
	B	22	6	0.273	33	4	0.121
24-Jul	T	22	0	0.000	33	1	0.030
	M	22	6	0.273	33	2	0.061
	B	22	11	0.500	33	4	0.121
31-Jul	T	22	1	0.045	33	3	0.091
	M	22	3	0.136	33	6	0.182
	B	22	4	0.182	33	0	0.000

4-Aug	T	22	0	0.000	33	1	0.030
	M	22	0	0.000	33	0	0.000
	B	22	0	0.000	33	0	0.000
13-Aug	T	22	0	0.000	33	0	0.000
	M	22	1	0.045	33	0	0.000
	B	22	0	0.000	33	1	0.030
20-Aug	T	22	1	0.045	33	0	0.000
	M	22	0	0.000	33	0	0.000
	B	22	0	0.000	33	0	0.000

Chapter 4 - Suitability of spectral response properties for identifying and characterizing *Dectes texanus* LeConte (Coleoptera: Cerambycidae) infestations in soybean (*Glycine max*, L.)

Introduction

Dectes texanus LeConte (Coleoptera: Cerambycidae) is a native, univoltine species that is an important pest in Kansas soybean (Michaud and Grant 2005, Buschman and Sloderbeck 2010, Sloderbeck and Buschman 2011). The lifecycle of the *D. texanus* is well known (Patrick 1973; Laster 1981; Hatchet et al. 1975; Michaud and Grant 2005; Niide 2009; Buschman and Sloderbeck 2010). Adults emerge in mid-June to early July and soon after begin to mate and females deposit eggs into the pith of soybean petioles. Upon hatching, early instars tunnel into stems and petioles and feed on the pith, gradually moving into the main stem where larvae continue to feed throughout late instar stages. Toward the end of the growing season, larvae will move to the base of the stem and girdle or cut the soybean plant approximately 5 cm above the soil line. This behavior prepares an overwintering chamber and prevents conspecifics from reaching the base of the stem. Due to the tunneling activity and girdling behavior, larvae are considered the most damaging stage to soybean plants, causing a 7-11% decrease in seed weight or up to 10% overall yield loss per plant (Richardson 1975, Buschman et al. 2006). Additionally, a study by Daugherty and Jackson (1969), found that fields with nearly 100% infested

plants resulted in 17% stalk lodging and measurable yield loss due to harvestability or mechanical issues.

Managing *D. texanus* in soybean production fields is difficult due to the tunneling activity and girdling behavior of late-instar larvae (Crook et al. 2004; Michaud and Grant 2005). Historically, recommendations for managing *D. texanus* included cultural controls (i.e., tillage, crop rotation, trap crops) and use of foliar insecticides (Campbell and van Duyn 1977, Michaud and Grant 2005, Michaud et al. 2007, Sloderbeck and Buschman 2011); however, such practices are considered outdated, impractical or costly for *D. texanus* control (Michaud et al. 2007, Sloderbeck and Buschman 2011). Management of *D. texanus* is further compounded by the difficulty to efficiently detect *D. texanus* infestations in soybean fields. Currently, detection of *D. texanus* (adults and larvae) relies on ground surveys to determine infestation levels. Detecting highly active adults in dense soybean canopies is also difficult, especially if the soybean field is large (> 12 ha). Sampling for larvae is time-consuming since larvae are protected within the main stem and require destructive sampling techniques that involve splitting open a soybean stem to positively identify larvae. To overcome some of these survey limitations, the use of novel methods for detecting *D. texanus*, such as remote sensing, may prove beneficial for developing new and effective sampling strategies (Maret and Johnson 1999, Ma et al. 2005, Prabhakar et al. 2013).

There is an increased interest in using remote sensing platforms to assess crop conditions (i.e., "plant health," yield, pest pressure) in real-time (Hatfield and Pinter 1993). Specifically, aerial photography using near infrared (NIR) sensors is becoming a valuable tool for identifying and monitoring pest populations (Pinter et al. 2003). Near

infrared sensor technologies can be used to collect highly temporal data (measurements with respect to time) while maintaining quality spatial resolution, which is determined by the number of pixels used to construct an image (Riley 1989, Pinter et al. 2003). Both attributes are ideal for monitoring pest populations, due to the temporal and spatial capabilities, because insect populations can change quickly through time. More recent advances in NIR sensor technologies include modifications to commercially available "point-and-shoot" cameras, which make the technology more affordable and/or accessible for pest management. For these applications, the camera sensors are modified to filter out red light (580 nm – 680 nm) and capture images in the NIR region, which ranges from 780 to 1400 nm (Cicek et al. 2010, Van der Merwe and Price 2015). As such, modified NIR cameras have been used to monitor the growth and health status of many crops like corn (Wallen et al. 1976), wheat (Elliot et al. 2009), rice (Zhao et al. 2013), and cotton (Lan et al. 2013). This is primarily because NIR cameras are easily operated, relatively inexpensive, and can be used in a range of environmental settings and agriculture systems. Since NIR cameras utilize the NIR region, use of vegetation indices is highly advantageous for detecting certain plant responses. Chlorophyll is highly reflective in the NIR region, allowing researchers to identify changes in the spectral properties of healthy and maturing green vegetation, which is also correlated to plant phenology and stress (Kollenkark et al. 1982, Jensen 2007). Due to the relationship between chlorophyll and the NIR region, numerous vegetation indices such as the Normalized Difference Vegetation Index (NDVI), Green Normalized Difference Vegetation Index (GNDVI) and Enhanced Vegetation Index (EVI) have been developed (Jensen 2007). Such indices are sensitive to changes in green vegetation and can assist in

predicting yields in field crops and can be used to help identify variations in vegetation (Bannari et al. 1995, van Leeuwen et al. 2010, Rullan-Silva et al. 2013).

Insect feeding and subsequent plant damage changes how a crop canopy intercepts or reflects light. As damage accumulates and changes the reflectance of wavelengths penetrating the crop canopy correlations can be made to specific types of insect damage (Riley 1989, Hatfield and Pinter, Jr. 1993, Pinter et al. 2003). For example, Yang et al. (2007) identified spectral characteristics associated with brown planthopper (*Nilaparvata lugens* Stål) and leaffolder (*Cnaphalocrosis medinalis* Guenee) damage in rice and used NDVI and GNDVI to differentiate between levels of damage severity. Ma et al. (2005) used vegetation indices to correlate specific wavelengths to locusts (*Locusta migratoria manilensis*) outbreak in East Asia. By identifying specific wavelengths characteristic to locust infestation, locust damage is monitored in real-time to produce responsive management decisions. Under field conditions, Lestina et al. (2016) used the enhanced vegetation index (EVI) to more narrowly predict the habitat for stem sawfly (*Cephus cinctus* Norton). In this study, EVI provided additional information for identifying ecological responses due to the capacity of the index to measure variation in host phenology, which are limited by other indices (Lestina et al. 2016). Given the above examples, biologically meaningful correlations between insect pests and measurable parameters are possible and such indices may be useful in detection and monitoring of *D. texanus* larva in soybean plants. Although stem-boring insects may not directly affect the green vegetation, they may indirectly impact the vegetation by feeding on soybean pith. In general, stem boring due to insects (i.e. dogwood borer (*Thamnosphecia scitul* (Harris)), woodwasp (*Sirex noctilio* (F)), European corn borer (*Ostrinia nubilalis*

(Hiibner))) affect photosynthetic capacity due to damage of the vascular tissue within the pith, thus disrupting xylem and phloem functions (Heichel and Turner 1973, Madden 1977, Godfrey et al. 1991, Trumble et al. 1993). However, the effect of pith boring in soybean and subsequent effects on how leaves absorb or reflect light is not known.

Soybean vegetation indices have been used to model changes in soybean phenology and plant health through the growing season (van Leeuwen et al. 2010, Buma et al. 2013). We wanted to determine if changes in soybean spectral response due to *D. texanus* feeding was measurable under field conditions. Our objective was to investigate the utility of vegetation indices as a method to detect soybean infested with *D. texanus*. In 2013 and 2014, we conducted two field experiments comparing varying densities of both natural and artificial *D. texanus* larval infestations to investigate whether vegetation indices can be used to detect *D. texanus* infestations in soybean. We hypothesized that soybean infested with *D. texanus* will have lower leaf reflectance due to physiological stress from larval feeding and subsequent tunneling of the main stem. In addition to affecting the soybean spectral response, larval feeding may also alter the growth and development of the soybean plant, which has been observed for other soybean pests. For example, fields heavily infested with soybean aphid can result in plant stunting, leaf distortion and a reduced number of pods (Hill et al. 2004). Therefore, we also examined characteristics of soybean plant response to *D. texanus* larval infestations under field conditions. We hypothesized that *D. texanus* infestation would negatively affect soybean growth and overall size. We predicted that infested plants would be stunted (i.e. smaller stem diameter, fewer nodes, and shorter in height) and have lower seed weights and reduced yield compared to non-infested plants.

Methods and Material

Study Sites

We conducted two field studies: the first used exclusion cages and the second was an open-plot design. In the exclusion cage study, other arthropods were excluded from soybean plants using cages, while plants in cages were artificially infested with a known number of adult *D. texanus*. Using a range of infestation levels in this study allowed us to quantify changes in soybean plant responses (e.g., vegetation indices values, yield, plant height, etc.) in the absence of other pest species. The exclusion cage study was conducted in 2013 and repeated in 2014 at the Ashland Bottoms Research Farm, Manhattan, KS (Riley County, 39.3333° N, 96.7000° W). Dryland production fields were selected based on consistent management practices between years. Soybeans (var. KS3406RR) were planted in late May both years of the study at 356K seeds per ha using a row-spacing of 76.2 cm (30”); field size was 7 and 9.5 ha in 2013 and 2014, respectively. The open-plot study measured soybean plant responses to naturally occurring *D. texanus* infestations under field conditions. The study was conducted in 2014 at the Kansas Agricultural Experiment Irrigation Station near Scandia, KS (Republic Co., 39.8000° N, 97.6333° W) in a soybean field with historically high *D. texanus* populations. Soybeans (var. P39T67R, Pioneer®) were planted on 20 May 2014 with a 76.2 cm (30”) row-spacing at 356K seeds per ha. The field was flood-irrigated as needed. All locations used for the cage and open-plot studies were managed using reduced tillage and were on a corn-soybean rotation.

Exclusion Cage Study

Experimental units (exclusion cages) were arranged in a randomized complete block design (RCBD; block size = 10). The experiment was initiated when all soybeans reached the V1 growth stage (Fehr and Caviness 1977). The exclusion cage design used by McCarville and O'Neal (2012) was modified in size and used for this experiment. Cage frames were constructed using 19 mm (3/4") diameter Polyvinyl Chloride (PVC) pipe (JM Eagle: Los Angeles, CA). Cage frames measured 0.6 × 0.6 × 1.2 m (length × width × height) and were covered with white No-See-Um Mesh (Quest Outfitters: Sarasota, FL). Netting prevented *D. texanus* adults from escaping while limiting oviposition by natural populations of *D. texanus* and excluding defoliators from surrounding areas around the soybean field. Cages were placed over a single row of soybean within the field in a 5 × 10 grid; cages were spaced approximately 3 and 1.5 m within and between rows, respectively. Areas in the field were scouted to maintain as even a plant density as possible within each cage; however due to over-pruning during cage placement soybean plant densities varied within each cage from 1 – 11 plants.

There were five infestation levels used to model changes in spectral response by soybean: two controls (non-infested and defoliation only), and 4, 16, and 30 individual *D. texanus* per cage, hereafter referred to as "low," "medium," and "high" infestation levels, respectively. All adult *D. texanus* used for artificial infestation in the study were collected by sweep netting soybean fields and naturally occurring stands of giant ragweed (*Ambrosia trifida*, L.), which is a native host of *D. texanus*, along roadside ditches neighboring the study sites. Before placing adults within the exclusion cages, adult *D. texanus* were sexed based on the last abdominal sternite (see Chapter 1, Hatchett et al.

