DEVELOPMENT OF SOYBEAN HOST PLANT RESISTANCE AND OTHER MANAGEMENT OPTIONS FOR THE STEM BORER, *DECTES TEXANUS* LECONTE

by

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B.Ag., Tokyo University of Agriculture, 1988
M.Sc., West Texas A&M University, 2004

AN ABSTRACT OF A DISSERTATION

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Abstract

Several studies were conducted to develop soybean management options that could provide protection from the soybean stem borer, *Dectes texanus* LeConte. Selected soybean genotypes were screened for host plant resistance against *D. texanus*. Soybean plants were grown in a footprint that could be covered by a field cage. When beetles were flying in the fields they were collected and placed in the field cages to increase the insect feeding pressure on the test plants. A susceptible commercial soybean variety treated with the systemic insecticide fipronil was used as a positive antibiosis check. Both commercial soybean varieties and plant introductions (PIs) obtained from the USDA National Soybean Germplasm Collection in maturity groups (MG) VI to VIII were tested over a four-year period. Since the number of ovipositions per plant could not be controlled, the ratio of oviposition punctures (OP's) per live larvae (OP/ Lv) was used as a novel index of potential plant antibiosis to *D. texanus*. Field evaluations identified PI165673 as a genotype with a very high OP/ Lv ratio - similar to that for the fipronil antibiosis control. PI165673 appears to be potential source of resistance to *D. texanus*. Factorial analysis indicated that soybean maturity group was not significant factor in the expression of resistance. The OP/ Lv ratio appears to be more sensitive means of identifying antibiosis than other more conventional damage indices. The use of field cages demonstrated consistent plant responses from year to year during the multi-year study. Greenhouse-grown soybean plants, including transgenic plants containing the *Manduca sexta* chitinase gene, were not morphologically appropriate for successful *D. texanus* oviposition because the greenhouse-grown plants were poorly developed and had not produced enough pith in petioles. Therefore the greenhouse results were inconclusive. Analysis of the vertical distribution of *D. texanus* oviposition on soybean plants revealed that *D. texanus* oviposition was most likely to occur on leaf petioles on the upper five nodes of the plant canopy. Histomorphological observations of plant petioles indicated that the proportion of the petiole perimeter occupied by vascular bundles might be related to *D. texanus* oviposition. Both foliar and seed applications of fipronil suppressed *D. texanus* larval damage on soybean plants. The efficacy of these treatments was sustained for long periods, even until adult beetles were present in early August. The effects of

the fipronil seed-treatment and harvest date on grain yield were both significant while the interaction was not. A physiological yield loss of 8.2% and plant lodging losses of 2.9% were associated with *D. texanus* infestation.

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Table of Contents

List of Figures

List of Tables

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CHAPTER 1 - Introduction

1.1. Introdcution

Dectes stem borer is the larva of a longhorn beetle, *Dectes texanus* LeConte, order Coleoptera, family Cerambycidae, subfamily Lamiine and tribe Acanthocinini, formerly known as *D. brevis* Casey, *D. latitarsus* Casey, and *D. spinosus* Auctorum (Dillion 1956). The genus *Dectes* LeConte contains three widely distributed species *D. texanus* LeConte, *D. Sayi* Dillon & Dillon and *D. nigripilus* Chemsak & Linsley in the Northwestern hemisphere (Bezark 2008). *Dectes texanus* is a native species of North America, broadly distributed east of the Rocky Mountains and Northern Mexico (Bezark 2008).

Adult *D. texanus* is dark brown to black with dense short pale gray pubescence, elongate, and moderately slender, 6-10 mm long and 1.6-2.5 mm wide (Dillion 1956, Hatchett et al. 1975, Patrick 1973, and Rogers 1977). The female abdomen is slightly larger than that of the male. Antennae are longer than the body and the male has slightly longer antennae than does the female. The pronotum has prominent lateral spines near the base. Elytra are densely spotted with long erect black setae elongated above the pubescence. The last female abdominal sternite is rather pointed and elongated, whereas the male segment terminates abruptly. Legs have a dark ashy spot at the extreme apex of anterior surface on femora and its tibia and tarsi are broadly fuscous annulate. The egg of *D. texanus* is yellowish white when first deposited and it turns dark yellow near hatch. The length is ca. 1.5 mm and the shape is elongate, slightly narrow at each end. The chorion is smooth and shiny with no sculpturing. Each newly hatched larva is creamy white with amber head capsule with slightly darker mandibles. The mature larva is yellowish, narrow, cylindrical and slightly bent. Larval length is 1.5 mm in the first instar to ca. 15 mm in at the sixth instar. Larvae are legless and have strongly protuberant dorsoventral ampullae on the first seven abdominal segments. The exarate pupa is yellowish white at first to dark brown later, and similar to the adult in form. The length is 8.9-9.4 mm. Sex determination can be readily made on this stage by the presence on females of a pair of genital lobes located on the last segment of the abdominal sternite.

Dectes stem borers are univoltine, with a holometabolous development and a pupal stage (Hatchett et al. 1975, Patrick 1971). Females may produce ca. 50 eggs each in their lifetime.

Eggs are placed in expanded petioles mostly in the upper half of the plant and the incubation is 6-10 days. The head capsule of the pre-born larva becomes visible through the chorion near hatching out. In laboratory rearing observations (Hatchett et al. 1973), egg survival to the larval stage is ca. 35%. The adult sex ratio is ca. 1:1.

Larvae spend nearly 10 months inside the host plant (Campbell and VanDuyn 1977). Measurements of the larval head capsule width taken by Hatchett et al. (1975) indicated that there are six larval instars. Neonate larvae immediately start to feed on the petiole pith and interfascicular parenchyma of the host plant. The larva feeds in the petiole for 14-21 days, and when the petiole pith is depleted, second and third instar larvae bore through the base of the petiole into the primary or secondary stem (Laster et al. 1981). The infested trifoliate leaf and petiole wilts, dries up, and drops from the plant (Hatchett et al. 1975). A rust colored abscission scar often develops around larval entrance hole after the leaf drops from the plant. Most larvae have reached the third instar at this time. Larvae tunnel up and down the stem, however movement and feeding are limited to the internodes just below the node where larvae enter the stem (Patrick 1973). Larvae use the paired dorsoventral ampullae for moving up and down the stems.

Although recognized as a phytophagous insect (Hatchett et al. 1975), *D. texanus* larvae are cannibalistic, attacking each other on contact. Only one larva survives to reach the base of the plant for overwintering, although several eggs may be deposited in one plant. Larval roaming behavior probably causes an increase in the number of encounters between larvae in the stem, and thus increased larval mortality. When plants approach physiological maturity, the mature larvae move toward the lower portion of the stem, pack the tunnel with frass, and complete the tunneling activity in the living stem (Hatchett et al. 1975). Then as the plants approach maturity, larvae girdle the stem from the inside, usually ca. 3-10 cm above the ground. They block the girdled site with a small frass plug, and move to the root crown area to hollow out the lower 20-25 mm of the stem and overwinter in diapause. This girdling activity simultaneously occurs with plant senescence. Campbell and VanDuyn (1977) inferred that this behavior might protect overwintering larvae from natural enemies and adverse environmental conditions. Buschman et al. (2002) have suggested that this behavior reduces competition for the only overwintering site on the plant. Campbell (1980) reported that not all infested plants are girdled. In three sets of

samples taken in the same field with 36, 42 and 44% larval infestation, only 21, 27 and 27% of these stems were girdled. Hatchett et al. (1975) reported similar observations.

D. texanus larvae overwinter in plant stubble at or near or below the soil line, normally as fourth instars (Hatchett et al. 1975). When temperatures increase at the beginning of spring, overwintering larvae become active and begin to feed on woody tissue of the stubble. When feeding is completed, the larviform prepupa moves upward in the stem area of the stubble just above ground level, and cuts an exit hole for adult emergence. The prepupa then returns to the base of the stem, and transforms into a distinct foreshortened prepupa.

The duration of the *D. texanus* pupal stage is 10 to 15 days. During this time, pupae have ability to move within stems, eye pigments appear and the entire body darkens to light gray near adult emergence. After adult eclosion in the pupal cell, the tender adult stays in the stubble for 1-2 days. When the cuticle becomes rigid, adults emerge from exit holes just above the soil line and below the frass plug in stubble previously cut by the prepupa (Hatchett et al. 1975).

Adult *D. texanus* emergence occurs from early July to middle of August in Kansas (Kaczmarek et al. 2001c, Sloderbeck et al. 2003), late May through early July in Texas (Niide et al. 2006a, Phillips et al. 1973), end of May to early August in Tennessee (Patrick and White 1972), from late June to mid- August in Missouri (Hatchett et al. 1975), and from late June to September in North Carolina (Campbell 1980). Adults emerge over a 6-week period, and there appear to be two emergence peaks, e.g. a first peak in early July and a second in early August in Missouri. Emergence of the two sexes is synchronous and occurs between 6 am and 11 am (Hatchett et al. 1975). Adult longevity ranges from 28 to 56 days for females and from 14 to 21 days for males (Lentz 1994). Adults prefer to rest and feed on the epidermis of tender stems, leaf petioles and leaves in the upper one-third of the plant. Feeding occurs periodically during the day but adults apparently don't consume large amounts. Although adults are strong fliers, they passively fall to the ground when flight is disturbed and make a hissing-like sound (Hatchett et al. 1975). Campbell (1980) observed that sticky traps placed at 1 m above the ground collected 99% of the recorded adults, and concluded that the adults fly at soybean canopy height.

Both male and female *D. texanus* mate more than once (Hatchett et al. 1973, 1975, Patrick 1973). Patrick (1973) observed males mating with more than one female, but females mated with only one male multiple times. Mating took place as soon as 5 days after emergence, and pairs were observed in copulation for 5 to 30 minutes on plants, most frequently between 2

pm to 5 pm. *D. texanus* adults feed for 2 days before mating and feeding may be prerequisite to copulation. Hanks (1999) reported that all Lamiines seem to require feeding to mature the eggs, and they usually fed on the same species of plant as the larva. Disengagement and reengagement occur several times during mating but the male does not dismount until mating is completed. Crook et al. (2003) observed that *D. texanus* adult males regularly locate and recognize mates with antennal contact. The elongate male antennae, two or more times the body length, might improve the efficiency of males in locating females for mating. Adult males die within 2 to 3 weeks after mating (Patrick 1973).

D. texanus females begin ovipositing between the third and twelfth day after mating and the average preovipositional and ovipositional periods are 7 and 27 days, respectively (Hatchett et al. 1975). Oviposition occurs on both plant lateral branches and the main stem. When the female locates an oviposition site, she chews a small hole in the epidermis, moves beyond the opening, and probes with her ovipositor until it reaches the hole. She then thrusts her ovipositor into the hole and inserts a single egg into the pith. However, not all oviposition sites contain eggs (Hatchett et al. 1975). The entire process may last 10 min. Females produce ca. 50 eggs in their entire lives (Hatchett et al. 1975). Females are selective in ovipositing on soybean, where the majority of ovipositional punctures have observed in the petioles (83.6%). Much less oviposition occurs on secondary stems (8.6%) and primary stems (7.8%) (Hatchett et al. 1975). Egg deposition depends on whether the female can reach the pith with her ovipositor or whether pith is present in the stem.

Cocklebur, *Xanthium pennsylvanicum* Waller, and common and giant ragweed, *Ambrosia artemisifolia* L. and *A. trifid*a L. (Asteraceae [formerly Compositae]) are native wild *D. texanus* hosts (Piper 1978). *Dectes texanus* is recognized as an economic threat to both soybean, *Glycine max* L. Merr., and sunflower, *Helianthus annus* L. Reported alternative hosts of *D. texanus* are crested anoda, *Anoda cristata* L. Schlecht, cowpea, *Vigna unguiculata* L. Walp (Piper 1973) and lima bean, *Phaseolus lunatus* L. (Taylor and Whalen 2002).

Daugherty and Jackson (1969) reported serious *D. texanus* damage to soybean fields in Missouri, with some fields sustaining nearly 100% plant infestions and ca. 17% plant lodging (girdling). Similar observations on soybean have been reported from North Carolina and Arkansas (Falter 1969), Louisiana and Tennessee (Patrick and White 1972), Texas (in sunflower fields) (Phillips et al. 1973), Mississippi (Laster and Thom 1981), and Kansas (Bell 1985). In

some of these states, large areas were in soybean monoculture, soils had high organic matter, and original native host weeds, e.g. cocklebur and ragweeds, were widely present before the areas were reclaimed for soybean. *D. texanus* has adapted to soybeans and may readily move from weeds to soybeans and from soybeans to weeds. Campbell (1980) inferred that as more land was cleared for expanding soybean production, the numbers of native weed hosts are reduced and the feeding behavior of certain *D. texanus* populations seemed to change toward acceptance of soybean as a host.

Buschman and Sloderbeck (2007) conducted an email survey of the pest status of *D. texanus*, receiving responses from 29 of 40 states. Responses indicated that *D. texanus* appeared to have reached pest status in three major areas. These included the U. S. central plains across Texas, Kansas and into Nebraska; south central states along the Mississippi and Ohio rivers; and the Atlantic coast regions from South Carolina to Delaware. *D. texanus* was also recognized as a sunflower pest in seven states mainly in the Great Plain Region.

Buschman and Sloderbeck (2009) also reported on a detailed survey of *D. texanus* infestations in Kansas, where between 1985 and 2008 the number of counties infested with *D. texanus* increased from 5 to 64 out of 105. The number of fields with more than 50% plants infested increased from 3 in 1999 to 25 in 2008. Counties in north central Kansas had heavy infestations (ca. 80% of soybean fields and ca. 40% of soybean plants infested), but eastern counties had surprisingly few *D. texanus* although there were many acres of soybeans. A large increase in Kansas soybean acreage occurred from 1985 (1.5 mill acres) to 2008 (3.3 mill acres). In addition, the use of no-till farming practices increased from 2 % in 1989 to 21% in 2004 across Kansas. The authors suggested that these trends were favorable for *D. texanus* and might be associated with the observed increase in infestations of this pest insect in Kansas.