1975). Cages that received *D. texanus* adults had an equal number of males and females per treatment to increase the chances for successful oviposition events and subsequent larval development in caged soybean plants. Grasshoppers (family Acrididae; various spp.) are a generalist pest found in soybean and were used in all defoliation treatments to cause natural chewing damage, which was then compared to soybean damaged by *D. texanus* larval feeding. Grasshoppers were also collected from surrounding crops and vegetation using sweep nets.

In 2013, the low, medium, and high infestation cages were infested with 2, 8, and 15 mated pairs of *D. texanus*, respectively, on 1 Aug, 27 Jul, and 19 Jul. Cages were infested at different times due to time constraints and availability of target pests. All *D. texanus* cages were re-infested on 15 Aug 2013 with 4 adults (i.e., two mating pairs) to increase the likelihood of having soybean plants with developing larvae. Cages designated with the defoliator treatment were infested on 7 Aug 2013 with two adult grasshoppers to defoliate leaves but to not damage the plant beyond its ability to compensate, which was estimated at ≥ 75% defoliation (Haile et al. 1998). This study was repeated in 2014 using the same treatments described above. All cages were infested on 9 Jul 2014 with either adult *D. texanus* or defoliators. All cages infested with *D. texanus* were re-infested two weeks later with two more mating pairs, which was based on availability of adults. Since grasshopper die-off did occur, all defoliator-treated cages were continuously re-infested with 2 to 5 adults bi-weekly throughout the growing season to maintain defoliation throughout the duration of the experiment.

Open-Plot Study

The open-plot study was arranged in a generalized randomized complete block design (GRCBD, block size = 4) with two treatments: 1) untreated control and 2) insecticide-treated soybean to reduce effects of *D. texanus* and other soybean pests on the soybean canopy. Untreated plots allowed for natural infestation by adult *D. texanus*, which was essential to comparing spectral differences between infested and non-infested plots. Plots were 9.1 m long by four rows wide, with 76.2 cm (30") row spacing. There were 18 rows of soybean between each plot and a 10.7 m buffer area between each block to limit insecticide drift between plots. A spray regime similar to Buschman et al. (2005) was used in this study. Fipronil (Regent® 4SC, BASF Crop Protection, USA: Research Triangle Park, NC), a systemic insecticide, was applied to foliage in treated plots to control *D. texanus* in stems. Soybean foliage in insecticide-treated plots was sprayed with fipronil on 14 and 31 Jul 2014 at 438 (0.2 a.i. per acre) and 672 ml/ha (0.28 a.i. per acre), respectively. Within each plot, we designated 3, 1-m^2 subplots, which were spaced approximately 1.5 m apart within each plot and marked with wooden stakes. Soybean plants within the subplots were used for all plant and larval assessments.

We examined differences in *D. texanus* larval densities between treatments in the open-plot study twice during the growing season. Specifically, we collected 15, whole-plant subsamples on 29 Jul and 13 Aug 2014 from all plots post insecticide application. Individual soybean plants (subsamples) were collected within a plot but outside of the 3, 1-m^2 subplots described above to assess larval densities during the growing season. Larval densities within each subplot were only assessed at harvest to prevent plant removal from affecting the canopy structure within a subplot. Plants were transported to

the lab where the main stem and all petioles were cut open and examined for *D. texanus* larvae. The number of dead and alive larvae was recorded for each plant collected.

Larval presence and plant assessment

During the growing season, we measured plant height (cm) and recorded soybean growth stage (vegetative or V-stage and reproductive or R-stage) as described by Fehr et al. (1971), for all plants within the area of interest (exclusion cages or subplots) in all experiments. At the end of each experiment, and just prior to harvest, all plants within cages and subplots were removed and transported to the lab for dissection. Each plant was individually examined for the physical presence of *D. texanus* larvae or indirect presence by documenting larval entrance holes and oviposition scars on the main stem.

Plant biometric data was recorded for each plant and included stem diameter (mm), plant height (cm), seed size (g/100 seed), percent seed moisture, and yield (t/ha). Stem diameter for all collected soybean plants was measured in the lab using a digital caliper (Traceable® Digital Calipers, #CNC3416, Control Company: Mundelein, IL). Plant height (soil line to the base of the top most fully developed node) was measured in the field during the growing season with a final measurement taken in the lab after plants had been collected. Pods from each plant were also removed, counted, and threshed using a small-plot thresher (Almaco, MOD. LPR, Ser. No. 92008, Specialized Agricultural Equipment: Nevada, IA) to obtain seed size and yield estimates. The mean seed size (g per 100 soybean seeds) was calculated by measuring 3, 100-count seed weights for seed harvested from each cage or subplot. Yield was calculated and corrected to 13% moisture and presented as ton/ha.

Photogrammetric imaging of soybean canopy

A Canon Powershot S100 HS camera (LDP LLC MaxMax.com: Carlstadt, NJ) was used to capture images for the exclusion cage and open plot studies. This camera was modified to filter out visible red light (580 nm – 680 nm) and light above the NIR region (above 780 nm); resulting in images captured in the blue (450 to 495 nm), green (495 to 570 nm), and the NIR range (680-780 nm). All images were written in Joint Photographic Experts Group (JPEG) format. Images were acquired by manually holding the camera or by attaching the camera to a retractable camera mount, depending on the best method to obtain the entire cage and/or subplot within a single image. Regardless of method, camera height was kept constant for all images, which was ~170 cm above the canopy. Netting surrounding each PVC frame was opened and lowered to expose soybean plants prior to capturing images, and reclosed after images were captured. During image acquisition for the open-plot study, a white, 1 x 1 m^2 quadrat made of 19 mm (3/4") diameter PVC pipe (JM Eagle: Los Angeles, CA) was placed around each flagged subplot to easily identify sampling areas during image processing.

Depending on environmental conditions, images were captured weekly or biweekly to achieve high temporal data for the duration of each experiment. This allowed us to examine changes in the soybean canopy during the season, and consequently any differences in the rate of senescence due to *D. texanus* infestation. At the start of the exclusion cage study, soybeans were in the V14/R4 and V5/R1 stages in 2013 and 2014, respectively. Imaging in the open-plot study started when soybean were in the V8/R1 growth stage. At the completion of all studies, the soybeans were in the V14/R8 for both years of the exclusion cage study, and in the V13/R8 stage for the open-plot study. All

images were taken between 10:00 and 14:00 CDT when the sun was at its highest point during the day. This reduced variation in canopy shadow, which is known to affect the accuracy of vegetation indices. Images were taken on days with either no cloud cover or when cloud cover was < 10% and with minimal wind (< 10 mph), based on weather information provided by the National Oceanic and Atmospheric Administration (NOAA) from nearby weather stations.

The software program AgPixel™ (Goldfinch Technologies ™, LLC: Des Moines, IA) was used to extract mean brightness values. Each pixel was assigned a number ranging from 0 (bright white) to 255 or (black) for the green, blue, and NIR bands captured (Jensen 2007). These extracted values were used to calculate the vegetation indices for each cage and subplot. For each image, the canopy area was selected manually to extract brightness values. Non-canopy shadows, like those caused by cage frames, retractable pole, camera, or calibration panels were not selected for pixel value extraction. However, naturally occurring shadows within the soybean canopy were included in the canopy selection (Ranson et al. 1985). Extracted brightness values were corrected using a gray calibration panel captured within each image (Peddle et al. 2001), which corrected for variations in light intensity that may occur between images and/or collection dates. Consequently, the correction calculation was incorporated into the all vegetation indices formulas for the following indices: Green Normalized Difference Vegetation Index (GNDVI), Blue Normalized Difference Vegetation Index (BNDVI), Enhanced Normalized Difference Vegetation Index (EVI), 2-band Enhanced Normalized Difference Vegetation Index (EVI2), 3-band Enhanced Normalized Difference Vegetation Index (EVI3), Green-Blue Normalized Difference Vegetation Index

(GBNDVI), NIR Blue Ratio Vegetation Index (NIRBRVI), and NIR Green Diff. Vegetation Index (NIRGDVI) (Table 4.1). These indices were selected based on availability in AgPixel™, which has potential for broader public use. For both the exclusion cage and open-plot studies, mean vegetation index values were plotted through time to model changes in soybean phenology and to test for any differences in brightness values as it related to targeted *D. texanus* infestation levels.

Statistical Analysis

Larval counts. End of season larval counts per cage (exclusion cage study) and plot (open-plot study) were modeled using the proportion of infested plants per cage or pooled subplot, depending on study. Subplots were pooled within a plot for the open-plot study, as there were no differences between subplots (data not shown). Since infestation data was not normally distributed, we used a generalized linear mixed model with a binomial distribution and logit link function. The exclusion cage study was subject to analysis of variance (ANOVA) (PROC GLIMMIX, SAS® version 9.4, SAS Institute Inc.: Cary, NC) with *D. texanus* infestation level (proportion of plants infested) as the fixed effect and block as the random term. In using PROC GLIMMEX and non-Gaussian distribution, the studentized residuals are no longer interpretable since the model is generated on the link (not the identity; logit in this case) scale; checking normality for non-Gaussian variables modeled with PROC GLIMMEX is unnecessary. The open-plot study was also analyzed using an analysis of variance (PROC GLIMMIX) with *D. texanus* infestation level as the fixed effect and block and interaction of block by treatment as the random term. The least square means were estimated for the fixed effects on the logit scale using the *LS Means* statement and back-transformed using the ilink

option. Treatment comparisons were made using a Tukey's adjustment method at $\alpha = 0.05$ for both studies.

In the open-plot study, we also examined the infestation level after each insecticide application during the season using 15 individually collected soybean plants per plot after each application; recall, these were outside the subplots areas. Larval densities from select plants were used to determine whether insecticide applications were effective at reducing larval survival in treated plots compared to untreated controls. Differences in infestation levels were analyzed using an ANOVA (mass, aov() function, RStudio©) in a 2 × 2 factorial design; factors consisted of insecticide application (treated versus untreated) and timing (date 1 and date 2). Model significance comparing the insecticide applications were determined significant at $\alpha = 0.05$.

Vegetation indices. All vegetation indices values from the exclusion cage experiments were analyzed using a mixed model, repeated measures ANOVA (PROC MIXED, SAS® version 9.4, SAS Institute Inc., Cary, NC). In the exclusion cage study, fixed effects were days after infestation (DAI), treatment, and the interaction of treatment by DAI, with block as the random term. The fixed effect of days is defined as time since the experiment began and was used to examine the change through time in vegetation indices (GNDVI, BNDVI, EVI, EVI2, EVI3, GBNDVI, NIRBRVI, NIRGDVI). Plant density (number of plants per m row) within a cage was treated as a covariate in the model to account for the influence of plant density on vegetation indices. Cage was the repeated measure, since measures were recorded within these experimental units through time. A heterogeneous compound symmetry (CSH) covariance structure was selected based on the lowest Akaike information criterion (AIC) values. Significant interactions

were further analyzed using the slice options of the *LS Means* statement in PROC MIXED and determined significant at $\alpha = 0.05$. Based on results from the repeated measures analysis stated above, vegetation indices calculated for the exclusion cage study were used to compare infested versus non-infested cages using a mixed model repeated measures analysis of covariance (PROC MIXED). The fixed effects were infestation status (infested or non-infested cages), days after infestation (DAI), and the interaction of infestation status and DAI. We used plant density as a covariate to account for the influence of this variable on vegetation indices. Cage was the repeated measure and a heterogeneous compound symmetry (CSH) covariance structure was selected based on lowest AIC values. Significant interactions were further analyzed using the slice options of the *LS Means* statement in PROC MIXED with significance determined at $\alpha = 0.05$.

The vegetation indices used in the open-plot study were analyzed using a repeated measures ANOVA (PROC MIXED). The day since experiment start (DSS), insecticide treatment, and the interaction between insecticide treatment and DSS were all fixed effects; block was a random effect. DSS was used since the open-plot study relied on natural *D. texanus* infestations and exact infestation dates were not known; DSS begins on the first day that images were acquired. The variable of 'plant' was the combined total number of plants collected within each area of interest (the pooled 1 m sections collected per plot) and was used as a covariate to help explain variations in the vegetation indices analysis. Plot was designated as the repeated measure, with heterogeneous compound symmetry (CSH) covariance structure as the error structure of the repeated measures and was selected based on lowest AIC value. Significant interactions were further analyzed

using the slice options of the *LS Means* statement in PROC MIXED, with the level of significance set at $\alpha = 0.05$.

Soybean growth and biometric response. Soybean growth stage data recorded at each sample date were compared to the mean vegetation index values to identify any relationships between soybean development and mean vegetation indices values over the growing season. Multiple linear regression was used to model the relationship between calculated means values for the vegetation indices and soybean vegetative and reproductive stages using the mass package, lm() function (RStudio© version 0.99.3441, The R Foundation: Vienna, Austria). Regression analyses were conducted separately for the 2013 and 2014 exclusion cage studies, and the 2014 open-plot study. Model significance was determined at $\alpha = 0.05$.

Differences in biometric responses were analyzed and included soybean stem diameter, height, number of nodes as well as seed size, and yield. We first analyzed the effect of *D. texanus* infestation on soybean growth. Plant height and vegetative stage from sample dates prior to the field observations of oviposition scaring were analyzed using an ANOVA (mass, aov() function, RStudio©). Model significance comparing the treatments were determined significant at $\alpha = 0.05$. For this analysis only the exclusion cage studies (2013 and 2014) were used because oviposition scar observations were not conducted in the open-plot study due to time constraints. Soybean biometric data collected at the end of the season, in both the cage study and open-plot study, were compared between infested and non-infested plants. The plants were examined on an individual plant basis because all plants may not have been infested within the cage or subplot. Soybean stem diameter, height, and number of nodes for infested and non-

infested planted were analyzed using a Welch two-tailed *t*-test (mass, RStudio© version 0.99.3441, The R Foundation: Vienna, Austria) with significance determined at $\alpha = 0.05$. Lastly, multiple regression analyses were used to examine relationships of seed size (g/100 seeds) and yield (ton/ha) with *D. texanus* infestation for each cage and sub-plot using the MASS package lm() function in RStudio©. Model significance was determined at $\alpha = 0.05$.

Results

Exclusion Cage Study

Larval counts. *Dectes texanus* larval densities at the end of the season in the 2013 exclusion cage study were significantly different between the five infestation levels (non-infested, defoliation only, low, medium, and high) ($F = 10.3$; $df = 4, 36$; $P < 0.0001$). The mean number of larvae per cage for control and defoliation treatments were not significantly different from one another, but both had significantly fewer larvae per cage than the three artificially infested *D. texanus* treatments. This was expected, since the cages were not artificially infested with adult *D. texanus*; however, there was low level of natural infestation (0.02 ± 0.1 to 0.05 ± 0.2 control larvae per cage) within our control treatments non-infested and defoliator only, respectively. There were no significant differences between the three-artificial infestation levels (Fig. 4.1a). In 2014, there was also a significant difference between the five infestation levels ($F = 12.30$; $df = 4, 36$; $P < 0.0001$). There were no significant differences between *D. texanus* densities in the non-infested, defoliation only, and low-infestation *D. texanus* treatments. Larval densities in low infestation level cages (0.27 ± 0.8) were significantly lower than the medium ($0.54 \pm$

0.7) *D. texanus* infestation level but were not statistically different from larval densities in the high infestation treatment (0.44 ± 0.5) (Fig. 4.1b).

Vegetation indices. In 2013, all vegetation indices (Table 4.1) showed a significant fixed effect of sample day, where estimated index values either increased or decreased through time (Table 4.2). Although there was a significant effect of sample day, there were no significant interactions of *D. texanus* infestation level by sample day for any of the vegetation indices (Table 4.2). Moreover, there were no differences in the vegetation indices between infestation levels within a sample day (Table 4.2). Some control cages (noninfested and defoliation only treatments) were unintentionally infested by naturally occurring adult *D. texanus*, which resulted in plants with larvae at the end of the season. The vegetation indices values were reanalyzed excluding these cages (n = 3). Consequently, there remained no significant differences between infestation levels within a sample day. Not surprising, there was still a significant difference through time for all indices, but there was no significant interaction between infestation level and sample day for any of the vegetation indices tested. Due to infested control cages, all infested cages were pooled together within year and were compared to all non-infested cages (i.e., no *D. texanus* larvae recorded at the end of the season). As with previous analyses there was a significant sample day effect for all vegetation indices examined, which corresponded with plant growth and development over the season. However, there was no significant interaction between treatments (infested vs. non-infested) and sample day for any of the indices examined; vegetation indices mean values changed equally through time in 2013, regardless of *D. texanus* infestation (Table 4.3).

In the 2014 exclusion cage study, there were no significant differences between the five treatments on a given sample day for any of the vegetation indices used (Table 4.2). There was a significant difference in the vegetation indices mean values for each treatment across sample days as soybeans matured through time (Table 4.2). There were no significant treatment by sample day interactions for any of the vegetation indices examined; plants senesced equally through time. In the 2014 cage study, there were five cages (two non-infested and three defoliation cages) that had naturally occurring *D. texanus* larvae at the end of the year. These cages were removed from the analysis, but it did not affect treatment differences or corresponding interaction terms. Due to unintentional infestation in the control cages, all infested cages were pooled together and compared to all non-infested. The vegetation indices mean values between infested and non-infested treatments were significantly different for ENDVI ($F = 5.05$; $df = 1, 52$; $P = 0.03$), ENDVI2 ($F = 5.66$; $df = 1, 53.4$; $P = 0.02$), NIRBRVI ($F = 5.28$; $df = 1, 51.5$; $P = 0.03$) and ENDVI3 ($F = 4.82$; $df = 1, 52.9$; $P = 0.03$) (Table 4.3). There was a significant difference between sample day as soybean plants matured through time for all vegetation indices (Table 4.3). There was a significant interaction between treatment and sample day for the ENDVI ($F = 3.48$; $df = 11, 212$; $P = 0.0002$), ENDVI2 ($F = 3.45$; $df = 11, 212$; $P = 0.0002$), ENDVI3 ($F = 3.04$; $df = 11, 212$; $P = 0.0008$), GBNDVI ($F = 2.52$; $df = 11, 212$; $P = 0.005$), NIRBRVI ($F = 3$; $df = 11, 214$; $P = 0.001$) and NIR Green Diff. ($F = 1.86$; $df = 11, 505$; $P = 0.04$) (Table 4.3); the soybeans were not developing equally through time. Although on some sample days the average V and R stages were the same, on multiple occasion the average V and R stages were further along in development as the season progressed for infested cages than non-infested cages (Table 4.4).

Soybean growth and biometric response. Mean vegetation indices values were plotted through time for each treatment to examine changes attributed to *D. texanus* infestation. In 2013, the mean brightness value plotted patterns were similar for all five treatments, and across all the vegetation indices. Although there were differences in the peak timing, the soybeans still had similar plotted patterns as the plants matured during the season. The GNDVI values for soybean plants within cages peaked ($GNDVI_{Peak}$) on 7 August for defoliation, medium and high infestation treatments and 14 August for non-infested and low-infestation treatments. During the $GNDVI_{Peak}$, mean plant growth stages were V14 (±0.38) and R3 (± 0.07) for 7 August and V15 (± 0.21) and R4 (± 0.04) on 14 August. There was a gradual decline in the vegetation indices mean values until 26 August, where the plants were maturing from R4 to R6. GNDVI measurements after 26 August indicate a steady decline in the mean values as pods reached full maturity and soybean plants senesced (Fig. 4.2a; Table 4.4). For the BNDVI values, soybean plants within cages peaked ($BNDVI_{Peak}$) on 14 August for all treatments, when the plants were at the growth stages of V15 (± 0.21) and R4 (± 0.04) (Fig. 4.2b). All the remainder vegetation indices (ENDVI, ENDVI2, ENDVI3, GBNDVI, NIRBRVI and NIR Green Diff.) peaked on 1 August; no plant biometric data was collected on 1 August 2013 (Fig. 4.2c – Fig. 4.2h). There was a significant relationship between all the vegetation indices calculated and soybean vegetative and reproductive stages, where mean brightness value decreased as the soybean plant matured. The regression model showed that 1 to 8% of the variation in the vegetation indices was due to changes in vegetative stage and 3 to 76% was due to changes in soybean reproductive stage (Table 4.5).

In 2014, the plotted patterns of the vegetation mean brightness values were similar across all five infestation treatments through time. All vegetation indices mean values peaked in activity from 9 July (V6 (± 0.09), R2 (± 0.00)) to 1 August (V12 (± 0.16), R3 (± 0.00)) (Fig. 4.3a – Fig. 4.3h). There was more variation in peak times compared to 2013. There was a decline in the vegetation mean brightness values from 1 to 11 August as the pods were entering the R5 stage and where beginning to develop seeds. Mean brightness values increased after 11 August but before 4 September as the plants entered the R6 stage and began to fill pods. By 6 September all treatments were in the R6 (full seed) stage, which was followed with a steady decline in mean brightness values and the plants senesced. There was an increase in GNDVI values as soybean plants reached full maturity by 29 September (Fig. 4.3a; Table 4.6), which was different than in the 2013 study. In the 2014 exclusion cage study there was also a significant relationship between all mean vegetation indices values and vegetative and reproductive stages. The results indicate that as soybean plants mature, regardless of the vegetation indices used, mean brightness values decrease. Regression models explained 13 to 23% of the variation in the vegetation indices as it relates to vegetative stage and 4 to 46% is explained by changes in reproductive stage (Table 4.5).

For soybean biometric data, we first compared the height and vegetative stage to the treatments for sample dates prior to the observation of oviposition scars. Next, we compared infested and non-infested plants at the end of the season. In 2013, ANOVAs conducted on the measurements taken before *D. texanus* oviposition occurred indicated that infestation level had no significant impact on soybean plant height ($F = 0.33$; $df = 1,4$; $P = 0.86$, $F = 0.27$; $df = 1,4$; $P = 0.90$) or change in vegetative stage ($F = 0.48$; $df =$

1,4; $P = 0.75$, $F = 0.79$; $df = 1,4$; $P = 0.54$) for 7 August and 14 August, respectively (Table 4.6). Prior to the observance of oviposition, soybean plants were developing equally. Conversely, we found a significant difference in the number of nodes per plant ($t = -2.30$; $df = 126.85$; $P = 0.02$) when we compared infested and non-infested plants at the end of the season; infested plants had one more node per plant than non-infested plants. Infestation level (infested vs. non-infested) had no significant effect on mean soybean stem diameter ($t = -1.18$; $df = 102.56$; $P = 0.24$) or height ($t = -1.79$; $df = 123.80$; $P = 0.02$) (Table 4.7).

Total yield per cage and soybean seed size were also examined. Plants infested with *D. texanus* had a significant effect on seed size (g/100 seed) ($F = 6.64$; $df = 2, 47$; $P = 0.003$; $R^2 = 0.19$), where mean seed size per cage decreased as the number of *D. texanus* larvae per cage increased. The regression model only explains 18.72% of the variation in seed size (Table 4.8). However, there was no relationship between yield (ton/hectare) and *D. texanus* infestations ($F = 0.16$; $df = 2, 47$; $P = 0.85$; $R^2 = -0.03$) (Table 4.8).

In the 2014, ANOVAs conducted on the measurements taken before *D. texanus* oviposition occurred indicated that infestation level had no significant impact on soybean plant height ($F = 0.54$; $df = 1,4$; $P = 0.70$; $F = 0.66$; $df = 1,4$; $P = 0.62$) or vegetative stage ($F = 0.85$; $df = 1,4$; $P = 0.50$, $F = 2.0$; $df = 1,4$; $P = 0.11$) for 3 July and 9 July, respectively (Table 4.6). These results indicate that the soybean plants were developing equally, regardless of treatment, prior to oviposition. In comparing infested and non-infested plants at the end of the season, we found there was a significant difference in the number of nodes on the soybean plants ($t = -3.34$; $df = 187.55$; $P = 0.001$), with infested

plants having one node more than the non-infested plants. There was also a significant difference in stem diameter (t = -2.87; df = 178.31; P = 0.004) showing a 0.56 mm increase in infested plants; however, there was no difference in plant height (t = -0.78; df = 175.12; P = 0.44) between infested and non-infested plants (Table 4.7).