As soybean crops approach maturity, girdled plants are vulnerable to external forces such as high wind or heavy rain that may cause plants to break at the girdled point. This condition is known as lodging, and if it occurs before harvest, serious yield losses can occur. Adult *D. texanus* also cause considerable scaring of the plant stem which may not penetrate the cortex or encircle the stem, may also cause lodging. In Kansas, Higgins et al. (1999) reported losses of 672-1,545 kg/ ha caused by lodging when harvesting was delayed.

As previously mentioned, not all infested plants were girdled (Hatchett et al. 1975, Campbell 1980). In Tennessee, Campbell (1980) reported the percentage of infested stems that

are girdled averaged less than 50%, and ranging between 15 and 70%. However, there is a 10% loss in seed weight for soybean plants that are infested but not girdled. Tunneling in primary or secondary stems may also interfere with water and nutrient transport, and may cause soybean plants to have reduced productivity.

Hanks (1999) investigated the natural history and behavior of 81 species of Cerambycid beetles including *D. texanus* and concluded that reproductive behavior was correlated with the condition of the larval host. Species were assigned to four groups based on the condition of the larval host plants at the time of colonization. *D. texanus* was grouped into the Healthy Host (HH) species group in which larvae feed in the stems of herbaceous plants, and require living hosts. Larvae may not be able to complete development if the hosts die, however larvae of this species may sometimes weaken the host to a crucial degree. Females of the HH group girdle the stems of herbaceous plants or branches of woody hosts prior to oviposition or the larvae internally girdle branches of herbaceous and woody plants. Choice of oviposition hosts by adult cerambycid females is crucial since larvae are legless and incapable of moving between host plants. Females of HH species oviposit on the same host that the larvae feed on, but only on vigorous host plants. Hanks (1999) reported that all Lamiines to require maturation feeding and that they usually feed on the same host species, as did their larvae. It homogenizes their behaviors with adults often feeding, mating, and ovipositing on the same host plants. The proximity of adult feeding, mating and ovipositing sites for HH species accounts for the relatively sedentary nature of adults of both sexes.

Host plant resistance is an essential factor in integrated pest management (IPM). Plant resistance to arthropods is defined as the sum of the constitutive, genetically inherited qualities that result in a plant of one cultivar or species being less damaged than a susceptible plant lacking these qualities (Smith 2005). VanEmden (1991) stated that the aim of plant resistance was to reduce the losses in yield caused by pests. The economic benefits of the use of resistant cultivars in IPM are that the cost of the resistant cultivar is limited to the cost of purchasing the seeds and that resistant plants may lead to reduced or eliminated use of insecticides, resulting in decreased production costs (Smith 1989). Plant resistance also has advantages as a stand-alone control tactic over other major tactics, i.e. chemical control and biological control, in IPM. In most cases, biological control depends on a sustained host/ prey density, however resistant plants may stand alone and are not density dependent. Plant resistance can reduce or eliminate the use

of insecticides. In addition, because insecticides often kill pest and beneficial insects, the compatibility between biological and chemical control is less, compared to that of plant resistance/ biological control and plant resistance/ chemical control combinations.

Based on the three widely used categories of the plant resistance to arthropods (Painter 1951), Smith (1989) defined these as; 1.) antixenosis - adverse plant effects on insect behavior; 2.) antibiosis - adverse plant effects on insect survival affecting physiology of the insect; and 3.) tolerance - a plant's ability to withstand, repair or recover from insect damage. Antixenosis can be expressed in a cultivar through either allelochemical or morphological traits. Allelochemical antixenosis and antibiosis characteristics give a plant greater numbers of potential resistance factors (Smith 1989) because thousands of combinations of chemical compounds may mediate resistance. On the other hand, characteristics of morphological antixenosis have been found that impair feeding behavior and may be a first line of plant defense (Pedigo 1999). However, Pedigo (1999) also noted that the use of some antixenosis characteristics may be limited by cultural environments in a practical sense. Many cultivars may show antixenosis if alternate host plants are grown in proximity. However in the absence of alternate hosts, antixenosis may break down.

Antibiosis usually affects the metabolic processes of arthropods, and insect and plant factors are involved in antibiosis mechanisms (Pedigo 1999). Antibiotic effects of a resistant plant range from mild to lethal. Lethal effects may be acute, in which case they often affect young larvae. Chronic effects of antibiosis often lead to mortality in older larvae and prepupae that fail to pupate, and in pupae and adults which fail to eclose. Individuals that survive from the direct effects of antibiosis may also suffer the debilitating effects of reduced body size and weight, prolonged periods of development, and reduced fecundity (Smith 2005). Because of the difficulty in designing experiments that can distinguish between the antixenosis and antibiosis, these two categories of plant resistance often seem to be overlapping.

Because only a plant response is involved in tolerance, unlike antixenosis and antibiosis, some plant scientists have not considered tolerance a form of resistance (Beck 1965). However, an important advantage of tolerance is that it places no selective pressure on the insect populations. Pest population levels are not diminished by exposure to tolerant plants, as they are on plants exhibiting antibiosis/ antixenosis, so their virulence gene frequencies remain unchanged. The selection pressure placed on them is lower than the high levels of selection

placed on them by antibiosis. Thus, the potential for development of resistance-breaking biotypes is greatly diminished through the use of tolerant cultivars (Smith 2005).

To evaluate the potential host plant resistance for development into new resistant cultivars, Smith (2005) emphasized that plant resistance to an insect pest should always be measured on a relative scale, with the degree of resistance based on a comparison with susceptible control plants that are severely damaged or killed under similar experimental conditions, as well as resistant control plants with a known, accepted, predetermined level of resistance. Insect responses to potential host plants sometimes differ between free-choice and non-choice evaluations. Smith (1989) mentioned that the parallel use of choice and no-choice screening is essential to provide reliable identification and measurement of host plant resistance against pest insects. A non-choice test reduces the possibility that uneven distribution of test insects may lead to an unbalanced infestation across the test plants as may occur in a free-choice test. This could cause an erroneous conclusion in the identification of resistance. When combined with the use of cages, a non-choice test can exhibit these advantages more clearly. Cages can protect test insects from predation and parasitism and they can limit emigration from plants being evaluated. Smith et al. (1994) also pointed out that greenhouse experiments could allow the researcher to make large-scale evaluations of seedlings in a relatively short period of time.

Although efforts have been made to detect resistance to *D. texanus* in soybean, no usable resistance has been identified in the Midwest and there are no resistant varieties available for use in Kansas. Richardson (1975) conducted field and laboratory tests in North Carolina to examine a wide range of soybean genotypes for *D. texanus* resistance. In three years of screening he tested a total of 618 soybean plant introductions from maturity groups V-VII and identified 18 lines with what appeared to have moderate levels of resistance. However, ratings were based on plant girdling, which is confounded by maturity group and environmental conditions. Later maturing varieties appear to be girdled less frequently or later, even when they were infested. For example, Richardson (1975) reported that the average infestation for maturity groups V, VI, VII was 56.5, 49.4, and 41.2%, respectively, and that many of the plant introductions with low borer infestations also had a procumbent growth habit, which may have contributed to the low ratings because the plants had reduced exposure to beetles. In additional evaluations of commercial varieties from maturity groups V-VIII, Richardson (1975) found that the variety with the highest

rate of plant girdling was Lee in maturity group VI (57.5%), and the variety with the lowest rate of girdling was Hampton, in maturity group VIII (7.4%). Campbell (1976) reported similar results, and demonstrated that group VIII soybeans had 8% *D. texanus* girdling compared to 44% girdling for soybeans in groups IV-VII.

Kaczmarek et al. (2001a) assessed *D. texanus* resistance in commercial soybean varieties from maturity groups II-V in Kansas under irrigated and dry land conditions, and found wide ranges of tunnelled plants (0-93%), larval infestation (0-87%), and girdling (0-60%). These results suggested that there must be significant differences in soybean response to *D. texanus*. However, intensive reexamination of these varieties the following season and in other locations revealed no consistency in varietal resistance across these trials. The average lodging across all varieties in the irrigated plots averaged nearly 37% but only 17% in dryland plots.

Whalen et al. (1998), working in Delaware in 1996 and 1997, evaluated soybean cyst nematode (SCN), *Heterodera glycines*, resistant and susceptible varieties in maturity groups III-V to determine if SCN resistance conferred any resistance to *D. texanus*. Resistant varieties had significantly lower stem lodging and lower numbers of infested stems compared with the susceptible varieties. In addition, maturity group III varieties exhibited significantly lower percentages of infested stems and stem lodging than did group IV and V varieties. During another three-year evaluation, Taylor and Whalen (2002) observed some soybean varieties resistant to SCN to have lower plant lodging and higher yields, however their results led them to conclude that the lowest stem lodging could be expected in maturity group IV varieties resistant to SCN. Nevertheless, their conclusions were inconsistent since their results varied from year to year. Higgins et al. (2003) assessed *D. texanus* resistance in several SCN resistant varieties in Kansas, and found no significant reductions in borer damage. In Texas, Niide et al. (2006b) evaluated eleven SCN resistant and susceptible soybean varieties in maturity groups III-V, but found no *D. texanus* resistance.

Chitin, one of the most abundant polysaccharides in nature, is found in two primary extracellular structures of insects - the exoskeleton (cuticle) and the gut lining (peritrophic membrane). Chitinase, a chitinolytic enzyme that catalyzes the hydrolysis of chitin, is found in chitin-containing organisms as well as in micro-organisms, plants, and other animals that do not have chitin. The enzymes derived from different sources have different biological functions; molting of the exoskeleton in insects, cell growth and division in fungi, utilization of chitin as

nutrition in bacteria, and defense against pest and pathogen attacks in plants (Choi et al. 1997, Flach et al.1992, and Kramer and Muthukrishnan 1997).

The potential use of chitinase as an enzyme to disrupt insect molting (production of a new chitin exoskeleton) or as an enhancer of the toxicity of entomopathogenic bacterium (e.g. *Bacillus thuringiensis*, Bt) has been explored for decades. There are many cases of success for microorganism-derived chitinase (microbial chitinase) enhancing *Bt* toxicity (Gongora et al. 2001, Liu et al. 2006, and Wang et al. 1996), but only a few successes of insect-derived chitinase as a plant resistance factor in transgenic plants (Ding et al. 1998, Fitchesa et al. 2004). Kramer and Muthukrishnan (2005) used a family 18 insect-derived chitinase as a host plant resistance factor in transgenic plants and as an enhancer protein for baculovirus toxicity in biopesticide development research. Although insecticidal activity of insect chitinase was not substantial enough for commercial development, insect-derived chitinase may have potential as a host plant resistance factor in transgenic plants. Ornatowski et al. (2004) successfully transformed embryogenic soybean cultures with a *Manduca sexta* chitinase (msc) gene using microprojectile bombardment.

Insecticidal control of *D. texanus* has proven to be difficult and ineffective. Insecticide sprays and granules targeting larvae in the stubble were ineffective since insecticides do not penetrate the stubble to contact the larvae. Adults can be controlled with contact insecticides (Kackzmarek 2003), but they are present for more than 5 weeks, requiring multiple insecticide applications. Effective control of adults also necessitates knowledge of annual adult emergence to provide accurate information for timing of insecticide applications. Scouting for adult *D. texanus* is been difficult, as visual plant inspection is time consuming and rarely successful. In Mississippi, aerial application of methyl parathion on a 5- to 7-day schedule for approximately 60 days reduced adult populations but soybean yields were no different in treated and untreated areas (Laster et al. 1981). Thus, control with foliar insecticide does not appear to be practical or economical.

Nonetheless, many efforts have been made to explore effective methods for the chemical control against *D. texanus* in Kansas. Kackzmarek et al. (2002) reported that lambda-cyhalothrin, permethrin and carbaryl were comparatively effective for controlling adult *D. texanus* under laboratory conditions, however Sloderbeck et al. (2004) concluded that more than one aerial application of lambda-cyhalothrin was needed to reduce larval infestations to acceptable levels in

field. The efficacy of several systemic insecticides has been evaluated as seed, soil and foliar treatments for *D. texanus* larval control in soybean plants. Imidacloprid and clothianidin did not provide adequate protection as seed treatments (Higgins et al. 2003, Kackmareck et al. 2001b), but fipronil applied as a soil or foliage treatment gave significant *D. texanus* larval control and provided a 10% increase in grain yield (Buschman et al. 2005, 2006). These results were the first to show positive effects of insecticides on *D. texanus* infestations and soybean yield response (Buschman and Sloderbeck 2006).

Fipronil, a phenyl pyrazole insecticide, is highly effective against a broad range of insect pests even when used at low doses. Fipronil interferes with the passage of chloride ions through the gamma-aminobutyric acid (GABA)- regulated chloride channel, disrupting the central nerve system. Fipronil binds with higher affinity to insect GABA receptor sites than vertebrate sites, a difference that accounts for selective fipronil toxicity (Gant et al. 1998, Scharf and Siegfried 1999). Fipronil is effective against other wood-boring cerambycids in the genera *Monochamus, Acanthocinus,* and *Stenocorus* (Grosman and Upton 2006) and against other coleopteran pests of corn and cotton (Maloney 2003, Mulrooney and Goli 1999).

To date, cultural controls are the only feasible measures of reducing losses from *D. texanus* since no resistant soybean cultivars have been developed and conventional chemical controls for this pest are not been economically feasible. Timely planting and harvesting, planting longer-season or late-maturing soybean varieties, avoiding planting in or adjacent to infested fields or next to areas with alternative hosts, good weed management, and destruction or burial infested stubble have all been suggested as beneficial management strategies (Campbell 1976, Laster and Thom 1981, and Sloderbeck et al. 1988).

Planting date influences the rate of *D. texanus* infestation. In North Calorina, Campbell (1976) reported that when soybeans were planted in May, *D. texanus* infestation was 14% while soybeans planted in June had an infestation rate of 85%. Since significant losses occur when plants are girdled and lodging occurs before harvest, early harvesting may also help to reduce girdling loss. Delaying the harvest until well after soybean maturity increases the occurrence of *D. texanus* stem girdling. When high percentages of infested stems are observed, harvesting should be done as soon as possible to avoid more girdling and lodging. Later-maturing varieties mature during relatively cooler weather, giving more time for harvest before plants are susceptible to lodging. Short-season varieties mature rapidly during the hot season, allowing

lodging to occur sooner than in longer-season varieties. Sloderbeck et al. (2003) stated that some consultants in Kansas advised growers to avoid early planted, short-season soybean varieties to reduce losses from this insect pest. The feasibility of these two recommendations is dependent on *D. texanus* phenological development in each region.

In North Carolina, Campbell (1980) reported that the proximity of a soybean field infested by *D. texanus* the previous year to significantly affect *D. texanus* infestation, and that *D. texanus* infestation decreased as the distance from the source of infestation increased. In addition, the numbers of adult *D. texanus* collected from ragweed were 2- to 4 times greater than those collected from adjacent soybean. These results lead recommendations to avoid planting the same (soybean) crops consecutively and practice good weed management in fallow fields and fencerows to reduce infestations. Fall tillage also reduces *D. texanus* winter survival. Campbell and VanDuyn (1977) evaluated methods and times of stem burial as a measure of reducing *D. texanus* adult emergence. Burial of infested stubble by deep plowing and row bedding reduced infestation from 20 to 52%. Burial of stems in the spring did not significantly increase larval mortality compared with the burial in the winter, but adult emergence decreased as the depth of stubble burial increase from 5 to 15 cm. Adult emergence was significantly reduced when the stubble was buried buried as soon after harvest as possible at a depth of 5 cm or more. Although these cultural methods offer a practical solution to *D. texanus* management, current residue cover legal requirements makes this option unacceptable. Findings of Sloderbeck et al. (2003) point out that the increase frequency of reduced tillage practices in the last few years may be one reason that the incidence of *D. texanus* has been increasing. Sloderbeck et al. (2003) further reported that soil type and moisture could be a factor in reducing *D. texanus* infestations, as hard-packed or crusty clay soils are suspected to inhibited adult emergence. Well-drained soil seem to harbor more larvae compared with wet soils. Stubble in low, wet areas led to a range of 50 to 70% larval mortality, while well-drained areas experienced a range of only 11 to 38% mortality.

Michaud et al. (2007) reported that *D. texanus* infestation in soybean fields could be reduced by 65% by planting sunflowers as a trap crop in the non-irrigated corners of a center pivot irrigated soybean field, presumably due to the fact that *D. texanus* adults have a strong feeding preference for cultivated sunflower over soybean (Michaud and Grant 2005). Sunflower plants can accumulate multiple eggs as an “oviposition sink” because *D. texanus* females do not

avoid ovipositing in plants already infested with their own eggs or those of conspecific females, and frequently oviposit multiple times into the same plant and even the same petiole (Michaud et al. 2007).

To date, there are no known natural enemies of *D. texanus*. However, Hatchett et al. (1975) reported some Hymenopteran parasites were identified and reared from larvae collected from giant ragweed. These were *Bracon cerambycidiphagus* (Muesbeck) and *Bracon* sp. (Braconidae); *Neocatolaccus tylodermae* (Ashmead); *Habrocytus* sp., *H. languriae* Ashmead, and *H. arkansensis* Girault (Pteromalidae); and *Melanicheumon brevicinctor* (Say) (Ichneumonidae). Recently, up to 2% of soybean plants containing *D. texanus* larvae contained a Hymenopteran parasite in 22% of infested soybean fields in Missouri (Tindall et al. 2009).

The rationale for this project was to develop techniques and if possible identify sources of resistance in soybeans to *D. texanus*. The objectives of this study were; 1.) to develop new techniques and procedures to identify potential antibiosis (reduced larval survival) and/ or antixenosis (oviposition non-preference) in soybean effective against *D. texanus*; 2.) to use these techniques to screen Kansas soybean varieties and soybean plant introductions from the USDA Soybean Germplasm Collection for antibiosis and antixenosis resistance; 3.) to determine vertical *D. texanus* oviposition preference in order to focus sampling on those plant regions; 4.) to investigate whether morphological differences in soybean varieties are associated with differences in *D. texanus* infestation and; 5.) to develop techniques to identify tolerance resistance in soybean varieties based on differences in seed yields to in insecticide treated- versus untreated soybean plots.

We hypothesized that; 1.) the ratio of *D. texanus* oviposition punctures/ larvae (OP/ Lv ratio) and the number of oviposition punctures (OP's) could be used to identify potential antibiosis and antixenosis in soybean germplasm; 2.) that variation exists in the level of *D. texanus* resistance exhibited by some soybean plant introductions and commercial soybean varieties; 3.) that we could describe the relationship between the vertical oviposition preference of *D. texanus* on plant petioles and the variation in petiole morphology during plant maturation; 4.) that resistant plant introductions would have a greater proportion of the stem perimeter occupied by vascular bundles; and 5.) that the yield response of different soybean varieties to *D. texanus* infestation varies between insecticide treated and untreated plots.

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CHAPTER 2 - Field Evaluation of Soybean Germplasm for Resistance to the Dectes Stem Borer, *Dectes texanus* LeConte

2.1. Abstract

No soybean cultivars exist to provide resistance to larval damage by the Dectes stem borer, *Dectes texanus* LeConte, in Kansas. In this study, selected soybean varieties and plant introductions (PIs) in maturity groups (MG) VI to VIII from the USDA National Soybean Germplasm Collection were evaluated for *D. texanus* resistance from 2005 through 2008. Field cages were used to confine beetles on test plants. The numbers of oviposition punctures (OP's) per plant were used as indicators of oviposition non-host preference or antixenosis and the ratio of OP's per live *D. texanus* larvae (OP/ Lv) was used as an indicator of plant antibiosis to larvae. A *D. texanus* susceptible variety treated with the systemic insecticide fipronil was used as a positive antibiosis control. Over the four years of screening, the plant introduction PI165673 had the highest OP/ Lv ratio (evidence of antibiosis resistance) and appeared to be a potential source of resistance to *D. texanus*. Factorial analysis of all soybean genotypes by maturity group indicated that maturity group was not a significant factor in the expression of resistance. The OP/ Lv ratio appeared to be a more sensitive indicator of *D. texanus* antibiosis resistance than other conventional damage indices. Field cages maintained pest population pressure on test plants and provided consistent results from year to year.

Key words:

Dectes stem borer, soybean plant introductions, maturity groups, OP/ Lv ratio, consistent plant response

2.2. Introduction

Host plant resistance is a key component of integrated pest management. Although efforts have been made to detect resistance in soybean to the Dectes stem borer, *Dectes texanus* LeConte, no usable resistance has been identified and there are no resistant varieties available for use in Kansas. Richardson (1975) conducted field and laboratory tests in North Carolina to examine a wide range of soybean genotypes for resistance to *D. texanus*. In three years of screening, he identified 18 lines that appeared to be moderately resistant out of 618 soybean plant introductions (PIs) from maturity groups V-VII. Some PIs had a procumbent growth habit, so it was not clear if the responses were true resistance or lack of exposure. Maturity group also confounded resistance ratings since most resistant lines were in the longest maturity groups. The average percent infestation for maturity groups V, VI, and VII averaged 57, 50, and 41%, respectively (Richardson 1975). The group VI variety "Lee" had the highest rate of girdling, with 58% plants infestation. The group VIII variety "Hampton" had the lowest rate of girdling. Similar results were observed by Campbell (1976), who reported that an average of 8% of plant girdling in soybean varieties in maturity group VIII compared to an average of 44% for varieties in maturity groups IV-VII.

In Kansas, Kaczmarek (2003) assessed soybean varieties in maturity groups II-V for *D. texanus* susceptibility under irrigated and dryland conditions. In 2000, there were significant differences in the number of girdled plants and the number of larvae in plants among several varieties in maturity groups III and IV, such as Asgrow AG3302, AG3702, Delta Pine DR3478, Garst D308, K-Soy Stressland, Midland 8393, NC+ 4N26, Midwest G3644s, Pioneer 94B01, and Z-Public K1380. However, tunneling ranged from 0 to 93% among varieties, the number of plants containing larvae ranged from 0 to 87%, and the number of plants girdled ranged from 0 to 60%. Lodging among all varieties in irrigated plots averaged nearly 37% compared to 17% in dryland plots, but there were no consistent differences among varieties in 2001.

Whalen et al. (1998) evaluated Delaware soybean varieties in maturity groups III-V with cyst nematode (SCN), *Heterodera glycines*, resistance to determine if SCN resistance conferred resistance to *D. texanus*. The SCN resistant varieties had significantly less lodging and infested stems compared to susceptible varieties, and maturity group III varieties exhibited significantly less lodging and fewer infested stems than did varieties in groups IV and V. However Taylor

and Whalen (2002) reported that there was reduced borer-related lodging in SCN-resistant maturity group IV varieties. Higgins et al. (2003) concluded that there was no relationship between *D. texanus* damage and SCN resistance in Kansas. Similar result was reported by Niide et al. (2006) in Texas.

The studies described above used borer-related stem girdling, lodging or tunneling to evaluate soybean susceptibility to *D. texanus*, and all of these parameters appear to have led to variable results. In the present study, an attempt was made to develop other biological parameters to screen soybean accessions and varieties for *D. texanus* resistance. The numbers of oviposition punctures were used to estimate antixenosis (reduced plant acceptability for oviposition) and the numbers of live larvae were used as a measure of antibiosis (suppression of larval survival or development). These measurements were taken early in the growing season to avoid the confounding effects of larval cannibalism. Since the number of surviving larvae may be determined by the number of plant oviposition scars, the ratio of oviposition punctures/ larvae was used to correct for plants that received fewer oviposition scars. Large field cages installed over the experimental plantings were used to confine hundreds of field-collected *D. texanus* adults on field-grown soybean entries to ensure sufficient insect infestation pressure on experimental plants to make useful evaluations. Since no known source of *D. texanus* resistance exist, fipronil-treated plants were used as a positive antibiosis controls. Fipronil is a systemic insecticide that provides effective control of *D. texanus* larvae (Buschman et al. 2005).

2.3. Materials and Methods

In 2005 and 2006, 15 soybean genotypes, including six Kansas varieties and nine plant introductions (PIs) were evaluated for *D. texanus* resistance. Varieties included the most resistant and most susceptible varieties in maturity groups II to IV, based on results of Khajuria et al. (2005). The PIs included several identified by Richardson (1975) as resistant to *D. texanus* and others known to have resistance to various other insect pests of soybean, including the Mexican bean beetle, *Epilachna varivestis* Mulsant (VanDuyn et al. 1971, 1972), bean leaf beetle, *Cerotoma trifurcata* (Forster), striped blister beetle, *Epicauta vittata* (F.) (Clark et al. 1972) banded cucumber beetle, *Diabrotica balteata* LeConte (Layton et al. 1987) and the corn earworm *Helicoverpa zea* Boddie, (Smith and Brim 1979, Smith et al. 1979) and Asian soybean

defoliating pests (Talekar et al. 1988). Seed of varieties were obtained from Dr. William T. Schapaugh, Soybean Breeder, Kansas State University, and seed of PIs were obtained from Dr. Randall L. Nelson, USDA ARS Soybean Germplasm Collection, Urabana, IL. In 2006, the susceptible conventional variety 93M50, received a soil treatment of the systemic insecticide fipronil (BASF Corporation, Research Triangle Park, NC) (Regent 4SC) (290.5 g/ ha) to simulate antibiosis. A 30 cm barrier (corrugated metal roofing) was installed around the fipronil-treated plants to reduce the chance of insecticidal effects on surrounding plants. In 2007 and 2008, soybean seed was treated with fipronil (Regent 500TS at 25 [2007] or 100 [2008] mg AI/ 100 kg) as a positive control for antibiosis.

In 2007, the number of lines evaluated was reduced to increase the number of plants that could be evaluated for each line. Plant introductions PI165673, PI171451 and PI165676 were selected for further research based on 2005 and 2006 results. The 2007 results suggested that the antibiosis and antixenosis observed in the three PIs might be associated with their maturity groups (MG VI-VIII). Therefore, in 2008 three commercial varieties with similar maturities were included along with the susceptible Kansas commercial variety 93M92 treated and untreated with the systemic insecticide at 100 mg AI (fipronil, Regent 500TS) / 100 kg. Untreated 93M92 served as a susceptible check. Seed of commercial varieties tested in 2007 and 2008 were obtained from Pioneer Hybrids Inc.

Fourteen plants from each of 15 soybean entries were planted 5 cm apart with 76 cm rows apart on June 2, 2005 and May 20, 2006 at the KSU North Central Kansas Experiment Field near Scandia, Kansas (Figure 2.2). Soybean entries were spaced to fit inside the footprint of a commercial screen cage that was placed over the plants later in the season. The placement of the entries within each cage was a stratified random design to insure each entry was included in the center positions at the same frequency. Plantings included five replications in 2005 and six replications in 2006. Supporting posts 1 m high with horizontal cords were employed to support procumbent PI plants to the height of varieties. Entries were planted May 31, 2007 and June, 30 2008, respectively, as before, but in a footprint to accept the screened tents. In 2007, six replications were included in three screened cages, and in 2008, six replications were included in six cages.