There was no relationship between seed size (g/100 seed) and *D. texanus* infestation (F = 0.26; df = 2, 47; P = 0.77; R^2 = -0.03), where the seed size was consistent between cages no matter the level of infestation. There was also no correlation between yield and *D. texanus* infestations (F = 1.70; df = 2, 47; P = 0.19; R^2 = 0.03) (Table 4.8).

Open Plot Study

Larval counts. There was a significant difference in the larval densities between insecticide-treated and non-treated plots (F = 34.82; df = 520; P = 0.00). Not surprisingly, there was a reduction in mean number of larvae found per plot in treated plots after the first and second insecticide applications from12.4 ± 0.8 to 2.3 ± 0.4, respectively. Larval densities in untreated plots after the first and second applications were 16.4 ± 0.3 and 12 ± 0.2, respectively; our treatments were successful at reducing *D. texanus* larvae during the growing season. There was also a significant difference in the number of larvae per subplot at the end of the season (F = 232.33; df = 1, 11; $P < 0.0001$); non-treated plots (39.86 ± 2.27) had 13.5 fold more total *D. texanus* larvae than insecticide-treated plots (3.13 ± 0.65) (Fig. 4.4).

Vegetation indices. For the open-plot study there were no significant differences in mean vegetation indices between untreated control and insecticide treated plots (F = 1.87; df = 1, 136; P = 0.17), albeit there were significant differences in larval infestation levels at various time points. There was a significant difference in sample date for all

vegetation indices as the plants matured through time (Table 4.2). There was no significant treatment by sample date interaction indicating that the plants senesced the same through time regardless of infestation level (Table 4.2).

Soybean growth and biometric response. Mean vegetation index values were plotted through time for both treatments (treated and non-treated) and all vegetation indices. All the vegetation indices and treatments peaked in activity on 18 July (V9/R2) except for the GNDVI (Fig. 4.5a), which indicated the peak in mean brightness value was on 28 July (V11/R3) (Fig. 4.5a). However, all vegetation indices followed the same patterns through time. After the peak in mean brightness values, the values steadily declined as the plants matured from the R3 to R5 stages. Then as plants reached R6 the mean brightness values increased until 17 September, where they decreased again. However, in the green/blue indices the untreated plots continued to increase in mean brightness value while the treated plants decreased as the pods reach full maturity or R8 (Fig. 4.5f; Table 4.4). There was also a significant relationship between all vegetation indices and soybean vegetative growth stages, where mean vegetation index values decreased as plants matured (Table 4.5). The regression model explained 15-42% of the variation in the vegetation indices mean values and were due to changes in soybean vegetative stages, and 10-24% was due to the changes in reproductive stage (Table 4.5). There was no significant relationship between the reproductive stages and the mean values of the GBNDVI ($F = 3.39$; $df = 1, 158$; $P = 0.08$; $R^2 = 0.01$).

When evaluating soybean response to *D. texanus* infestation, there was a significant difference in the number of nodes present per plant ($t = -5.62$; $df = 618.35$; $P < 0.01$). More specifically, *D. texanus* infested plants had one more node than non-infested

plants. In addition, infested soybean plants had 1% larger stem diameters (t = -6.59; df = 589.56; $P < 0.01$) and were 3% taller (t = -6.10; df = 596.77; $P < 0.01$) than non-infested plants (Table 4.7). The regression indicated a relationship between seed size (g per 100 seeds) and *D. texanus* infestation (F = 6.01; df = 2, 47; P = 0.0047; R^2 = 0.17) (Table 4.8). In the regression model, 16.98% of the variance in seed size was explained by the number of plants used for analysis. There was no relationship between yield (ton/hectare) and *D. texanus* infestations (F = 1.22; df = 2, 45; P = 0.30; R^2 = 0.0093) (Table 4.8).

Discussion

During 2013 and 2014, we conducted two field studies using exclusion cages and open plots to characterize the effects of natural and artificial *D. texanus* populations on soybean plant response. These investigations provided a basis for evaluating the potential for remote sensing to detect *D. texanus* in soybean using established vegetation indices. Our experimental design also provided significantly different larval densities to test our hypotheses; however, results were inconsistent between years and we observed that larval feeding and tunneling in the main stem did not always cause a change in soybean leaf reflectance values (vegetation indices). We hypothesized that successfully infested plots and cages, where larvae tunneled soybean plants, would result in biotic stress; therefore, we expected to see a reduction in leaf reflectance values (Jensen 2007). In our studies, several vegetation indices were used to quantify *D. texanus* larval feeding damage (Table 4.1). Interestingly, only the 2014 exclusion cage study showed a significant difference between *D. texanus* infested and non-infested cages for several indices, including ENDVI, ENDVI2, ENDVI3, GBNDVI, NIR Green Diff. and NIRBRVI.

Where indices had a significant difference between treatments, the common band utilized was the blue band, but could also incorporate green and NIR bands. The blue band of light has shown to be important in plant biological functions. Plants use chlorophyll a and b to absorb the majority of the blue light reaching the plant, which aides in photosynthesis, but the blue band has also been quantitatively linked to chloroplast movement within the plant cell and stomatal conductance (Sharkey and Raschke 1981, Jarillo et al. 2001, Jensen 2007). Since xylem assists in transporting water throughout the plant, *D. texanus* pressure in 2014 may have been the only year where pressure was high enough to disrupt the xylem enough to affect the stomate conductance. *Dectes texanus* larvae feed primarily on the pith within the petioles shortly after egg hatch and continue to feed on pith of the main stem in the soybean plant as larvae develop (Patrick 1973, Laster et al. 1981, Niide et al. 2012). In order to use vegetation indices to detect larval presence, larvae need to disrupt the xylem and/or phloem functions (Haile et al. 1999, Macedo et al. 2003, Nabity et al. 2008). Although *D. texanus* pressure varied between treatments, it is possible that feeding damage was too low for detection. Interestingly, the BNDVI and GNDVI, which only utilizes the blue or green band in conjunction with the NIR band, did not detect differences between the infested and non-infested plants. This suggests that the blue band, used in combination with another band, may be necessary for detecting insects in soybean, specifically *D. texanus*. Although there was tunneling and feeding in the main stem during both years and experiments, infestation levels did not produce consistent significant differences between treatments. Future studies designed to quantify amounts of xylem, phloem, and pith consumed by a larva within a soybean plant and resulting effects of such feeding on

soybean photosynthetic capabilities would help explain whether any change in the spectral response was a result of direct feeding or if there were other factors influencing spectral responses.

A limitation of the current study is the limited number of spectral bands and defined wavelengths, which therefore likely limited the number of predictive indices we could use for *D. texanus* detection. Use of more sensitive instruments like spectroradiometers, could be used in future studies to find specific wavelengths more indicative of *D. texanus* damage. Spectroradiometers are portable devices and are used to measure the amount of energy reflected from an object of interest (i.e. soybean leaves) over different wavelengths (Peddle et al. 2001). These tools can also utilize a broader range of wavelengths than satellites or modified cameras are capable of, which may be necessary for identifying differences in *D. texanus* infested and non-infested plants (Jensen 2007). There have been studies that successfully used spectroradiometers for detecting characteristic wavelengths to insect damage (Lan et al. 2013, Zhao et al. 2013). For example, Zhao et al. (2013) used spectroradiometer data to identify characteristics bands to rice leaf folder damage for use in vegetation indices. Characteristic to the name, the rice leaf folder folds inside the rice leaf where they will feed, creating longitudinal white and transparent streaks on the blade (Zhao et al. 2013). Feeding damage of the two spotted spider mite, by both immatures and adults, is caused by extracting fluids from plant cells (Lan et al. 2013). The feeding by the target pests in these studies had a direct effect on plant photosynthetic pathways by causing damage to critical energy-producing tissues. Since spectroradiometers utilize more wavelengths than modified cameras, specific wavelengths that are more sensitive to *D. texanus* feeding could be tested. Future

studies examining wavelengths beyond the infrared, green, red and blue bands may provide the indices required to model *D. texanus* feeding effects on soybean.

The vegetation indices significantly decreased as plants matured, which was observed in the 2013 and 2014 cage studies, and open plot studies. This was expected as plants decrease in chlorophyll content and reduce photosynthetic activity as the plants senesce through time. In the exclusion cage and open plot studies in 2014, we observed a significant increase in the mean brightness values from V5-V7 and then a decline as plants were developing pods from R3 to R5. During this time, the adult *D. texanus* populations were declining (see Chapter 2) and larvae within plants were feeding on the pith of the main stem moving towards the bottom of the plant to prepare overwintering chambers. This could likely explain the observed trends of the plotted mean brightness values in conjunction with the soybean plants preparing and allocating resources for pod development. As pod size increases, chlorophyll concentration, plant respiration, and photosynthesis decrease within the plant (Andrews and Svec 1975, Sambo et al. 1977). This likely explains the changes in the vegetation indices mean brightness values (increases and decreases during the season) we observed; as pods begin to develop, photosynthetic activity increases in the pods and decreases in the leaves, causing the changes detected in the vegetation indices.

D. texanus affected soybean growth and development over the course of our experiments. Soybean plants in the open plot study infested with *D. texanus* had significantly more nodes than non-infested plants, while seed size slightly decreased as the number of larvae per cage increased in the 2013 exclusion cage and 2014 open plot studies. Although these effects were observed, it did not result in any significant yield

responses. The lack of physiological yield loss is likely contributed to the ability of soybean plants to compensate from biotic and abiotic stresses, which has been found with other soybean pests. Koch and Rich (2015), found that brown marmorated stink bug (*Halyomorpha halys*) feeding reduces the number of seeds per pod, but this pest had no effect on the overall number of pods per plant. In their study, compensation occurred by increasing the weight of the remaining seeds and thus yield was unaffected by *H. halys* (Koch and Rich 2015). Similar responses have been reported for other stink bug species including *Nezara viridula* (Russin et al. 1987, Corrêa-Ferreira and De Azevedo 2002), *Acrosternum hilare*, *Euschistus servus* (Russin et al. 1987)*, Piezodorus guildinii*, and *Euschistus heros* (Corrêa-Ferreira and De Azevedo 2002). Considering that soybean has the capacity to compensate to insect damage, this would likely explain the increase in node numbers when *D. texanus* are present.

The results of this study provide insight into the utilization of remote sensing techniques as detection methods for *D. texanus*. Regardless of infestation level, *D. texanus* did not always alter the leaf reflectance and consequently mean values for the vegetation indices tested. More research is needed to determine if the changes brightness values were linked to infestation or other biotic or abiotic factors (i.e. water, nutrition deficiency, disease, sunlight) as well. A major finding in this study was the plant response to *D. texanus* infestation, where infested plants had larger stem diameters and more nodes than non-infested plants. In identifying these differences in plant biometrics, the information may assist by serving as indicators of at-risk areas in the field. Although subtle differences in plant height are difficult to distinguish by humans, emerging technologies capable of detecting changes in altitude of objects and compatible software

to identify height differences may show promise for pest detection. To better understand the yield impact of *D. texanus* on soybean, yield loss studies need to include mechanical yield loss due to lodging in the future; that was not a focus of the current study. Given that we were able to detect changes in crop phenology through time, there is great potential in using remote sensing methods to determine optimal times to harvest soybean before *D. texanus* infestations lodge plants. Such an application would require further investigation.