When beetles were present in the field, polyester screen tents (North Pole USA, Washington, MO) were installed over the experimental plants (Figures 2.3, 2.4). Adult beetles

were collected from surrounding soybean fields using sweep nets, and a total of 550, 300, 315 and 110 beetles were released into each screen tent in 2005, 2006, 2007 and 2008, respectively (Figures 2.5, 2.6). Plant height and maturity was recorded near the time when tents were installed. Tents were left in place for 28 d (2005) or 10 d (2006) before removal. In 2007 and 2008, beetles were confined in the cages for at least 15 d and the cages were opened to release any remaining beetles. Plants were collected for infestation observations starting 7 d after the cages were opened to allow eggs time to hatch.

Plants from one or two replicates were collected at a time and processed in the laboratory. When possible, three plants per replicate were collected from each of the 15 entries in 2005 and 2006, and five plants per replicate were collected from each of five entries in 2007 and eight entries in 2008. For each plant, the number of oviposition punctures, live larvae, eggs and entry nodes were recorded separately for each petiole and internode on the plant. The length of the main stem and the plant development stage were also recorded. Plants were dissected to count eggs and larvae still inside stems and petioles. The ratio of oviposition punctures to live larvae (OP/ Lv) was also calculated. Data were analyzed using SAS PROC GLM (Colette and Robinson 2000) and means were compared with Duncan's Multiple Range Test ($\alpha = 0.05$).

2.4. Results and Discussion

Heavy rainstorms destroyed two of five replications in 2005 and one of six replications in 2006, yielding 3 and 5 replications for data collection. In 2005, the number of oviposition punctures (OP's) ranged from 134 to 196 per plant on among plant genotypes (Table 2.1). There was a significant difference ($F = 3.74$; df = 16, 110; $P < 0.0001$) and broad overlap across entries, but PI171444, PI171451 and PI227687 had the fewest OP's, from 49 to 62 OP's per plant. All the commercial varieties tended to have very high numbers of live larvae, from 4.3 to 7.5 larvae per plant (Table 2.1), and 93M50 had the largest number of live larvae (7.5 larvae per plant). The fewest number of larvae (1.4 larvae per plant) were found in PI227687 and PI82312 (Table 2.1). There was significant difference ($F = 3.30$; df = 16, 110; $P < 0.0001$) among the entries. The number of eggs found was so small that there were no significant differences among the entries ($F = 1.29$; df = 16, 110; $P = 0.2147$) (Table 2.1). Varieties had larger numbers of larval entry nodes (1.6 to 5.0) than did plant introductions (0.0 to 2.1) (Table 2.1). Among stems

of plant introductions, there appeared to be fewer larvae that were successful in developing to the stage where they could tunnel into the main stem or side branches. The ratio of oviposition punctures to live larva (OP/ Lv) was highly significant among the soybean entries ($F = 3.86$; df = 16, 93; $P < 0.0001$) (Table 2.1). PI165673 had the highest OP/ Lv ratio (99.3), which was significantly greater than all other entries.

In 2006, PI82312 had the highest number of OP's (110), followed by the susceptible variety, 93M50 (55.1) (Table 2.2). The fipronil-treated NEX2403K control had the fewest OP's (23.7). The mean number of OP's per plant among the remaining 12 entries was significantly different ($F = 5.32$; df = 18, 199; $P < 0.0001$) and ranged from 25.3 to 53.9. The largest numbers of live larvae per plant were found in X3727NRS (9.4) (Table 2.2), and the varieties tended to have higher numbers of live larvae, ranging from 6.1 to 9.4 larvae per plant. The PIs averaged 3.3 to 9.3 larvae per plant, but there was broad overlap across the entries. The fipronil treated NEX2403K control and PI165673 had the fewest live larvae (0.4 and 3.3, respectively). There was significant difference on the numbers of live larvae ($F = 5.17$; df = 18, 199; $P < 0.0001$) across the entries.

PI82312, PI228065 and KS4704RR had significantly more eggs (1.2 to 1.8 eggs per plant) than PI165676, PI165673, KS4404RR, NEX2403K (0.3 eggs per plant) (Table 2.2). The largest number of entry nodes (4.2 per plant) was found in KS4404RR, which was significantly larger than the fipronil treated NEX2403K control plants (0) (Table 2.2). With the exception of the NEX2403K positive control, commercial varieties had comparatively larger numbers of entry nodes (1.3 to 4.2) than the plant introductions (0.4 to 1.3). The OP/ Lv ratio was highly significant ($F = 9.84$; df = 18, 186; $P < 0.0001$), and the ratios in the fipronil treated NEX2403K control plants (26.8) and PI165673 plants (20.5) were significantly greater than those for all other entries (Table 2.2).

In 2005 and 2006, PI165673 had a significantly higher level of antibiosis, as measured by the OP/ Lv ratio, than all other entries tested (Tables 2.1, 2.2). Interestingly, PI165673 does not appear to have antixenotic effects on *D. texanus* oviposition. PI165676 also exhibited significant antibiotic effects against *D. texanus* compared to the other genotypes tested. PI171451 has well-known insect resistance (Hatchett et al. 1976, VanDuyn et al. 1971, 1972) and appeared to exhibit antixenosis toward *D. texanus*, as it sustained only 49.1 OP's per plant in 2005 (Table 2.1). However the OP/ Lv ratio in PI171451 was significantly lower than that of PI165673. Thus,

PI165673, PI165676, and PI171451 showed preliminary evidence of *D. texanus* resistance. The varieties did not exhibit antibiosis. NEX2403K with fipronil successfully served as a positive control, and exhibited strong antibiosis in the 2006 experiment (Table 2.2).

In 2007, the largest number of OP's was found in PI165673 (25.3 per plant) (Table 2.3), while PI171451 and PI165676 had comparatively fewer OP's (15.6 and 15.3, respectively). There were significant differences ($F = 14.97$; df = 4, 5; $P < 0.0001$) across the tested entries. All plant introductions had significantly fewer live larvae per plant (0.6 to 0.9) than the fipronil treated 93M50 (1.7 per plant) (Table 2.3). No eggs were found in untreated 93M50 plants (Table 2.3), but the three plant introductions received 0.03 to 0.11 eggs per plant. The largest number of entry nodes was found in untreated 93M50 plants (1.92 per plant) (Table 2.3), which was significantly more than all other entries. The OP/ Lv ratio was significantly larger ($F = 5.38$; df = 9, 85; $P < 0.05$) (Table 2.3) for PI165673 and fipronil treated 93M50 (23.5 to 25.4 per larva) than the other three entries.

In 2008, the largest number of OP's was again found on PI165673 plants and on fipronil-treated 93M92 plants with 24.2 per plant (Table 2.4). Significantly fewer OP's were found on PI171451, untreated 93M92, and Prichard (12.0 to 13.6 per plant) (Table 2.4). NC-Roy and untreated 93M92 had significantly more live larvae (2.7 per plant) than all three PIs, the variety Prichard, or fipronil-treated 93M92 (Table 2.4) even though untreated 93M92 received very few oviposition punctures. The number of eggs and larval entry nodes were too small to give useful information and there were no significant differences (eggs $F = 1.04$; df = 7, 5; $P = 0.4091$, entry nodes $F = 0.99$; df = 7, 5; $P = 0.4442$, respectively) between genotypes. The plants were apparently collected before the larvae were able to develop enough to tunnel into the main stems, so there were few larval entry nodes. Differences in the OP/ Lv ratio were highly significant ($F = 7.59$; df = 7, 5; $P < 0.0001$). PI165673 and treated 93M92 had significantly higher OP/ Lv ratios (16.0 and 15.7, respectively) than all other entries except PI165676 (Table 2.4). Untreated 93M92 plants had a significantly smaller OP/ Lv ratio (4.6) than all other genotypes except PI171451.

The factorial analysis conducted for the 2008 data (Table 2.5) indicates that maturity group was not a significant factor for any of the infestation variables, except for entry node where the numbers were too small to give meaningful results. The factorial analysis probabilities (P) on the OP's, live larva, egg, entry node and OP/ Lv ratio among maturity groups were 0.0978,

0.0912, 0.4071, 0.0227 and 0.1819, respectively. There were significant differences on the OP/ Lv ratio (F = 19.75; df = 3, 1; $P < 0.0001$) between the resistant entries and non-resistant entries, however there was no difference (F = 1.65; df = 3, 1; P = 0.1819) among the maturity groups. Interactions on the OP/ Lv ratio were significant (P = 0.0002), indicating that not all the entries assumed to be resistant were truly resistant. Maturity group VII and VIII entries were not significantly different from each other (Table 2.4), so there does not appear to be resistance across these entries. Antibiosis in PI165673 seemed to be as strong as antibiosis in the fipronil-treated plants (90 to 100% control) (Tables 2.3, 2.4). PI171451 also appeared to have some antixenosis-based resistance.

In all four years of experiments, PI165673 appeared to have the greatest level of antibiosis (highest OP/ Lv ratio) against *D. texanus* compared to all other plant introductions and varieties evaluated (Tables 2.1, 2.2, 2.3, and 2.4). Interestingly, PI165673 showed the lowest level of antixenosis (highest number of oviposition punctures) for genotypes tested in 2007 and 2008 (Tables 2.3, 2.4) and the second highest in 2005. PI165676 appeared to have a moderate antibiotic effect, as it exhibited an OP/ Lv ratio similar to PI165673 in 2005 and 2008. PI171451 appeared to have significant antixenotic effects (lower numbers of oviposition punctures) relative to the susceptible controls 93M50, in 2007, and 93M92, in 2008. Although PI171451 has well-known insect resistance, the OP/ Lv ratio was not close to that of PI165673. PI165673, PI165676 and PI171451 may be useful as parents for creating *D. texanus* resistant genotypes in soybean breeding programs.

The OP/ Lv ratio appears to be a better determinant of antibiosis than variables such as numbers of larvae, eggs, or entry nodes. 93M50 and 93M92 plants treated with fipronil successfully functioned as positive controls, as both exhibited OP/ Lv ratios no different than those for PI165673. The residual activity of fipronil appeared to remain effective during the entire period of larval feeding. Using large field cages to confine adult *D. texanus* for the purpose of maintaining oviposition pressure on tested plants consistently provided similar plant resistance responses over the four years of the experiments in these trials (Figure 2.1).

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Table 2.1 Mean ± SEM number of *Dectes texanus* oviposition punctures, eggs, larvae, soybean entry node, and oviposition punctures per larva on 15 soybean genotypes, 2005

Soybean genotype (Maturity group)	Oviposition punctures per plant	Live larvae per plant	Eggs per plant	Entry node per plant	Oviposition punctures/ larva ratio
R2803RR (II)	134.0 ± 13.9 abcd	6.9 ± 1.2 ab	0.1 ± 0.1 a	5.0 ± 1.0 a	22.3 ± 2.5 bc
DG31M25 (II)	96.1 ± 11.9 cdef	5.4 ± 1.3 abcd	0.0 ± 0.0 a	4.1 ± 1.2 ab	29.2 ± 11.7 bc
93M50 (III)	160.0 ± 41.5 ab	7.5 ± 1.9 a	0.2 ± 0.1 a	3.6 ± 1.2 abc	24.9 ± 8.7 bc
X3727NRS (III)	75.1 ± 8.9 cdef	4.7 ± 0.7 abcde	0.2 ± 0.6 a	1.6 ± 0.6 bcd	20.3 ± 5.1 bc
KS4404RR (IV)	65.6 ± 18.3 def	4.3 ± 1.1 abcde	0.7 ± 0.6 a	2.2 ± 1.0 bcd	16.5 ± 1.9 c
KS4704RR (IV)	69.3 ± 12.4 def	4.7 ± 0.4 abcde	0.7 ± 0.4 a	2.7 ± 0.7 abcd	15.4 ± 2.6 c
PI82312 (VI)	196.0 ± 52.2 a	6.4 ± 2.6 abc	0.8 ± 0.6 a	0.0 ± 0.0 d	39.8 ± 11.9 bc
PI165673 (VI)	175.0 ± 19.7 a	2.3 ± 0.7 de	0.1 ± 0.1 a	1.3 ± 0.4 cd	99.3 ± 30.2 a
PI171444 (VI)	61.7 ± 18.7 ef	4.1 ± 1.2 abcde	0.1 ± 0.1 a	1.9 ± 0.9 bcd	15.3 ± 2.8 c
PI171451 (VII)	49.1 ± 7.4 f	2.6 ± 0.7 de	0.0 ± 0.0 a	1.8 ± 0.5 bcd	24.4 ± 8.2 bc
PI228065 (VII)	131.0 ± 26.9 abcde	3.7 ± 1.5 bcde	0.3 ± 0.2 a	1.4 ± 0.4 cd	46.5 ± 15.7 bc
PI229358 (VII)	85.8 ± 11.7 cdef	3.2 ± 0.5 cde	0.0 ± 0.0 a	2.1 ± 0.6 bcd	29.1 ± 4.3 bc
PI323275 (VII)	85.3 ± 27.7 cdef	1.4 ± 0.7 e	0.2 ± 0.2 a	2.0 ± 0.7 bcd	3.1 ± 23.1 b
PI165676 (VIII)	143.0 ± 15.5 abc	2.1 ± 0.7 de	0.1 ± 0.1 a	1.3 ± 0.6 cd	54.0 ± 9.9 b
PI227687 (VIII)	61.6 ± 11.1 ef	1.4 ± 0.5 e	0.0 ± 0.0 a	1.2 ± 0.5 cd	35.0 ± 7.9 bc

Mean ± SEM followed by the same letter in a columm are not significantly different ($P < 0.05$).

Duncan's Multiple Range test.

Table 2.2 Mean ± SEM number of *Dectes texanus* oviposition punctures, eggs, larvae, soybean entry node, and oviposition punctures per larva on 15 soybean genotypes, 2006

Soybean genotype (Maturity group)	Oviposition punctures per plant	Live larvae per plant	Eggs per plant	Entry node per plant	Oviposition punctures/ larva ratio
NEX2403K* (II)	23.7 ± 2.8 d	0.4 ± 0.2 e	0.3 ± 0.2 c	0.0 ± 0.0 de	26.8 ± 4.6 a
DB32C25 (II)	27.0 ± 4.7 cd	6.1 ± 1.1 abcd	0.5 ± 0.2 bc	3.2 ± 0.9 a	4.6 ± 0.5 ef
93M50 (III)	55.1 ± 8.5 b	6.5 ± 0.9 abcd	0.7 ± 0.3 bc	1.9 ± 0.4 b	9.7 ± 1.3 de
X3727NRS (III)	37.3 ± 4.3 bcd	9.4 ± 1.7 a	0.6 ± 0.2 bc	1.3 ± 0.3 bcde	4.6 ± 0.4 ef
KS4404RR (IV)	43.8 ± 3.2 bcd	6.6 ± 0.5 abcd	0.3 ± 0.1 c	4.2 ± 0.7 a	7.2 ± 0.7 def
KS4704RR (IV)	27.9 ± 2.9 bcd	8.1 ± 1.1 ab	1.2 ± 0.3 ab	1.7 ± 0.5 bc	3.9 ± 0.4 ef
PI82312 (VI)	110.0 ± 43.5 a	9.3 ± 2.8 a	1.8 ± 0.7 a	0.6 ± 0.3 cde	11.7 ± 2.5 cd
PI165673 (VI)	49.3 ± 5.6 bcd	3.3 ± 0.5 de	0.3 ± 0.1 c	1.3 ± 0.4 bcd	20.5 ± 3.4 b
PI171444 (VI)	25.3 ± 2.6 d	5.3 ± 0.8 bcd	0.9 ± 0.3 bc	0.4 ± 0.1 de	5.7 ± 0.7 ef
PI171451 (VII)	30.5 ± 4.5 bcd	4.3 ± 0.7 cd	0.1 ± 0.1 c	1.1 ± 0.3 bcde	7.9 ± 1.1 def
PI228065 (VII)	46.1 ± 9.6 bcd	7.0 ± 1.2 abc	1.2 ± 0.3 ab	0.9 ± 0.2 bcde	7.6 ± 1.0 def
PI229358 (VII)	25.5 ± 2.5 d	4.5 ± 0.6 cd	0.5 ± 0.2 bc	1.1 ± 0.5 bcde	6.7 ± 0.9 def
PI323275 (VII)	38.4 ± 4.3 bcd	4.7 ± 0.6 cd	0.9 ± 0.3 bc	0.6 ± 0.3 bcde	7.8 ± 0.7 def
PI165676 (VIII)	53.9 ± 5.5 bc	5.5 ± 0.9 bcd	0.3 ± 0.1 c	1.1 ± 0.4 bcde	14.5 ± 2.7 c
PI227687 (VIII)	47.4 ± 6.8 bcd	8.5 ± 1.8 ab	0.8 ± 0.3 bc	0.6 ± 0.3 cde	6.7 ± 0.6 def

Mean ± SEM followed by the same letter in a columm are not significantly different ($P < 0.05$).

Duncan's Multiple Range test.

Table 2.3 Mean ± SEM number of *Dectes texanus* oviposition punctures, eggs, larvae, soybean entry node, and oviposition punctures per larva on three soybean introductions and the cultivar 93M50, 2007

Soybean genotype (Maturity group)	Oviposition punctures per plant	Live larvae per plant	Eggs per plant	Entry node per plant	Oviposition punctures/ larva ratio
PI165673 (VI)	25.3 ± 2.2 b	0.9 ± 0.2 c	0.07 ± 0.05 b	0.30 ± 0.09 b	23.5 ± 2.9 a
PI171451 (VII)	15.6 ± 1.8 c	0.9 ± 0.2 c	0.03 ± 0.03 b	0.27 ± 0.09 b	15.1 ± 1.8 b
PI165676 (VIII)	15.3 ± 2.2 c	0.6 ± 0.2 c	0.11 ± 0.06 ab	0.37 ± 0.13 b	12.7 ± 3.1 b
93M50 Fipronil treated (III)	35.8 ± 2.5 a	1.7 ± 0.2 b	0.27 ± 0.09 a	0.57 ± 0.17 b	25.4 ± 3.4 a
93M50 untreated (III)	24.8 ± 2.9 b	2.3 ± 0.3 a	0.00 ± 0.00 b	1.92 ± 0.24 a	12.8 ± 1.6 b

Mean ± SEM followed by the same letter in a columm are not significantly different ($P < 0.05$).

Duncan's Multiple Range test.

Table 2.4 Mean ± SEM number of *Dectes texanus* oviposition punctures, eggs, larvae, soybean entry node, and oviposition punctures per larva on three soybean introductions and the cultivar 93M92, 2008

Soybean genotype (Maturity group)		Oviposition punctures per plant	Live larvae per plant	Eggs per plant	Entry node per plant	Oviposition punctures/ larva ratio
PI165673	(VI)	24.2 ± 4.0 a	1.2 ± 0.3 b	0.2 ± 0.1 a	0.0 ± 0.0 a	16.0 ± 2.2 a
NC-Roy	(VI)	19.1 ± 2.6 ab	2.6 ± 0.5 a	0.1 ± 0.1 a	0.0 ± 0.0 a	10.2 ± 1.7 bc
PI171451	(VII)	12.0 ± 2.1 b	1.5 ± 0.3 b	0.2 ± 0.1 a	0.0 ± 0.0 a	8.2 ± 1.0 cd
Santee	(VII)	20.6 ± 2.8 ab	1.9 ± 0.3 ab	0.7 ± 0.7 a	0.0 ± 0.0 a	10.2 ± 1.2 bc
PI165676	(VIII)	16.3 ± 3.9 ab	1.2 ± 0.3 b	0.1 ± 0.1 a	0.0 ± 0.0 a	13.0 ± 2.0 ab
Prichard	(VIII)	13.1 ± 2.5 b	1.2 ± 0.3 b	0.0 ± 0.0 a	0.1 ± 0.1 a	9.4 ± 1.4 bc
93M92 Fipronil treated	(III)	24.2 ± 2.7 a	1.4 ± 0.2 b	0.6 ± 0.3 a	0.7 ± 0.7 a	15.7 ± 1.7 a
93M92 untreated	(III)	13.6 ± 2.0 b	2.7 ± 0.4 a	0.2 ± 0.1 a	0.2 ± 0.1 a	4.6 ± 0.5 d

Mean ± SEM followed by the same letter in a columm are not significantly different ($P < 0.05$). Duncan's Multiple Range test.

Table 2.5 Factorial analysis of the evaluation of soybean plant introductions and varieties for resistance to *Dectes texanus*, 2008

Comparison items		Oviposition punctures per plant	Live larvae per plant	Eggs per plant	Entry node per plant	Oviposition punctures/ larva ratio
Maturity group	(III)	18.4 ± 1.9 a	2.1 ± 0.3 a	0.4 ± 0.2 a	0.11 ± 0.05 a	10.2 ± 1.3 a
	(VI)	21.7 ± 2.4 a	1.9 ± 0.3 a	0.1 ± 0.1 a	0.00 ± 0.00 b	12.4 ± 1.4 a
	(VII)	16.3 ± 1.9 a	1.7 ± 0.2 a	0.4 ± 0.3 a	0.00 ± 0.00 b	9.2 ± 0.8 a
	(VIII)	14.7 ± 2.3 a	1.2 ± 0.2 a	0.1 ± 0.0 a	0.03 ± 0.03 b	11.0 ± 1.2 a
Resistant*		18.9 ± 1.7 a	1.4 ± 0.1 b	0.3 ± 0.1 a	0.00 ± 0.00 b	13.0 ± 0.9 a
Susceptible		16.5 ± 1.3 a	2.1 ± 0.2 a	0.2 ± 0.2 a	0.07 ± 0.03 a	8.6 ± 0.7 b
Probability						
Maturity group		0.0978	0.0912	0.4071	0.0227	0.1819
Resistance		0.2628	0.0027	0.8875	0.0207	< 0.0001
Interaction		0.0126	0.1593	0.3166	0.0287	0.0002

Mean ± SEM followed by the same letter in a columm are not significantly different ($P < 0.05$). Duncan's Multiple Range test.

* Piant introductions and fipronil treated 93M92 are considered as resistant entries; conventional varieties and fipronil untreated 93M92 are considered as susceptible

Figure 2.1 Mean number of *D. texanus* oviposition punctures per live larvae (OP/ Lv) for select soybean plant introductions and fipronil treated- and untreated susceptible variety controls (each PI compared to variety of similar maturity in 2008). Bars followed by a different letter differ significantly (DMRT, $P < 0.05$)

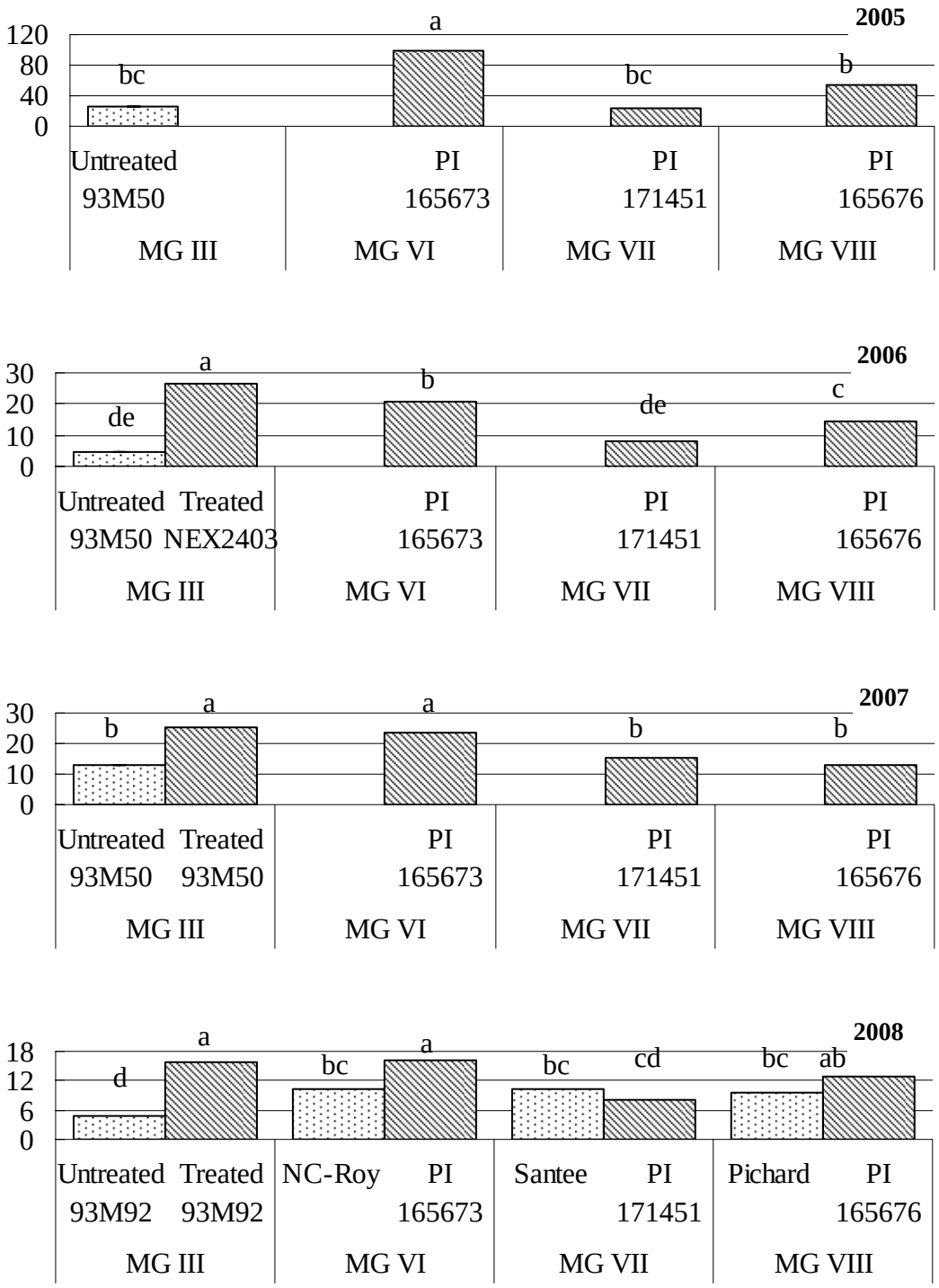

Figure 2.2 Field plots

Figure 2.3 Assembling field cages

Figure 2.4 Field cages

Figure 2.5 Releasing collected adult beetles

Figure 2.6 Collected adult beetles

CHAPTER 3 - Greenhouse Evaluation of Soybean Germplasm for Resistance to Dectes Stem Borer, *Dectes texanus* LeConte

3.1. Abstract

Greenhouse evaluations of host plant resistance can allow for large-scale, year-round evaluation of germplasm in a relatively short period of time under controlled conditions. No-choice greenhouse evaluations were conducted in 2005 and 2006 to identify resistance against the Dectes stem borer, *Dectes texanus* LeConte, in soybean plant introductions and in transgenic soybean plants containing the *Manduca sexta* chitinase gene. Plants were exposed to *D. texanus* beetles in plant cages. However, very few *D. texanus* oviposition punctures (OPs) produced live larvae (Lv), and as a result the OP/ Lv ratio was too small to yield significant differences between genotypes. Greenhouse-grown plants appeared to be morphologically inappropriate for successful *D. texanus* oviposition or larval survival, which depend on an amount of petiole and stem pith sufficient for *D. texanus* oviposition.