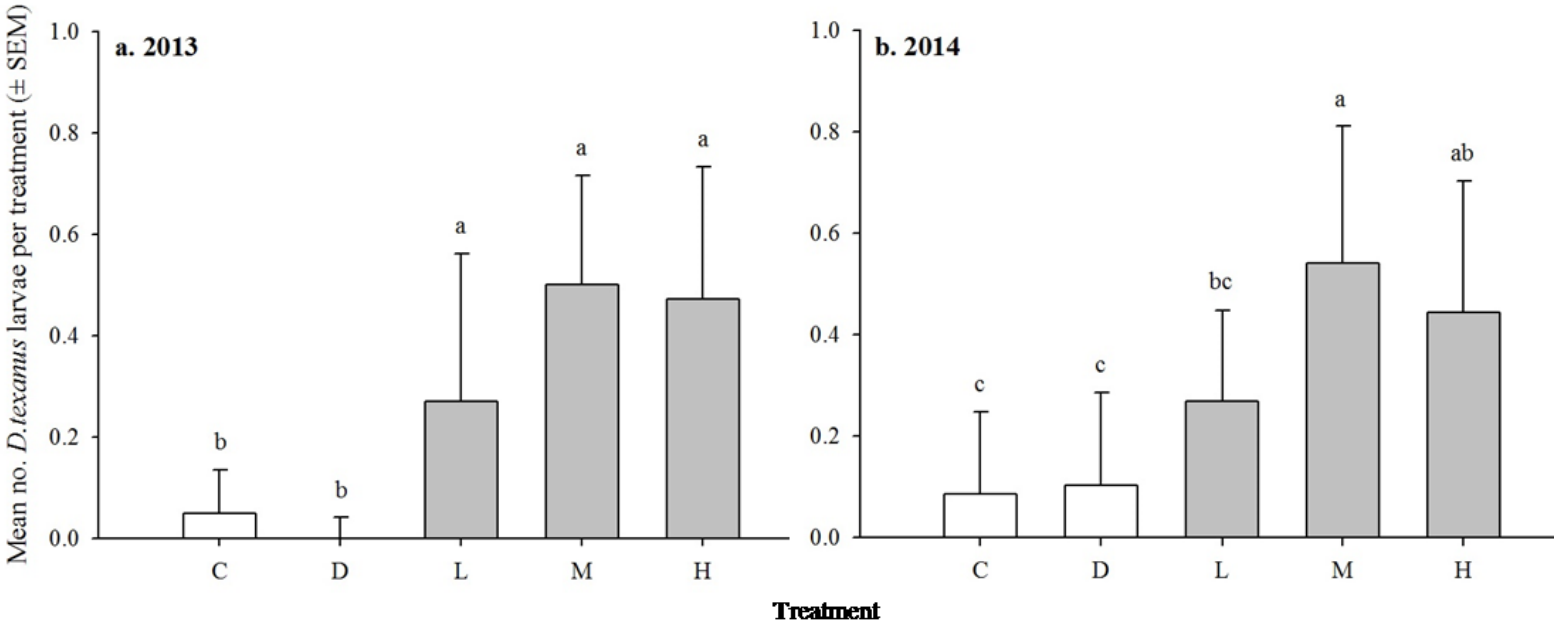

Figure 4.1. The end of season *D. texanus* larvae total counts ± SEM for the 2013 (a) and 2014 (b) cage studies. The treatments used for both studies included: control or insect free (C), defoliators only (D), and three levels of *D. texanus* infestation, low (L; 2 mating pairs), medium (M; 8 mating pairs), and high (H; 15 mating pairs).

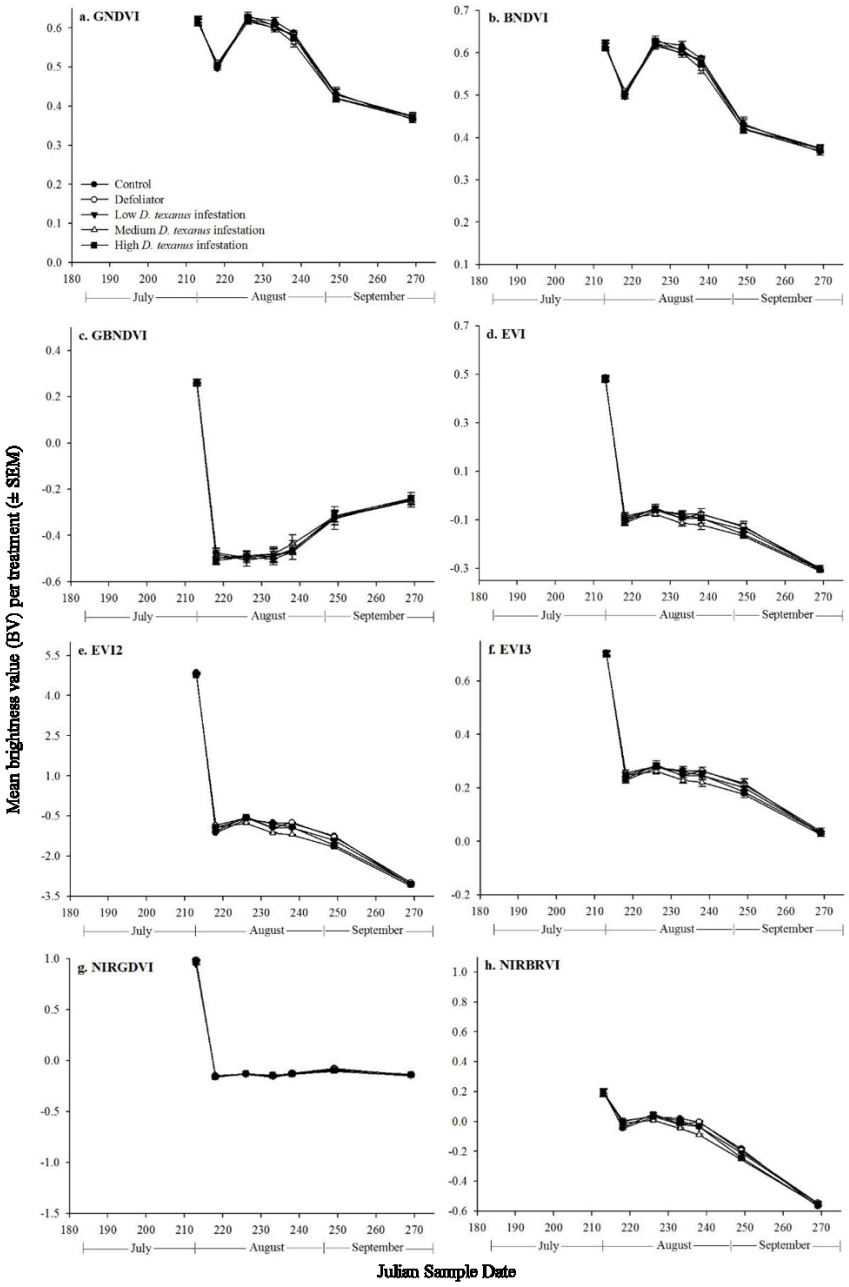

Figure 4.2. The GNDVI (a), BNDVI (b), GBNDVI (c), EVI (d), EVI2 (e), EVI3 (f), NIRGDVI (g) and NIRBRVI (h) plotted mean brightness values (BV; ± SEM) for the 2013 cage study by treatments. The treatments include control or insect free, defoliators only, and three levels of *D. texanus* infestation, low (2 mating pairs), medium (8 mating pairs), and high (15 mating pairs).

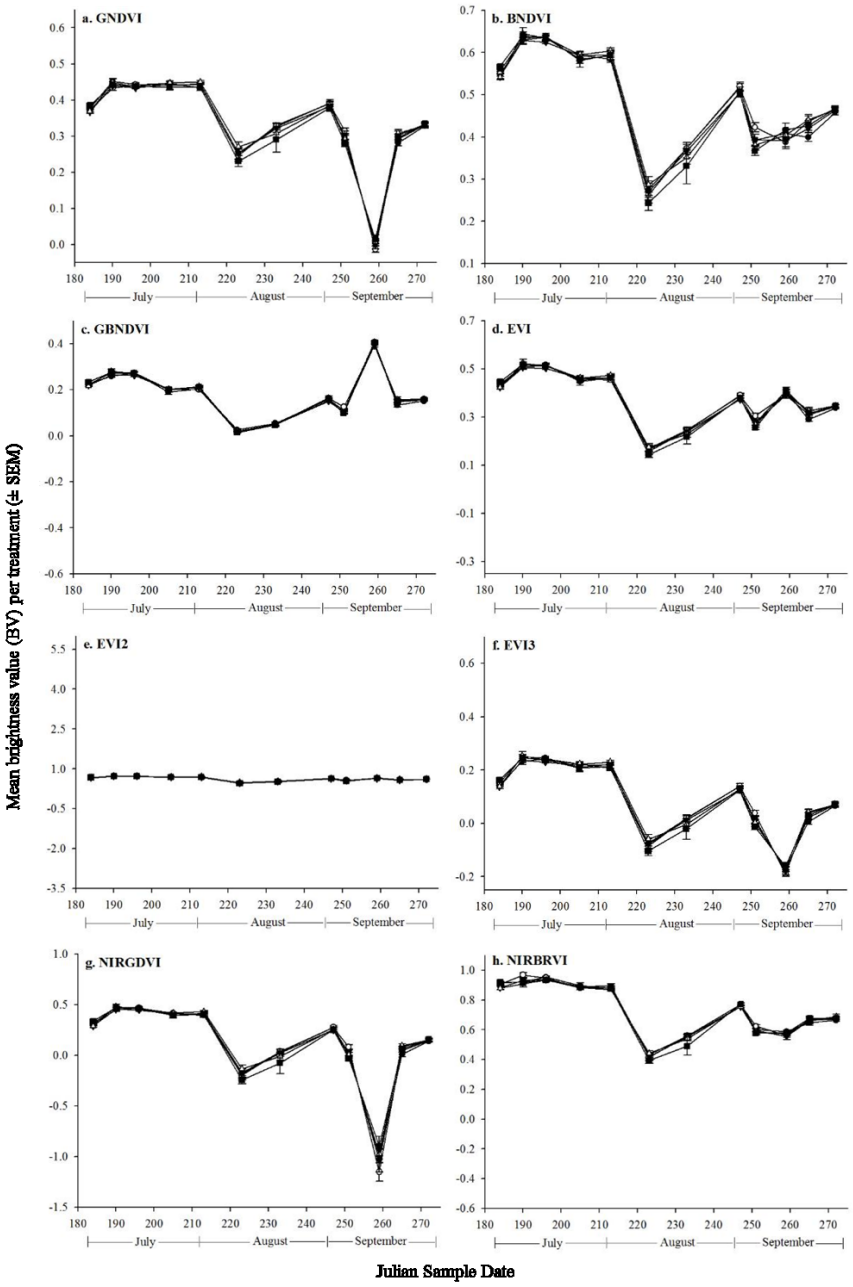

Figure 4.3. The GNDVI (a), BNDVI (b), GBNDVI (c), EVI (d), EVI2 (e), EVI3 (f), NIRGDVI (g) and NIRBRVI (h) plotted mean brightness values (BV; ± SEM) for the 2014 cage study by treatments. The treatments include control or insect free, defoliators only, and three levels of *D. texanus* infestation, low (2 mating pairs), medium (8 mating pairs), and high (15 mating pairs).

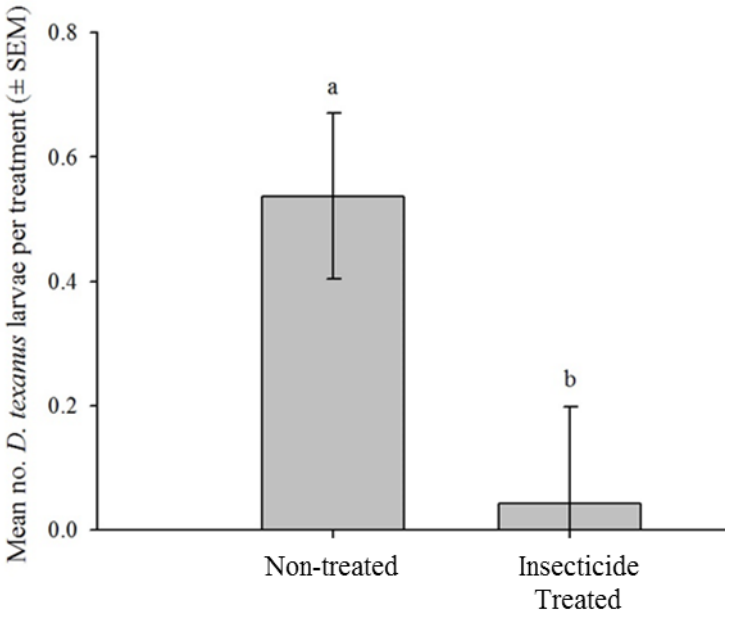

Figure 4.4. The end of season mean *D. texanus* larvae (± SEM) per treatment for the 2014 open plot study. The plots were allowed to be naturally infested during the course of the study. The two treatments used in this study included: non-infested (plots that were allowed to be naturally infested with *D. texanus*) and treated (plots treated with insecticide).

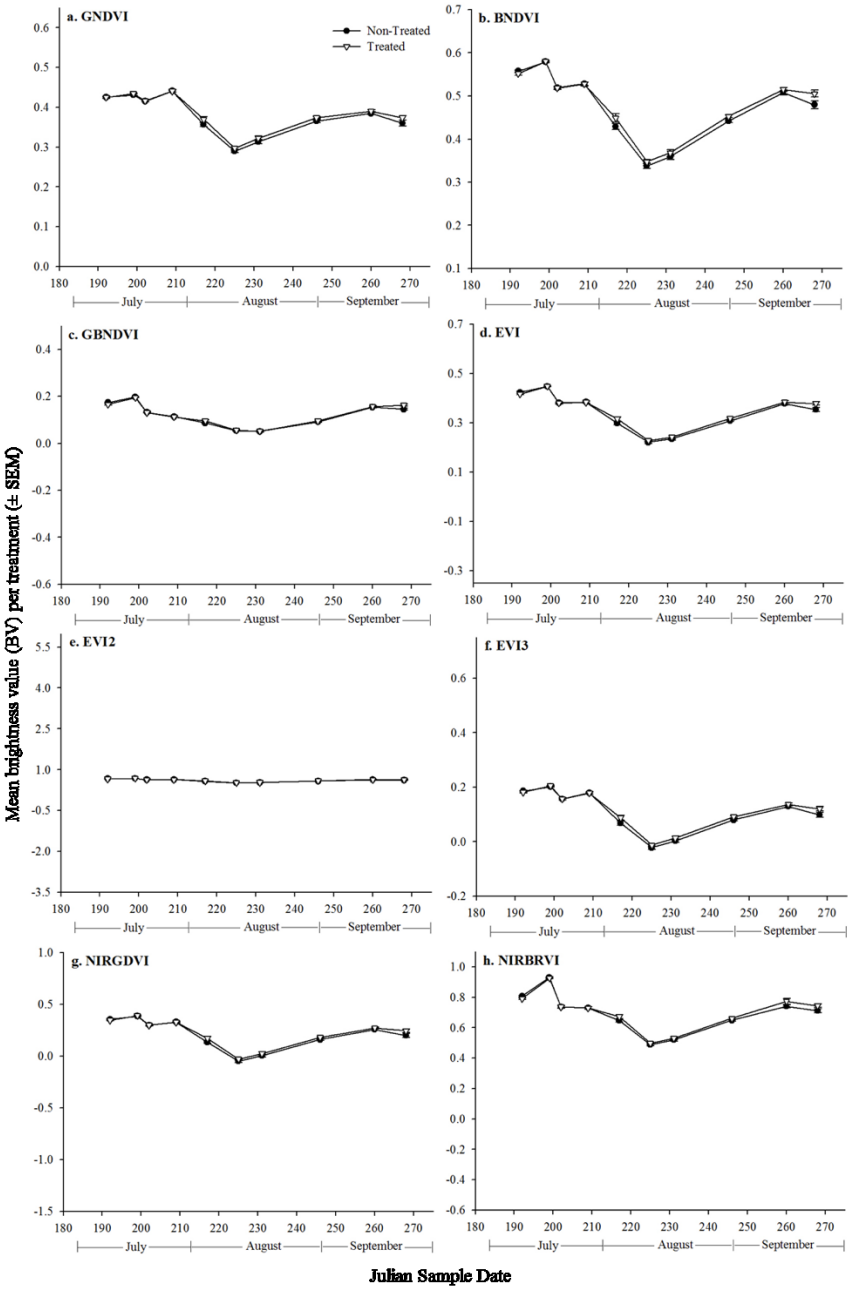

Figure 4.5. The GNDVI (a), BNDVI (b), GBNDVI (c), EVI (d), EVI2 (e), EVI3 (f), NIRGDVI (g) and NIRBRVI (h) plotted mean brightness values (BV; ± SEM) for the 2014 open plot study by treatments. The treatments include non-treated or no insecticide application and treated.

Table 4.1. Vegetation indices formulas used for the cage and open plot studies. Formulas were selected based on availability through the program AgPixel™ and modified to include the gray panel for calibration.

Vegetation Index	Equation
Green Normalized Difference Vegetation Index (GNDVI)	$GNDVI = \left(\frac{\bar{X}_{NIR}}{\bar{X}_{GrayPanel}}\right) - \left(\frac{\bar{X}_{green}}{\bar{X}_{GrayPanel}}\right) \Big/ \left(\frac{\bar{X}_{NIR}}{\bar{X}_{GrayPanel}}\right) + \left(\frac{\bar{X}_{green}}{\bar{X}_{GrayPanel}}\right)$
Blue Normalized Difference Vegetation Index (BNDVI)	$BNDVI = \left(\frac{\bar{X}_{NIR}}{\bar{X}_{GrayPanel}}\right) - \left(\frac{\bar{X}_{blue}}{\bar{X}_{GrayPanel}}\right) \Big/ \left(\frac{\bar{X}_{NIR}}{\bar{X}_{GrayPanel}}\right) + \left(\frac{\bar{X}_{blue}}{\bar{X}_{GrayPanel}}\right)$
Enhanced Normalized Difference Vegetation Index (EVI)	$ENDVI = \left(\frac{\bar{X}_{NIR}}{\bar{X}_{GrayPanel}}\right) + \left(\frac{\bar{X}_{green}}{\bar{X}_{GrayPanel}}\right) - 2\left(\frac{\bar{X}_{blue}}{\bar{X}_{GrayPanel}}\right) \Big/ \left(\frac{\bar{X}_{NIR}}{\bar{X}_{GrayPanel}}\right) + \left(\frac{\bar{X}_{green}}{\bar{X}_{GrayPanel}}\right) + 2\left(\frac{\bar{X}_{blue}}{\bar{X}_{GrayPanel}}\right)$
2-band Enhanced Normalized DifferenceVegetation Index (EVI2)	$ENDVI2 = \left(\frac{\bar{X}_{NIR}}{\bar{X}_{GrayPanel}}\right) + \left(\frac{\bar{X}_{green}}{\bar{X}_{GrayPanel}}\right) - \left(\frac{\bar{X}_{blue}}{\bar{X}_{GrayPanel}}\right) \Big/ \left(\frac{\bar{X}_{NIR}}{\bar{X}_{GrayPanel}}\right) + \left(\frac{\bar{X}_{green}}{\bar{X}_{GrayPanel}}\right) + \left(\frac{\bar{X}_{blue}}{\bar{X}_{GrayPanel}}\right)$
3-band Enhanced Normalized DifferenceVegetation Index 3 (EVI3)	$ENDVI3 = \left(\frac{\bar{X}_{NIR}}{\bar{X}_{GrayPanel}}\right) - \left(\frac{\bar{X}_{green}}{\bar{X}_{GrayPanel}}\right) - \left(\frac{\bar{X}_{blue}}{\bar{X}_{GrayPanel}}\right) \Big/ \left(\frac{\bar{X}_{NIR}}{\bar{X}_{GrayPanel}}\right) + \left(\frac{\bar{X}_{green}}{\bar{X}_{GrayPanel}}\right) + \left(\frac{\bar{X}_{blue}}{\bar{X}_{GrayPanel}}\right)$
Green-Blue Normalized Difference Vegetation Index (GBNDVI)	$GBNDVI = \left(\frac{\bar{X}_{green}}{\bar{X}_{GrayPanel}}\right) - \left(\frac{\bar{X}_{blue}}{\bar{X}_{GrayPanel}}\right) \Big/ \left(\frac{\bar{X}_{green}}{\bar{X}_{GrayPanel}}\right) + \left(\frac{\bar{X}_{blue}}{\bar{X}_{GrayPanel}}\right)$
NIR Blue Ratio Vegetation Index (NIRBRVI)	$NIRBRVI = \left(\frac{\bar{X}_{NIR}}{\bar{X}_{GrayPanel}}\right) - \left(\frac{\bar{X}_{blue}}{\bar{X}_{GrayPanel}}\right)$
NIR Green Diff. Vegetation Index	$NIR\,Green\,Diff. = \left(\frac{\bar{X}_{NIR}}{\bar{X}_{GrayPanel}}\right) - \left(\frac{\bar{X}_{green}}{\bar{X}_{GrayPanel}}\right) - \left(\frac{\bar{X}_{blue}}{\bar{X}_{GrayPanel}}\right) \Big/ \left(\frac{\bar{X}_{NIR}}{\bar{X}_{GrayPanel}}\right) - \left(\frac{\bar{X}_{green}}{\bar{X}_{GrayPanel}}\right) + \left(\frac{\bar{X}_{blue}}{\bar{X}_{GrayPanel}}\right)$

Table 4.2. Result from the 2013 and 2014 cage study and the 2014 open plot study testing of fixed effect (PROC MIXED) of the all the mean brightness values for each vegetation indices. Significance was determined at $\alpha = 0.05$.

		Cage Study						Open Plot Study		
		2013			*2014*			*2014*		
Vegetation Index	**Factor**	***F***	***df***	***P***	***F***	***df***	***P***	***F***	***df***	***P***
GNDVI	Treatment	2.35	4, 48.7	0.07	0.89	4, 45.4	0.48	2.11	1, 137	0.15
	Day	824.61	6, 126	≤0.01*	950.62	11, 193	≤0.01*	299.39	9, 137	<0.0001*
	Treatment x Day	0.77	24, 187	0.77	0.97	44, 320	0.54	0.68	9, 137	0.72
BNDVI	Treatment	0.89	4, 48.3	0.48	0.57	4, 43.6	0.69	2.74	1, 137	0.10
	Day	775.7	6, 126	≤0.01*	548.63	11, 193	≤0.01*	890.45	9, 137	<0.0001*
	Treatment x Day	0.61	24, 187	0.93	1.14	44, 320	0.27	1.34	9, 137	0.22
EVI	Treatment	2.16	4, 47.1	0.09	0.55	4, 37.5	0.70	2.47	1, 137	0.12
	Day	4030.54	6, 127	≤0.01*	596.11	11, 193	≤0.01*	1151.71	9, 137	<0.0001*
	Treatment x Day	0.68	24, 190	0.87	1.14	44, 320	0.26	1.31	9, 137	0.24
EVI2	Treatment	2.23	4, 48.3	0.08	0.59	4, 40.1	0.67	2.48	1, 137	0.12
	Day	4447.75	6, 126	≤0.01*	574.87	11, 193	≤0.01*	1067.16	9, 137	<0.0001*
	Treatment x Day	0.72	24, 189	0.83	1.13	44, 320	0.27	1.32	9, 137	0.23
EVI3	Treatment	0.36	4, 36.3	0.84	0.61	4, 42.7	0.66	2.67	1, 137	0.10
	Day	3527.85	6, 125	≤0.01*	756.59	11, 193	≤0.01*	666.18	9, 137	<0.0001*
	Treatment x Day	0.66	24, 186	0.88	1.07	44, 320	0.36	1.13	9, 137	0.34
GBNDVI	Treatment	1.41	4, 50.1	0.24	1.13	4, 37	0.36	1.1	1, 137	0.30
	Day	996.56	6, 122	≤0.01*	1462.82	11, 192	≤0.01*	982.82	9, 137	<0.0001*
	Treatment x Day	0.68	24, 185	0.86	1.02	44, 317	0.45	1.25	9, 137	0.27
NIRBRVI	Treatment	1.37	4, 52.8	0.26	1.03	4, 33.2	0.41	3.19	1, 137	0.08
	Day	1990.87	6, 128	≤0.01*	789.19	11, 196	≤0.01*	2376.54	9, 137	<0.0001*
	Treatment x Day	0.62	24, 192	0.92	1.14	44, 322	0.26	1.57	9, 137	0.13
NIRGD	Treatment	0.8	4, 68.2	0.53	0.24	4, 58.4	0.92	2.75	1, 137	0.10
	Day	1372.95	6, 118	≤0.01*	484.24	11, 193	≤0.01*	588.02	9, 137	<0.0001*
	Treatment x Day	0.75	24, 185	0.79	1.12	44, 321	0.29	1.23	9, 137	0.28

*: Indicates significant differences at $\alpha = 0.05$

Table 4.3. Result from the 2013 and 2014 cage study testing the fixed effects (PROC MIXED) of the all the vegetation indices for infested vs non-infested cages. Significance was determined at $\alpha = 0.05$.