Key words:

D. texanus larval damage, no-choice greenhouse experiments, soybean plant introductions, transgenic soybean, *Manduca sexta* derived chitinase gene

3.2. Introduction

Insect responses to potential host plants sometimes differ between free-choice and non-choice assays. Smith (1989) encouraged the parallel use of choice and no-choice screening methods to provide reliable identification of host plant resistance against pest insects. A non-choice test reduces the problem of uneven distribution of insects that may lead to uneven infestation across the test plant genotypes, which may occur in a free-choice test. This could cause inaccurate conclusions about potential resistance in evaluated genotypes. The non-choice cage test can avoid this problem because each plant has the same number of insects introduced. Cages can also protect test insects from predation and parasitism and they can limit emigration of test insects from the plants being evaluated. Smith et al. (1994) also points out that greenhouse experiments allow the researcher to make large-scale evaluations of many plants in a relatively short period of time.

Richardson (1975) conducted field and laboratory tests in North Carolina to examine a wide range of soybean genotypes for resistance to the stem borer, *Dectes texanus* LeConte. In three years of screening, a 618 soybean plant introductions (PIs) from maturity groups V-VII were evaluated and 18 lines were identified with what appeared to be moderate *D. texanus* resistance. However, ratings were based on plant girdling, a measurement confounded by maturity group and environmental conditions. Later maturing varieties appear to have lower rates of girdling, even when they were equally infested. Richardson (1975) reported average infestations (identified by the larval entry hole) for maturity groups V, VI, VII of 57, 50, and 41% respectively, and reported that many PIs with low *D. texanus* infestations had a procumbent growth habit, which may have contributed to low ratings, as a result of reduced exposure. In additional evaluations of varieties in maturity groups V-VIII, the variety with the highest rate of plant girdling (58%) was Lee in maturity group VI and the variety with the lowest rate of girdling (7%) was Hampton in maturity group VIII. Campbell (1976) reported that only 8% of plants in maturity group VIII were girdled compared to 44% in groups IV-VII.

Kaczmarek (2003) assessed commercial soybean varieties from maturity groups II-V for *D. texanus* susceptibility in Kansas under irrigated and dryland conditions. Infestations ranged from 0 to 93% for tunneling, 0 to 87% for larval presence, and 0 to 60% for girdling and lodging, suggesting significant differences in varietal response. However, intensive re-examination of the

same varieties revealed no evidence of plant resistance to *D. texanus* in these commercial varieties. The average lodging across all varieties in the irrigated plots averaged nearly 37% but only 17% in dryland plots.

Whalen et al. (1998) evaluated potential resistance to *D. texanus* in Delaware in soybean varieties in maturity groups III-V with resistance to the soybean cyst nematode, *Heterodera glycines*. The nematode-resistant varieties had significantly lower *D. texanus*-related lodging compared to nematode-susceptible varieties. In addition, soybean maturity group III varieties exhibited significantly less stem lodging and stem borer infestation than did varieties in groups IV and V. However, Taylor and Whalen (2002) found that *D. texanus* infestation rates for *H. glycines* resistant varieties varied annually, and maturity group was not responsible for resistance to *D. texanus* populations. Higgins et al. (2003) assessed *D. texanus* resistance in several *H. glycines* - resistant varieties in Kansas, and found no relationship between *D. texanus* resistance and *H. glycines* resistance. Niide et al. (2006) evaluated 11 *H. glycines* resistant and susceptible varieties in maturity groups III-V in Texas and observed similar results.

The potential use of chitinase as an insect management tool has been explored for decades (Flach et al. 1992, Kramer and Muthukrishnan 2005). They have sought to use chitinase as a toxin in transgenic plants to produce host plant resistance. Chitinase is an enzyme that can disrupt an insect's chitin exoskeleton and the insects' ability to molt. Chitinase can also be used as an enhancer of the toxicity of the entomopathogenic bacterium *Bacillus thuringiensis* (Bt). Chitin is one of the most abundant polysaccharides in nature and it is a principal component of the cuticle and the gut peritrophic membrane. Chitinolytic enzymes that catalyze the hydrolysis of chitin have been found in chitin-containing organisms as well as other microorganisms, plants, and animals that do not have chitin. The enzymes derived from different sources have different biological functions such as molting of the exoskeleton in insects, cell growth and division in fungi, chitin utilization as nutrition in bacteria, and defense against pest and pathogen attacks in plants (Choi et al. 1997, Flach et al.1992, and Kramer and Muthukrishnan 1997).

Microorganism-derived chitinase (microbial chitinase) has been used to enhance the toxicity of *Bt* preparations (Gongora et al. 2001, Liu et al. 2006, and Wang et al. 1996). Insect-derived chitinase may also play a role in host plant resistance in transgenic plants (Ding et al. 1998, Fitchesa et al. 2004). Kramer and Muthukrishnan (2005) used a family of 18 insect-derived chitinases for host plant resistance in transgenic plants or as an enhancer protein for

baculovirus toxicity in biopesticide development. Unfortunately, the insecticidal activity in these applications of insect chitinase has not been substantial enough for commercial development. However, insect-derived chitinase may still have potential as a host plant resistance factor in transgenic plants against other pests.

In the present study, a series of no-choice greenhouse experiments were conducted in 2005 and 2006 to evaluate a number of varieties, plant introductions and transgenic soybean plants containing the *M. sexta* chitinase gene for resistance to *D. texanus* larval damage.

3.3. Materials and Methods

In 2005, two Kansas varieties and nine plant introductions (PIs) were evaluated for *D. texanus* resistance, and in 2006, seven Kansas varieties and nine soybean PIs were evaluated. The commercial varieties included several of the most resistant and susceptible varieties to *D. texanus* identified by Khajuria et al. (2005). The PIs included several identified by Richardson (1975), others known to have resistance to various other Coleopteran pests of soybean (Hatchett et al. 1976, VanDuyn et al. 1971, 1972) and those recommended by Drs. C. M. Smith (KSU) and N. S. Talekar, Asian Vegetable Research and Development Center (AVRDC), Taiwan. Varieties were obtained from Dr. W. T. Schapaugh (KSU) and PIs were obtained from R. L. Nelson, USDA, ARS Soybean Germplasm Collection, Urbana, IL.

Seeds were planted and grown in PRO-MIX BX, (Premier Tech, Canada) in 16.2 cm diameter plastic pots. Plants were watered as required and fertilized ca. every 21 d with Peters Professional 20-20-20 fertilizer (Scotts Co., Marysville, OH) (250 cc of 360 ppm N per pot). Seeds were planted on four different dates from early March to the middle of May to ensure plants of optimum developmental stage (V-4 or later) were available when adult *D. texanus* were available for testing (Reynolds and Smith 1985). Insecticidal soap (Safer Insect Killing Soap, Woodstream Corporation, Lititz, PA) and yellow sticky-traps were used to suppress aphids and mites. The placement of the entries was a randomized block design with 4 replications.

Genetically transformed soybean seed expressing the *M. sexta* chitinase (msc) gene were supplied by Dr. H. N. Trick, Dept. of Plant Pathology, Kansas State University. Transgenic and non-transgenic seeds were produced from soybean cultivars ‘Jack’ and ‘Fayette’ (Ornatowski et al. 2004, Trick et al. 1997). Two sets of 12 plants of each transgenic and non-transgenic isoline

were planted on May 26 and June 9 in the KSU greenhouse facilities at Manhattan to produce plants of V-4 or later (Reynolds and Smith 1985), to coincide with the availability of *D. texanus* beetles. Seeds were planted and grown in a mixture of peat moss, soil, perlite and osmocote fertilizer in 16.2 cm diameter plastic pots. Plants were watered and fertilized as required.

The transgenic plant tissue was tested by polymerase chain reaction (PCR) analysis to determine which plants were expressing the chitinase gene (Erlich 1989, Innis 1990). For the first set of plants seven of eight plants tested positive for expression of the chitinase gene and the non-transgenic plants tested negative for expression of the gene. In the second set of plants, seven transgenic plants tested positive and the seven non-transgenic plants tested negative. Pots were placed with a randomized design.

D. texanus infested soybean stubble was collected in the fall of 2004 and 2005 from the KSU Southwestern Research & Extension Center in Garden City, Kansas, and/ or the KSU North Central Kansas Experimental Field near Scandia, Kansas. Collection of stubble was facilitated by searching for girdled stems which had a smooth concave cut end, compared to the jagged edges of other broken stems. Collected stubble was held in a cold room (5.6 °C) until April and then placed in a cage in a greenhouse at Kansas State University in Manhattan, KS for adult *D. texanus* emergence (Figure 3.1). Water was sprinkled on the stubble and emerging adults were collected daily, after 11:00 am (Hatchett et al. 1975), sexed, and placed separately in 60 mm Petri dishes with 1.3 cm sections of fresh green bean *Phaseolus vulgaris* L., seed pod, (Hatchett et al. 1973). According to Hatchett et al. (1975), feeding for 2 d was a prerequisite to mating. The two-day-old adults were paired to confirm sex determination, and pairs of copulating beetles were maintained in the oviposition cages in 60 mm Petri dishes with access to fresh pods until needed.

A funnel-shaped mesh oviposition cage (commercial paint strainer, 60 cm high, 50 cm wide) was placed over a potted plant and supported with three bamboo stakes (45 cm tall) placed around the perimeter of the pot to seal the cage (Figure 3.2). The elastic band at the opening of the cage was tightened around the pot. Two pairs of mating beetles were added to each oviposition cage (Figure 3.3). Dead beetles were replaced as needed and live beetles were allowed to feed and oviposit for 7 d on the test plants.

Genetically engineered soybean test plants were placed in double plexiglass rearing cages (Bug Dorm-1, Megaview Science Education Services, Taiwan) to expose them to adult

D. texanus (Figures 3.4, 3.5). The first set of plants was infested during the early *D. texanus* emergence period, so only two adult females and one adult male were added to each cage. For the second set of plants, two pairs of mating beetles were added to each cage. Dead beetles were replaced immediately and living beetles were allowed to feed and oviposit for 7 d on test plants. Before caging, the height and developmental stages of each caged plant was recorded.

Immediately after termination of the infestation and removal of the cages, the number of oviposition punctures on each leaf petiole and stem was recorded. Eggs in plants were allowed to hatch and left undisturbed for at least 14 d before the plants were dissected to record the number of larvae, eggs and larval tunnels. The *D. texanus* infestation data, the OP/ Lv ratio and the plant measurements were analyzed to determine whether differences existed between the genotypes for *D. texanus* infestation in the plants in the no-choice experiment that could be used to identify the resistance to *D. texanus*. Data were analyzed using PROC GLM SAS (Colette and Robinson 2000) and means are compared using Duncan's Multiple Range test ($\alpha = 0.05$).

3.4. Results and Discussion

Of the soybean plant introductions and varieties evaluated, in the greenhouse, there were no significant differences for numbers of oviposition punctures in 2005 ($F = 1.18$; df = 12, 27; $P = 0.3471$), but in 2006, differences were significant ($F = 2.40$; df = 16, 52; $P = 0.0091$). Very few oviposition punctures were successful and thus very few live larvae were present. As a result, the number of eggs, larvae, entry nodes and oviposition punctures per larva were very small and meaningless (Table 3.1).

In the first set of plants in the genetically engineered plant evaluations, the non-transgenic isoline had ca. 5 times more oviposition punctures than the transgenic line (Table 3.2). In the second set of plants, transgenic plants had ca. 1.7 times more oviposition punctures than the non-transgenic isoline plants (Table 3.2). Nevertheless, there were no significant differences ($F = 0.09$; df = 1,1; $P = 0.7698$) between transgenic and non-transgenic isolines for any infestation parameter. Again, there were few tunnels, entry nodes, or live larvae, and the OP/ Lv ratio was too small to yield significant differences between genotypes.

Greenhouse experiments with transgenic soybeans were conducted in June and July under temperatures exceeding 39 ^{o}C, which were probably responsible for the death of many

adult *D. texanus* beetles. It was difficult to determine whether the reduction in reproductive activity was caused by the environmental conditions or by plant resistance. In addition, greenhouse-grown plants were not morphologically appropriate for successful *D. texanus* oviposition and/ or survival. Michaud and Grant (2005) noted that greenhouse-grown plants were not morphologically appropriate for successful oviposition or larval survival. Hatchett et al. (1975) also noted that successful oviposition depended on the existence of pith in the petioles and stem and that the *D. texanus* females appeared to have difficulty inserting the ovipositor to penetrate into the pith of greenhouse-grown plants.

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Table 3.1 Mean ± SEM number of *Dectes texanus* oviposition punctures, live larvae, eggs, larval entry nodes and oviposition punctures/ larva ratio in soybean introductions and varieties evaluated for *D. texanus* resistance

Soybean genotype (Maturity group)	Oviposition punctures per plant		Live larvae per plant		Eggs per plant		Entry node per plant		Oviposition punctures/ larva ratio	
	2005	2006	2005	2006	2005	2006	2005	2006	2005	2006
S29-C9 (II)	3.8 ± 1.1 a	1.7 ± 1.2 c	0.3 ± 0.3 a	0 a	0	0 a	0.3 ± 0.3 a	0 b	5.0 ± .	.
R2803RR (II)	n/a	6.3 ± 1.8 b,c	n/a	0.3 ± 0.3 a	n/a	0 a	n/a	0 b	n/a	7.0 ± .
DG31M25 (II)	n/a	4.3 ± 1.9 b,c	n/a	0.0 ± 0.0 a	n/a	0 a	n/a	0 b	n/a	.
93M50 (III)	n/a	10.3 ± 7.8 b,c	n/a	0 a	n/a	0 a	n/a	0 b	n/a	.
X3727NRS (III)	4.8 ± 2.8 a	4.0 ± 0.6 b,c	0 a	0 a	0	0 a	0 a	0 b	.	.
KS4404RR (IV)	n/a	8.3 ± 2.3 b,c	n/a	0.3 ± 0.3 a	n/a	0 a	n/a	0 b	n/a	4.0 ± .
KS4704RR (IV)	n/a	23.7 ± 20.7a,b,c	n/a	1.3 ± 1.3 a	n/a	0 a	n/a	0.3 ± 0.3a	n/a	16.3 ± .
PI-82312 * (VI)	.	.	.	.	.	.	.	.	.	.
PI-165673 (VI)	13.3 ± 5.4 a	36.3 ± 10.4 a	0.5 ± 0.5 a	0 a	0	0 a	0.8 ± 0.5 a	0 b	10.0 ± .	.
PI-171444 (VI)	0.3 ± 0.3 a	6.0 ± 2.1 b,c	0 a	0 a	0	0.2 ± 0.2a	0 a	0 b	.	.
PI-171451 (VII)	14.0 ± 10.5a	10.3 ± 3.7 b,c	0 a	0.2 ± 0.2 a	0	0 a	0.3 ± 0.3 a	0 b	.	26.0 ± .
PI-228065 (VII)	8.5 ± 2.5 a	7.7 ± 3.9 b,c	0 a	0.5 ± 0.3 a	0	0 a	0 a	0 b	.	10.5 ± 2.5
PI-229358 (VII)	6.5 ± 2.9 a	14.7 ± 5.3 a,b,c	0 a	0.2 ± 0.2 a	0	0 a	0 a	0 b	.	10.0 ± .
PI-323275 (VII)	5.3 ± 0.6 a	14.5 ± 4.3 a,b,c	0 a	0.2 ± 0.2 a	0	0 a	0 a	0 b	.	17.0 ± .
PI-165676 (VIII)	8.8 ± 3.8 a	25.5 ± 7.8 a,b,c	0 a	0.5 ± 0.3 a	0	0 a	0 a	0 b	.	19.0 ± 11.0
PI-227687 (VIII)	8.0 ± 3.1 a	26.0 ± 5.4 a,b	0 a	0 a	0	0 a	0 a	0 b	.	.

Mean ± SEM followed by the same letter in a columm are not significantly different ($P < 0.05$).

Duncan's Multiple Range test.

* Seed were planted but not used because of low plant germination/ emergence.

Table 3.2 Evaluation of genetically engineered soybean plants for resistance to *Dectes texanus*

Plant set 1.

Treatment/ check origin name	PCR result	Oviposition puncture	Live larva	Entry node	Tunnel	Oviposition punctures/ larva ratio
Trt-1 Jack	(+)	2	0	0	0	n/a
Trt-2 Jack	(+)	2	0	0	0	n/a
Trt-3 Jack	(+)	0	0	0	0	n/a
Trt-4 Jack	(+)	1	0	0	0	n/a
Trt-5 Jack	(+)	2	0	0	0	n/a
Trt-6 Jack	(-)	2	0	0	0	n/a
Trt-7 Jack	(+)	0	0	0	0	n/a
Trt-8 Fayette	(+)	11	1	0	1	11
Ck-1 Jack	n/a	25	0	0	0	n/a
Ck-2 Jack	n/a	6	0	0	0	n/a
Ck-3 Jack	n/a	9	0	0	0	n/a
Ck-4 Jack	n/a	18	1	4	1	18
Ck-5 Fayette	n/a	2	0	0	0	n/a
Ck-6 Fayette	n/a	31	0	1	1	n/a
Ck-7 Fayette	n/a	7	0	0	1	n/a
Ck-8 Fayette	n/a	7	0	0	0	n/a

Table 3.2 Evaluation of genetically engineered soybean plants for resistance to *Dectes texanus*

Plant set 2.

Treatment/ check origin name	PCR result	Oviposition puncture	Live larva	Entry node	Tunnel	Oviposition punctures/ larva ratio
Trt-1 Jack	(+)	30	0	0	0	n/a
Trt-2 Jack	(+)	36	1	1	1	36
Trt-3 Jack	(+)	18	0	0	0	n/a
Trt-4 Jack	(+)	32	0	0	0	n/a
Trt-5 Fayette	(+)	7	0	0	0	n/a
Trt-6 Fayette	(+)	6	0	0	0	n/a
Trt-7 Fayette	(+)	40	2	3	3	20
Ck-1 Jack	(-)	12	0	0	0	n/a
Ck-2 Jack	(-)	11	0	0	0	n/a
Ck-3 Jack	(-)	25	0	0	0	n/a
Ck-4 Jack	(-)	6	0	0	0	n/a
Ck-5 Fayette	(-)	13	0	0	0	n/a
Ck-6 Fayette	(-)	18	1	1	2	18
Ck-7 Fayette	(-)	15	1	6	1	15

Figure 3.1 *D. texanus* rearing cage

Figure 3.2 Tested plants covered with mesh oviposition cages

Figure 3.3 Paired beetles confinded in a cage

Figure 3.4 Plexigrass rearing cages

Figure 3.5 GE plant in a cage

CHAPTER 4 - Analysis of the Vertical Distribution of Dectes Stem Borer, *Dectes texanus* LeConte, Oviposition Punctures on Soybean

4.1. Abstract

Choice of an oviposition site by cerambycid females can be crucial to the survival of larvae because they are legless and incapable of moving far from the oviposition site. There is also considerable vertical variation in the maturity of the tissues of the plant. Plant leaf position is known to affect the growth of phytophagous larva feeding on soybean. The vertical distribution of oviposition punctures (OP's) of *Dectes texanus* LeConte on soybean plants was studied to compare the distribution among different soybean lines to understand the biology and behavior of the *D. texanus* oviposition in soybean. Plant introductions (PIs) with later maturity (VI to VIII) tended to show faster vegetative development but slower reproductive development compared to soybean varieties in maturity groups II to IV. Results indicated that *D. texanus* were ovipositing in the top four or five nodes of the growing plant. These findings are of significance to efforts aimed at improving the precision of foliar insecticide applications for *D. texanus* control.

Key words:

oviposition site, soybean plant structure, vertical distribution, oviposition punctures

4.2. Introduction

The choice of an oviposition site by the cerambycid *Dectes texanus* LeConte is crucial to the survival of larvae, which are legless and incapable of moving far from the oviposition site. Optimal oviposition sites are on vigorous host plants that provide larva tissue on which they can feed and grow. There is also considerable vertical variation in the maturity of tissues, so it is reasonable to expect females to oviposit on plant parts most favorable for larval survival. Effective soybean plant resistance to *D. texanus* should be located in these tissues. It is therefore important to identify *D. texanus* preferred sites for feeding, resting, mating, and oviposition, since such information can ensure that *D. texanus* control measures are applied correctly. Biological and behavioral studies of *D. texanus* are needed to better understand the biology and behavior of this soybean pest.

According to Hatchett et al. (1975), adult *D. texanus* feed on tender stems, leaf petioles, and leaves of soybean in the upper portion of the plants during the day. Although adults are strong fliers, they appear reluctant to fly and prefer to drop to the ground when disturbed. Hatchett et al. (1975) reported that *D. texanus* adults mated mainly on the plant and that 84% of the eggs were oviposited in the soybean petioles, 9% in secondary stems, and 8% in primary stems. Eggs were normally inserted into the pith in the center of the stem or petiole, depending on the presence of pith and/ or on whether the female could reach the pith with her ovipositor.

Campbell (1980) concluded that adults fly at soybean canopy height after observing that sticky traps placed 1.5 m above ground were ineffective for sampling adults, whereas traps placed at 1 m above ground collected 99% of recorded adults. Campbell (1980) also observed that adult *D. texanus* preferred to rest on the upper one-third of the plant and that eggs were laid in well developed petioles primarily in the upper half of the plant.

Plant leaf position is known to affect the growth of phytophagous larvae feeding on soybean. Reynolds and Smith (1985) reported a 2- to 6-fold difference in the growth rate of larvae of soybean looper, *Pseudoplusia includens* Walker, when fed leaves from different vertical positions on the plant, and that older leaves supported less growth than younger leaves. Similar results were reported for the bollworm, *Heliothis zea* (Boddie) by McWilliams and Beland (1977) who observed a larval feeding preference for young trifoliate leaves.

Cerambycid beetle oviposition site choice is crucial because larvae are legless and incapable of moving between plants. Oviposition occurs only on vigorous host plants. The proximity of feeding, mating, and ovipositing sites may account for the sedentary nature of adults. Hanks (1999) investigated the natural history and behavior of 81 cerambycid species including *Dectes texanus* LeConte, and concluded that reproductive behavior was correlated with the condition of the host plant. The beetle species were classified into four groups, based on the condition of the host plant at the time of larval colonization. The group including *D. texanus* girdles the stems of herbaceous plants or branches of woody hosts before oviposition, or larvae internally girdle branches of herbaceous and woody plants. A larva might not be able to complete development if the host dies, but larvae sometimes critically weaken the host plant.

The purpose of this study was to identify the vertical distribution of *D. texanus* oviposition punctures (OP's) on the soybean plant and to compare the distribution among different plant introductions (PIs) and Kansas soybean varieties. Identifying the oviposition preference site could be useful information for the improved management of *D. texanus*.

4.3. Materials and Methods

Soybean plant introductions (PIs) and Kansas varieties were evaluated for host plant resistance to *D. texanus* in 2006 and 2007. In 2006, six varieties and nine PIs were evaluated. Varieties were selected to include the most *D. texanus* resistant- and susceptible varieties in relative maturity groups II to IV (Khajuria et al. 2005). The PIs included several identified by Richardson (1975) and other PIs known to have resistance to various other Coleopteran pests of soybean (Hatchett et al. 1976, VanDuyn et al. 1971, 1972) and those recommended by Drs. C. M. Smith, Dept. of Entomology, Kansas State University, and N. S. Talekar, Asian Vegetable Research and Development Center (AVRDC), Taiwan. One variety susceptible to *D. texanus* (93M50) was soil-treated with fipronil (Regent 4SC, BASF Corporation, Research Triangle Park, NC) (290.5 g/ ha) and served as a positive control to simulate plant antibiosis. A 30.5 cm barrier (corrugated metal roofing) was installed 20.3 to 25.4 cm deep around the fipronil-treated plants to reduce the chances that the roots of other plants would absorb the insecticide.

In 2007, PI165673, PI171451 and PI165676 identified in 2005 and 2006 evaluations and the susceptible variety 93M50 were evaluated. 93M50 seed was treated with Regent 500TS

(fipronil) (BASF Corporation, Research Triangle Park, NC) 25 mg AI/ 100 kg seed to serve as a positive control. The PI's were obtained from R. L. Nelson, USDA, ARS Soybean Germplasm Collection, Urabana, IL. The commercial seed variety was obtained from Pioneer Seeds (Central BU, Johnston, IA).

Seed of each variety (15 in 2006, 5 in 2007) was planted 5.1 cm apart in 76.2 cm rows 76.2 cm apart in a 4.3 m x 3.1 m ft foot print on 20 May, 2006 or 5.1 cm apart in a 3.7 m x 3.7 m footprint on 31 May, 2007. Entries were carefully spaced to fit inside a commercial patio screen cage fitted over the plants later in the season. Placement of the entries within each cage was a stratified random design with some modification to insure each entry was placed in the center position at the same frequency. Six replications were grown in six cages in 2006 and six replications were grown in three field cages in 2007. A 1 m high trellis was constructed to support the procumbent PIs. Experiments were conducted at the KSU North Central Kansas Experiment Field near Scandia, Kansas.

When beetles were present in the field, large screen tents were installed over the experimental plots (Ozark Trail 4.3 m x 3.1 m Polyester Screen House, North Pole USA, Washington, MO in 2006, First-up Outdoor Shelters 3.7 m x 3.7 m Screen House, North Pole USA, Washington, MO in 2007). A heavy rainstorm destroyed one of the six replications in 2006 leaving five replicates, and forcing reinstallation of screen tents 7 d after the initial installation. Tents were left in place for 10d after the second installation. Plant maturity was recorded 53 d and 79 d after seeding in 2006 and 69 d after seeding in 2007. *D. texanus* beetles were collected from surrounding soybeans fields and released into each screen tent (300 in 2006, 315 in 2007). In 2007, beetles were confined in cages for 15 d, when the cages were opened to release any remaining beetles. Then cages were kept closed for 7 d more in 2007. Plant collections for infestation observations began 7 d after beetle release to allow eggs sufficient time to hatch.

Plants from each entry were collected and taken to the laboratory for dissection and data collection. In most cases, leaf petioles were present on nodes above the third node at the time of dissection. In 2006, three plants from each replicate of the 15 entries were collected, and in 2007, five plants from each replicate of five entries were collected. Numbers of oviposition punctures on each petiole and internode were recorded on each plant. Oviposition punctures on main stem internodes were recorded with the lower petiole. Development stages of the plants were also recorded based on the number of fully expanded leaves (Pedersen 2004).

In 2006, the plant growth stage when the cage was first installed was recorded shortly before cage installation (July 12), but not for the cage installations (July 17 and 25). Plant growth stage was recorded when the first sets of three plants from each replicate of the 15 entries were collected (August 8) in 2007. The plant growth stage at cage installation was estimated, based on growth stage records, which allowed the calculation of the number of days required to develop one leaf stage and then calculating the stage when the cage was opened. Data were analyzed using SAS, PROC GLM (Colette and Robinson 2000) and mean observations per node were compared with Duncan's Multiple Range Test ($\alpha = 0.05$).