		2013			2014		
Vegetation Index	**Factor**	***F***	***df***	***P***	***F***	***df***	***P***
GNDVI	Treatment	0.23	1, 51	0.64	0.22	1, 54.6	0.64
	Day	883.94	6, 135	≤0.01*	980.48	11, 212	≤0.01*
	Treatment x Day	0.25	6, 135	0.96	1.39	11, 212	0.18
BNDVI	Treatment	0.08	1, 50.7	0.78	0.19	1, 56	0.67
	Day	821.99	6, 134	≤0.01*	529.6	11, 212	≤0.0001*
	Treatment x Day	0.57	6, 134	0.76	1.69	11, 212	0.08
EVI	Treatment	0.46	1, 50.3	0.50	5.05	1, 52	0.03*
	Day	4314.2	6, 136	≤0.01*	301.85	11, 212	≤0.01*
	Treatment x Day	0.18	6, 136	0.98	3.48	11, 212	0.0002*
EVI2	Treatment	0.42	1, 51.6	0.52	5.66	1, 53.4	0.02*
	Day	4695.8	6, 135	≤0.01*	290.21	11, 212	≤0.01*
	Treatment x Day	0.15	6, 135	0.99	3.45	11, 212	0.0002*
EVI3	Treatment	0.98	1, 50.3	0.33	4.82	1, 52.9	0.03*
	Day	3602.5	6, 136	≤0.01*	371.79	11, 212	≤0.01*
	Treatment x Day	0.17	6, 136	0.98	3.04	11, 212	0.0008*
GBNDVI	Treatment	0.03	1, 53.6	0.87	2.14	1, 50	0.15
	Day	1044.7	6, 130	≤0.01*	736.54	11, 212	≤0.01*
	Treatment x Day	0.14	6, 130	0.99	2.52	11, 212	0.005*
NIRBRVI	Treatment	0.03	1, 56.2	0.87	5.28	1, 51.5	0.03*
	Day	2128.7	6, 137	≤0.01*	390.54	11, 214	≤0.01*
	Treatment x Day	0.52	6, 137	0.79	3	11, 214	0.001*
NIRGD	Treatment	0.16	1, 131	0.69	3.4	1, 209	0.07
	Day	120.4	6, 284	≤0.01*	286.81	11, 505	≤0.01*
	Treatment x Day	0.19	6, 284	0.98	1.86	11, 505	0.04*

*: Indicates significant differences at $\alpha = 0.05$

Table 4.4. Plant measurements collected from the 2013 and 2014 exclusion cage study and the 2014 open plot study. The information includes the total number of plants examined for each treatment at each sample date as well as the total number of *D. texanus* larvae collected from each treatment at the end of the growing season. Other information included is the mean stem diameter taken at the end of the season, mean plant height, mean vegetative stage and mean reproductive stage the plants were in for each treatment at the sample date.

Study	Year	Date	Treatment	Mean Stem Diameter (mm)	± SEM	Mean Plant Height (cm)	± SEM	Mean V stage	± SEM	Mean R stage	± SEM
Cage	2013	7-Aug	C	9.17	0.10	94.46	8.96	15	0.52	4	0.13
			D	9.46	0.13	91.57	3.98	14	0.84	4	0.15
			L	8.75	0.09	91.38	3.07	15	0.54	4	0.15
			M	9.25	0.42	92.33	3.47	15	0.48	4	0.10
			H	9.21	0.10	91.63	3.26	15	0.71	4	0.10
		14-Aug	C	9.17	0.10	103.98	3.89	15	0.74	4	0.13
			D	9.46	0.13	104.62	2.86	16	0.70	4	0.16
			L	8.75	0.09	100.30	3.11	14	0.90	4	0.15
			M	9.25	0.42	103.25	2.97	15	0.70	4	0.16
			H	9.21	0.10	102.95	3.27	16	0.56	4	0.10
		21-Aug	C	9.17	0.10	101.69	3.45	15	0.56	5	0.00
			D	9.46	0.13	98.17	4.18	15	0.49	5	0.00
			L	8.75	0.09	100.20	3.85	14	0.52	5	0.00
			M	9.25	0.42	98.58	4.32	15	0.74	5	0.00
			H	9.21	0.10	101.73	3.40	13	0.84	5	0.00
		26-Aug	C	9.17	0.10	100.55	2.61	14	0.44	6	0.00
			D	9.46	0.13	92.87	3.43	15	0.48	6	0.00
			L	8.75	0.09	96.39	4.16	16	0.79	6	0.00
			M	9.25	0.42	95.22	2.68	17	0.79	6	0.00
			H	9.21	0.10	95.35	2.61	16.50	0.37	6.10	0.10
		6-Sep	C	9.17	0.10	106.39	3.67	16.80	0.49	6.00	0.00
			D	9.46	0.13	113.19	4.46	16.30	0.44	6.00	0.00
			L	8.75	0.09	101.60	3.23	16.40	0.97	6.00	0.00
			M	9.25	0.42	103.19	2.70	16.90	0.60	6.00	0.00
			H	9.21	0.10	98.58	2.37	15.80	0.47	6.00	0.00

Cage	2014	3-Jul	C	7.61	0.10	22.26	0.33	4.90	0.09	0.50	0.07
			D	7.19	0.09	21.21	0.20	4.90	0.04	0.50	0.06
			L	6.96	0.10	22.13	0.23	4.70	0.06	0.80	0.05
			M	7.08	0.10	21.34	0.30	4.60	0.07	0.70	0.06
			H	7.75	0.11	22.23	0.30	5.00	0.09	0.90	0.04
		9-Jul	C	7.61	0.10	25.02	0.27	6.20	0.08	2.00	0.00
			D	7.19	0.09	25.30	0.37	6.30	0.06	2.00	0.00
			L	6.96	0.10	24.48	0.18	5.90	0.09	2.00	0.00
			M	7.08	0.10	25.91	0.26	6.40	0.07	2.00	0.00
			H	7.75	0.11	25.94	0.37	6.70	0.11	2.00	0.00
		15-Jul	C	7.61	0.10	30.35	0.45	7.70	0.14	2.00	0.00
			D	7.19	0.09	30.86	0.42	7.90	0.09	2.00	0.00
			L	6.96	0.10	29.85	0.42	7.70	0.10	2.00	0.00
			M	7.08	0.10	29.40	0.40	7.70	0.15	2.00	0.00
			H	7.75	0.11	30.16	0.32	8.50	0.09	2.00	0.00
		23-Jul	C	7.61	0.10	41.32	0.42	9.40	0.12	3.00	0.00
			D	7.19	0.09	39.88	0.68	9.80	0.15	3.00	0.00
			L	6.96	0.10	39.39	0.50	9.80	0.16	3.00	0.00
			M	7.08	0.10	38.83	0.50	9.20	0.12	3.00	0.00
			H	7.75	0.11	40.32	0.44	10.00	0.13	3.00	0.00
		1-Aug	C	7.61	0.10	59.37	0.58	12.30	0.14	3.40	0.06
			D	7.19	0.09	53.02	0.84	11.80	0.15	3.20	0.05
			L	6.96	0.10	52.32	0.73	11.40	0.20	3.40	0.06
			M	7.08	0.10	55.05	0.37	12.10	0.19	3.60	0.07
			H	7.75	0.11	50.86	0.79	12.00	0.23	3.30	0.06
		11-Aug	C	7.61	0.10	68.45	0.76	13.20	0.16	5.00	0.00
			D	7.19	0.09	70.04	0.44	13.50	0.12	5.00	0.00
			L	6.96	0.10	65.85	0.69	13.20	0.13	5.00	0.00
			M	7.08	0.10	65.66	0.72	13.30	0.16	5.00	0.00
			H	7.75	0.11	66.36	0.77	13.40	0.17	5.00	0.00

21-Aug	C	7.61	0.10	--	--	14.40	0.22	5.20	0.05
	D	7.19	0.09	--	--	15.20	0.15	5.20	0.05
	L	6.96	0.10	--	--	14.60	0.12	5.00	0.00
	M	7.08	0.10	--	--	13.20	0.26	5.20	0.05
	H	7.75	0.11	--	--	14.00	0.23	5.20	0.05
4-Sep	C	7.61	0.10	70.96	0.80	14.80	0.14	6.00	0.00
	D	7.19	0.09	72.14	0.74	15.50	0.09	6.00	0.00
	L	6.96	0.10	68.74	0.79	14.70	0.16	6.00	0.00
	M	7.08	0.10	66.96	0.87	14.50	0.19	6.00	0.00
	H	7.75	0.11	68.01	0.88	14.90	0.11	6.00	0.00
8-Sep	C	7.61	0.10	72.26	0.75	15.60	0.15	6.00	0.00
	D	7.19	0.09	70.04	0.70	15.40	0.17	6.00	0.00
	L	6.96	0.10	68.01	0.80	15.20	0.17	6.00	0.00
	M	7.08	0.10	68.96	0.77	15.30	0.22	6.00	0.00
	H	7.75	0.11	69.28	0.76	14.80	0.18	6.00	0.00
16-Sep	C	7.61	0.10	69.50	0.98	14.40	0.23	7.00	0.00
	D	7.19	0.09	69.34	0.85	14.40	0.29	7.20	0.05
	L	6.96	0.10	65.21	0.98	14.90	0.19	7.20	0.05
	M	7.08	0.10	64.61	1.42	14.60	0.27	7.10	0.04
	H	7.75	0.11	65.21	0.63	14.50	0.20	7.10	0.04
22-Sep	C	7.61	0.10	72.01	0.75	15.90	0.18	7.30	0.06
	D	7.19	0.09	72.20	0.59	15.70	0.18	7.50	0.06
	L	6.96	0.10	69.47	0.68	15.30	0.14	7.30	0.06
	M	7.08	0.10	68.83	0.94	15.40	0.19	7.30	0.06
	H	7.75	0.11	69.91	0.74	16.30	0.16	7.30	0.09

Open-Plot	2014	11-Jul	NT	6.71	0.07	--	--	8.42	0.13	1.33	0.07
			T	6.65	0.06	--	--	7.92	0.14	1.54	0.08
		18-Jul	NT	6.71	0.07	37.60	0.54	8.96	0.19	2.00	0.00
			T	6.65	0.06	37.81	0.43	8.29	0.19	2.00	0.00
		21-Jul	NT	6.71	0.07	41.70	0.50	8.58	0.22	2.00	0.00
			T	6.65	0.06	40.59	0.52	8.38	0.19	2.00	0.00
		28-Jul	NT	6.71	0.07	51.62	0.39	10.50	0.19	3.00	0.00
			T	6.65	0.06	51.41	0.44	10.83	0.20	3.00	0.00
		5-Aug	NT	6.71	0.07	33.68	0.49	11.38	0.21	3.13	0.06
			T	6.65	0.06	32.76	0.63	11.67	0.20	3.33	0.07
		13-Aug	NT	6.71	0.07	76.52	0.70	14.83	0.19	4.58	0.07
			T	6.65	0.06	72.07	1.00	14.33	0.24	4.79	0.06
		19-Aug	NT	6.71	0.07	78.48	0.76	14.29	0.21	5.00	0.00
			T	6.65	0.06	73.10	0.95	14.04	0.23	5.00	0.00
		3-Sep	NT	6.71	0.07	79.02	0.88	14.71	0.25	6.00	0.00
			T	6.65	0.06	74.69	1.05	14.75	0.28	6.00	0.00
		17-Sep	NT	6.71	0.07	80.71	1.15	15.63	0.32	7.00	0.00
			T	6.65	0.06	75.05	1.02	14.58	0.22	7.00	0.00
		25-Sep	NT	6.71	0.07	81.35	1.09	16.54	0.29	8.00	0.00
			T	6.65	0.06	74.14	1.09	14.04	0.47	8.00	0.00

Table 4.5. Results of the multiple linear regression analysis on the correlation between the soybean vegetative and reproductive stages and the vegetation indices. The table includes information for both the 2013 and 2014 exclusion cage and open-plot studies.

			Vegetative (V) stage							
Study	**Year**		**BNDVI**	**GNDVI**	**EVI**	**EVI2**	**EVI3**	**GBNDVI**	**NIRBRVI**	**NIRGD**
Cage	2013	***F***	19.73	24.99	17.86	17.65	27.99	21.68	4.584	21.89
		df	1, 298	1, 298	1, 298	1, 298	1, 298	1, 298	1, 298	1, 298
		P	≤0.01*	≤0.01*	≤0.01*	≤0.01*	≤0.01*	≤0.01*	≤0.01*	≤0.01*
		R^2	0.06	0.07	0.05	0.05	0.08	0.06	0.01	0.07
Cage	2014	***F***	368.5	164.4	331.5	305.9	310.1	87.78	505.5	158.8
		df	1, 598	1, 598	1, 598	1, 598	1, 598	1, 598	1, 598	1, 598
		P	≤0.01*	≤0.01*	≤0.01*	≤0.01*	≤0.01*	≤0.01*	≤0.01*	≤0.01*
		R^2	0.38	0.21	0.36	0.34	0.34	0.13	0.46	0.21
Open-Plot	2014	***F***	76.4	117.7	68.94	67.9	98.96	28.18	80.88	87.09
		df	1, 158	1, 158	1, 158	1, 158	1, 158	1, 158	1, 158	1, 158
		P	≤0.01*	≤0.01*	≤0.01*	≤0.01*	≤0.01*	≤0.01*	≤0.01*	≤0.01*
		R^2	0.32	0.42	0.30	0.30	0.38	0.15	0.33	0.35
			Reproductive (R) stage							
			BNDVI	**GNDVI**	**EVI**	**EVI2**	**EVI3**	**GBNDVI**	**NIRBRVI**	**NIRGD**
Cage	2013	***F***	243.3	932.6	392.2	408.8	547.3	545	10.93	169.8
		df	1, 298	1, 298	1, 298	1, 298	1, 298	1, 298	1, 298	1, 298
		P	≤0.01*	≤0.01*	≤0.01*	≤0.01*	≤0.01*	≤0.01*	0.001	≤0.01*
		R^2	0.45	0.76	0.57	0.58	0.65	0.65	0.03	0.40
Cage	2014	***F***	365.2	311.6	257.2	234.5	453.7	28.11	520.6	276.7
		df	1, 598	1, 598	1, 598	1, 598	1, 598	1, 598	1, 598	1, 598
		P	≤0.01*	≤0.01*	≤0.01*	≤0.01*	≤0.01*	≤0.01*	≤0.01*	≤0.01*
		R^2	0.38	0.34	0.30	0.28	0.43	0.04	0.46	0.32
Open-Plot	2014	***F***	22.83	50.16	19.17	18.66	35.22	3.39	26.93	28.88
		df	1, 158	1, 158	1, 158	1, 158	1, 158	1, 158	1, 158	1, 158
		P	≤0.01*	≤0.01*	≤0.01*	≤0.01*	≤0.01*	0.08	≤0.01*	≤0.01*
		R^2	0.12	0.24	0.10	0.10	0.18	0.01	0.14	0.15

*: Indicates significant differences at $\alpha = 0.05$

Table 4.6. ANOVA comparing the height (cm) and vegetative stages (V) for all infestation treatments (low (2 mating pairs), medium (8 mating pairs), and high (15 mating pairs) before ovipositioning scars were present in the field. Data for this analysis was from the 2013 and 2014 cage studies.

		Height (cm)		
Year	**Sample Date**	***F***	***df***	***P***
2013	7-Aug	0.33	1,4	0.86
	14-Aug	0.27	1,4	0.90
2014	3-Jul	0.54	1,4	0.70
	9-Jul	0.66	1,4	0.62
		Vegetative Stage (V)		
		F	***df***	***P***
2013	7-Aug	0.48	1,4	0.75
	14-Aug	0.79	1,4	0.54
2014	3-Jul	0.85	1,4	0.50
	9-Jul	2.00	1,4	0.11

Table 4.7. Result from the Welch two-tailed t-test on the end of season number of soybean stem nodes, soybean stem diameter, and the soybean plant height. The analysis compared *D. texanus* infested plans to non-infested plants for the three different plant measurements for the exclusion cage study (Cage) in 2013 and 2014 and the 2014 open-plot study (Open-Plot).

		Mean Soybean Stem Nodes					
						Estimates	
Study	**Year**	***t***	***df***	***P***	**95% *CI***	**non-infested**	**infested**
Cage	2013	-2.30	126.85	0.023*	-1.52 – -0.12	14.59	15.41
	2014	-3.34	187.55	0.001*	-1.44 – -0.37	13.80	14.71
Open-Plot	2014	-5.62	618.35	≤0.01*	-1.34 – -0.65	13.34	14.33
		Mean Soybean Stem Diameter					
						Estimates	
Study	**Year**	***t***	***df***	***P***	**95% *CI***	**non-infested**	**infested**
Cage	2013	-1.18	102.56	0.239	-1.02 – 0.26	8.96	9.34
	2014	-2.87	178.31	0.005*	-0.94 – -0.17	7.08	7.64
Open-Plot	2014	-6.59	589.56	≤0.01*	-0.85 – -0.46	6.48	7.14
		Mean Soybean Plant Height					
						Estimates	
Study	**Year**	***t***	***df***	***P***	**95% *CI***	**non-infested**	**infested**
Cage	2013	-1.79	123.80	0.076	-6.45 – 0.33	90.22	93.28
	2014	-0.78	175.12	0.439	-3.18 – 1.38	63.23	64.13
Open-Plot	2014	-6.10	596.77	≤0.01*	-5.66 – -2.90	69.40	73.68

*: Indicates significant differences at $\alpha = 0.05$

Table 4.8. Results of the linear regression analysis on the correlation between *D. texanus* infestation to yield (ton/hectare) and seed weight (g/100 seed) for both the exclusion cage and open plot studies.

		Yield (ton/ha)					
Study	**Year**	**Slope**	**Intercept**	***F***	***df***	***P***	R^2
Cage	2013	0.08	26.2	0.16	2, 47	0.85	-0.04
	2014	0.12	18.9	1.70	2, 47	0.19	0.03
Open-Plot	2014	0.04	30.1	1.22	2, 45	0.30	0.01
		Seed Weight (g/100 seed)					
Study	**Year**	**Slope**	**Intercept**	***F***	***df***	***P***	R^2
Cage	2013	0.06	9.6	6.64	2, 47	0.003*	0.19
	2014	-0.05	13.6	0.26	2, 47	0.77	-0.03
Open-Plot	2014	-0.03	11.9	6.01	2, 47	0.005*	0.17

*: Indicates significant differences at $\alpha = 0.05$

Chapter 5 - Summary and Conclusions

This research involved three studies intended to address several knowledge gaps about *D. texanus* in field biology and behavior of *D. texanus* as well as the soybean plant responses to infestation. The first and second studies used large scale spatial sampling and protein marking methods, conducted in 2012, 2013, and 2014; eight commercial soybean fields of varying sizes were selected. The first experiment was designed to examine adult *D. texanus* activity within soybean fields to determine if and when *D. texanus* adults and/or larvae are aggregated during the growing season. This study did identify adult *D. texanus* peak activity in Kansas to be in late-June to mid-July with many fields having a prolonged time period of high adult activity. *Dectes texanus* adult and larval populations were also found to be sometimes aggregated at distinct times, typically observed before or after peak activity, during the growing season; however, there was not a consistent pattern either within fields over time or among fields. Similarly, *D. texanus* larvae were not typically aggregated within the fields at the end of the season; only fields 1 and 4 showed significant larval aggregations. It was also found that in continuously planted soybean fields, such as field 1 (2012) there were positive spatial associations between larvae and adults collected; farmers planting consecutive soybean crops, especially those that practice no-till, may have more overwintering of larvae and increased adulty activity the following season.

This study provides information about *D. texanus* in-field behavior that has potential implications for soybean management. Our findings were similar to other studies, in that peak adult activity occurred in a majority of our sample fields from the beginning to middle of July (Hatchett et al. 1975, Rystrom 2015); however, differed by

observing a single, prolonged, peak during July contrary to two distinct peaks in adult activity with the first in early July and the second in early August (Hatchett et al. 1975). The prolonged peak period is particularly beneficial when used with the current insecticide recommendation provided by Sloderbeck and Buschman (2011); treat adults during peak activity and then 10 days later. The variations between years and peak activity highlights the need to develop and incorporate degree-day models for predicting emergence and *D. texanus* adult activity in the field. The results of this study indicate that adult aggregation occurs during July when adult presence is at its highest (mid-late July). This information provided on peak activity time is valuable in determining effective timing of insecticide applications. This study provides support to further explore site-specific management as an option for *D. texanus*. This study was also limited to within a given soybean field and inconsistency in aggregation patterns may be attributed to the behavior of how *D. texanus* beetles disperse into a field. Future research aimed at quantifying the surrounding landscape, specifically areas with native hosts that may influence aggregation locations, could provide information to make accurate predictions as to which edges are more at risk of infestation and suitable for treatment.

The second study examined the dispersal capabilities of adult *D. texanus*. Using a protein-based, mark-capture technique developed by Jones et al. (2006) we were able to positively identify protein marked individuals, which allowed us to measure adult dispersal within a field. Although dispersal measurements were limited to the size of the field, our study found that *D. texanus*, on average, traveled between 52 to 389 m, which was previously unknown. This information is beneficial for future identification of fields that may be "at risk" of becoming infested; however future studies focusing on dispersal

across the landscape and factors that may be driving and influencing that dispersal will be key in making management decisions. Furthermore, based on the leaf disc analysis, we found that the methodology used in this study was appropriate for use in soybean. With minimal drift and no obvious cross-contamination between spray zones the protein application methods may be useful for marking other insects in soybean.

Lastly, the third study was designed to determine if changes in soybean spectral response due to *D. texanus* feeding was measurable under field conditions. This study was we conducted in 2013 and 2014 using two field experiments comparing varying densities of both natural and artificial *D. texanus* larval infestations. Although our experimental design provided significantly different larval densities to test our hypotheses, the results were inconsistent between years and studies and we observed that larval feeding and tunneling in the main stem did not always cause a change in soybean leaf reflectance values (vegetation indices). The results also showed that the 2014 exclusion cage study was the only study, and year, to have significant difference between *D. texanus* infested and non-infested cages for several indices, including ENDVI, ENDVI2, ENDVI3, GBNDVI, NIR Green Diff. and NIRBRVI. More research is needed to determine if the changes brightness values were linked to infestation or other biotic or abiotic factors (i.e. water, nutrition deficiency, disease, sunlight) as well. *Dectes texanus* did affect the soybean growth and development over the course of our experiments. Soybean plants in the open plot study infested with *D. texanus* had significantly more nodes than non-infested plants, while seed size slightly decreased as the number of larvae per cage increased in the 2013 exclusion cage and 2014 open plot studies. Although these effects were observed, it did not result in any significant yield responses. In identifying

these differences in plant biometrics, the information may assist by serving as indicators of at-risk areas in the field. Although subtle differences in plant height are difficult to distinguish by humans, emerging technologies capable of detecting changes in altitude of objects and compatible software to identify height differences may show promise for pest detection. To better understand the yield impact of *D. texanus* on soybean, yield loss studies need to include mechanical yield loss due to lodging in the future; that was not a focus of the current study. Given that we were able to detect changes in crop phenology through time, there is great potential in using remote sensing methods to determine optimal times to harvest soybean before *D. texanus* infestations lodge plants. Such an application would require further investigation.

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