4.4. Results and Discussion

In general, plant introductions (PIs) developed more nodes than the Kansas varieties. In 2006, the mean developmental stage for the PIs averaged 8.5 to 10.1 nodes at the preliminary plant measurements, but 16.3 to 22.5 nodes at the first dissections, depending on the entry (Table 4.1). The Kansas varieties averaged 8.1 to 9.7 nodes at the preliminary plant measurements, but 14.5 to 18.3 nodes at the first dissections, depending on variety. In contrast, observations in 2007 recorded smaller differences because the elapsed times were shorter. The mean developmental stage for the PIs at the first dissections averaged 18.3 to 20.6 nodes while the Kansas variety averaged 18.2 to 19.0 nodes (Table 4.2).

In 2006, the estimated developmental stage at the 1st cage installation (58 d after seeding) was 10.1 to 12.5 nodes for PIs and 9.7 to 11.3 nodes for KS varieties. At the 2nd cage installation (66 d after seeding), the estimated developmental stage for PIs ranged from 12.5 to 16.3 nodes and from 12.1 to 13.9 nodes for KS varieties. In 2007, the estimated developmental stage at the time of field cage installation (54 d after seeding) was 12.6 to 14.3 nodes for PIs and 12.6 to 13.3 nodes for 93M50. PI165673 had at least one more node than the other PIs and fipronil treated 93M50 plants appeared to have slightly more nodes compared to untreated 93M50.

In 2006, the number of days to develop a new node averaged from 2.1 to 3.4 for PIs and 3.0 to 5.4 for KS varieties. Fipronil treated NEX2403K had the lowest rate of development. In 2007, the number of days to develop a new node averaged 3.7 for PI165673, 3.9 for PI171451, 4.0 for fipronil treated 93M50, and 4.2 for PI165676 and untreated 93M50.

These results clearly indicate that *D. texanus* oviposit on the top four or five nodes of the growing plant. This can be seen most clearly in Table 4.2 where peak oviposition occurred on nodes 11 and 12, which was within two nodes of the top node when the cage was installed (nodes 13 to 14). Beetles did not appear to live long in the cages since the number of oviposition scars decreased quickly for the nodes that appeared while the plants were caged. 2006 results (Table 4.1) were more difficult to interpret because plants were caged twice and beetles were added twice. Peak oviposition was more variable, but was usually very close to the highest node present at either cage installation. Very little oviposition occurred below nodes 5 or 6 when the first cage was installed.

We conclude that *D. texanus* oviposition most likely occurs on soybean leaf petioles on the upper nodes of the plant canopy. Our conclusion is supported by results of behavioral studies conducted by Hatchett et al. (1975), who reported that adults fed on tender stems, leaf petioles, and leaves,that mating occurred mainly on the plant, and that > 80% of the eggs were oviposited in the pith of petioles. Our findings indicate that foliar insecticide applications should be applied to the upper parts of the plant.

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Table 4.1 Vertical distribution of the mean number of *D. texanus* oviposition punctures on selected soybean plant introductions, 2006

Plant node	Plant Introduction					
	PI 165673	[1]n	PI 165676	n	PI 82312	n
25 (top)	0.0 ± 0.0 e	1	.		0.0 ± . b	1
24	0.0 ± 0.0 e	2	0.0 ± . c	1	0.0 ± . b	1
23	0.0 ± 0.0 e	5	0.0 ± 0.0 c	2	0.0 ± 0.0 b	2
22	0.2 ± 0.2 e	6	0.0 ± 0.0 c	4	0.0 ± 0.0 b	3
21	0.4 ± 0.2 de	11	0.5 ± 0.5 c	6	0.0 ± 0.0 b	4
20	0.4 ± 0.3 de	13	0.9 ± 0.7 bc	7	0.5 ± 0.5 b	8
19	0.8 ± 0.3 de	13	1.7 ± 0.7 abc	7	1.6 ± 1.3 b	8
18	2.4 ± 0.5 bcde	13	1.5 ± 0.7 abc	11	2.6 ± 1.9 b	8
17	3.5 ± 0.9 bc	13	1.3 ± 0.7 abc	11	3.1 ± 2.4 b	8
16	6.8 ± 1.0 a	13	3.6 ± 0.8 ab	12	6.0 ± 2.8 ab	8
15	7.0 ± 1.5 a	13	4.1 ± 1.2 a	14	9.0 ± 3.9 a	8
14	6.8 ± 1.3 a	13	3.7 ± 1.1 ab	14	5.2 ± 2.0 ab	9
13	4.3 ± 0.9 b	13	3.5 ± 1.1 ab	15	5.8 ± 2.0 ab	9
12	2.6 ± 0.8 bcde	13	3.5 ± 0.9 ab	15	4.0 ± 0.7 ab	9
11	1.5 ± 0.5 cde	13	3.7 ± 0.8 ab	15	4.4 ± 1.3 ab	9
10	3.4 ± 0.9 bc	13	3.9 ± 1.0 a	15	2.9 ± 1.4 b	9
9	2.9 ± 0.8 bcd	14	2.3 ± 0.7 abc	15	1.9 ± 0.8 b	9
8	0.7 ± 0.3 de	15	1.2 ± 0.4 abc	15	1.0 ± 0.3 b	9
7	1.1 ± 0.4 cde	15	1.7 ± 0.5 abc	15	0.8 ± 0.3 b	9
6	0.4 ± 0.2 de	15	0.5 ± 0.3 c	15	0.6 ± 0.2 b	9
5	0.5 ± 0.2 de	15	0.4 ± 0.2 c	15	0.3 ± 0.2 b	9
4	0.1 ± 0.1 e	15	0.5 ± 0.2 c	15	0.1 ± 0.1 b	9
3 (bottom)	0.1 ± 0.1 e	15	0.4 ± 0.2 c	15	0.3 ± 0.2 b	9
Developmental stage (number of nodes):						
[2]Prelim.Obs.	10.0		9.5		9.8	
[3]1-install.	11.9		11.3		11.3	
[4]2-install.	15.0		14.1		13.7	
[5]Removal	18.8		17.7		16.8	
[6]Dissection	20.3		18.7		17.7	
[7]#days/node	2.6		2.8		3.3	
Statistical data:						
F	12.07		5.88		4.03	
df	29, 287		28, 270		29, 164	
p	< 0.0001		< 0.0001		< 0.0001	

Table 4.1 Vertical distribution of the mean number of *D. texanus* oviposition punctures on selected soybean plant introductions, 2006

Plant node	Plant Introduction					
	PI 171444	[1]n	PI 171451	n	PI 227687	n
25 (top)	0.0 ± 0.0 c	3	.		0.0 ± 0.0 f	4
24	0.0 ± 0.0 c	5	.		0.0 ± 0.0 f	5
23	0.1 ± 0.1 c	7	0.0 ± . e	1	0.3 ± 0.3 ef	7
22	0.1 ± 0.1 c	8	0.0 ± 0.0 e	4	0.0 ± 0.0 f	8
21	0.2 ± 0.2 c	10	0.0 ± 0.0 e	5	0.5 ± 0.4 ef	8
20	0.4 ± 0.3 bc	11	0.0 ± 0.0 e	6	0.5 ± 0.4 ef	11
19	0.9 ± 0.4 abc	11	0.0 ± 0.0 e	7	0.9 ± 0.4 def	13
18	1.5 ± 0.5 abc	11	0.2 ± 0.2 e	11	2.1 ± 0.7 abcdef	13
17	1.8 ± 0.7 ab	14	1.1 ± 0.5 bcde	12	2.5 ± 0.9 abcd	13
16	1.6 ± 0.4 abc	14	1.2 ± 0.4 bcde	13	2.2 ± 0.7 abcde	13
15	1.4 ± 0.4 abc	14	2.8 ± 0.7 ab	13	3.0 ± 0.9 abc	13
14	1.1 ± 0.3 abc	15	1.9 ± 0.6 abcde	15	3.7 ± 0.6 ab	13
13	1.5 ± 0.4 abc	15	1.7 ± 0.4 abcde	15	1.9 ± 0.7 bcdef	13
12	1.8 ± 0.4 ab	15	2.1 ± 0.5 abcd	15	3.4 ± 0.7 ab	13
11	2.3 ± 0.6 a	15	3.4 ± 0.7 a	15	4.0 ± 0.9 a	13
10	1.4 ± 0.5 abc	15	2.5 ± 0.6 abc	15	2.7 ± 1.0 abcd	13
9	1.1 ± 0.3 abc	15	2.4 ± 0.5 abc	15	2.8 ± 0.5 abcd	13
8	0.9 ± 0.3 abc	15	1.4 ± 0.5 bcde	15	1.4 ± 0.5 cdef	14
7	0.6 ± 0.3 bc	15	0.9 ± 0.4 bcde	15	0.9 ± 0.3 def	14
6	0.6 ± 0.5 bc	15	1.1 ± 0.6 bcde	15	0.4 ± 0.2 ef	14
5	0.1 ± 0.1 c	15	0.4 ± 0.2 de	15	0.1 ± 0.1 f	14
4	0.2 ± 0.1 c	15	0.3 ± 0.2 de	15	0.1 ± 0.1 f	14
3 (bottom)	0.0 ± 0.0 c	15	0.4 ± 0.2 de	15	0.1 ± 0.1 f	14
Developmental stage (number of nodes):						
[2]Prelim.Obs.	10.1		8.5		10.1	
[3]1-install.	12.1		10.4		12.5	
[4]2-install.	15.3		13.3		16.3	
[5]Removal	19.3		17.0		21.1	
[6]Dissection	20.5		18.0		22.5	
[7]#days/node	2.5		2.7		2.1	
Statistical data:						
F	3.79		4.54		6.74	
df	30, 303		27, 269		31, 286	
p	< 0.0001		< 0.0001		< 0.0001	

Table 4.1 Vertical distribution of the mean number of *D. texanus* oviposition punctures on selected soybean plant introductions, 2006

Plant node	Plant Introduction					
	PI 228065	[1]n	PI 229358	n	PI 323275	n
25 (top)	0.0 ± 0.0 f	3	.		0.0 ± . e	1
24	0.0 ± 0.0 f	5	0.0 ± . d	1	0.0 ± 0.0 e	2
23	0.3 ± 0.3 def	6	0.0 ± 0.0 d	2	0.0 ± 0.0 e	3
22	0.0 ± 0.0 f	7	0.5 ± 0.5 cd	2	0.0 ± 0.0 e	4
21	0.2 ± 0.1 ef	10	0.0 ± 0.0 d	5	0.1 ± 0.1 e	7
20	0.1 ± 0.1 f	11	0.0 ± 0.0 d	7	0.1 ± 0.1 e	13
19	0.6 ± 0.3 cdef	11	0.0 ± 0.0 d	11	0.2 ± 0.2 de	15
18	1.8 ± 0.7 abcdef	13	0.3 ± 0.1 cd	12	0.4 ± 0.2 cde	15
17	3.5 ± 1.1 a	13	0.7 ± 0.3 bcd	13	2.1 ± 0.7 abcde	15
16	2.8 ± 0.9 ab	13	1.5 ± 0.8 abcd	14	2.3 ± 0.4 abcd	15
15	2.5 ± 0.7 abc	13	1.9 ± 0.5 abcd	14	2.7 ± 0.5 ab	15
14	2.2 ± 0.4 abcde	13	2.0 ± 0.4 abcd	14	2.9 ± 0.5 a	15
13	3.0 ± 0.8 a	14	2.8 ± 0.7 ab	15	3.1 ± 0.6 a	15
12	2.2 ± 0.8 abcd	14	1.7 ± 0.6 abcd	15	3.6 ± 0.7 a	15
11	2.4 ± 0.7 abc	14	2.4 ± 0.7 abc	15	2.5 ± 0.6 abc	15
10	1.9 ± 0.6 abcdef	14	3.1 ± 0.6 a	15	1.6 ± 0.6 abcde	15
9	1.9 ± 0.7 abcdef	14	1.6 ± 0.5 abcd	15	1.7 ± 0.4 abcde	15
8	2.2 ± 0.6 abcde	15	1.7 ± 0.6 abcd	15	0.6 ± 0.3 cde	15
7	1.0 ± 0.4 bcdef	15	0.9 ± 0.3 bcd	15	0.7 ± 0.2 bcde	15
6	0.7 ± 0.4 cdef	15	0.9 ± 0.3 bcd	15	0.7 ± 0.3 bcde	15
5	0.3 ± 0.2 def	15	0.3 ± 0.2 cd	15	0.3 ± 0.2 de	15
4	0.3 ± 0.2 def	15	0.3 ± 0.2 cd	15	0.2 ± 0.1 de	15
3 (bottom)	0.4 ± 0.2 def	15	0.2 ± 0.1 d	15	0.1 ± 0.1 e	15
Developmental stage (number of nodes):						
[2]Prelim.Obs.	9.6		8.6		10.1	
[3]1-install.	11.8		10.1		12.1	
[4]2-install.	15.3		12.4		15.3	
[5]Removal	19.6		15.4		19.3	
[6]Dissection	20.7		16.3		20.3	
[7]#days/node	2.3		3.4		2.5	
Statistical data:						
F	4.12		4.27		7.92	
df	29, 293		28, 276		31, 300	
p	< 0.0001		< 0.0001		< 0.0001	

Table 4.1 Vertical distribution of the mean number of *D. texanus* oviposition punctures on soybean varieties 93M50, NEX2403K, and DB32C25, 2006

Plant node	Variety					
	93M50	[1]n	NEX2403K	n	DB32C25	n
25 (top)	.		.		.	
24	.		.		.	
23	.		.		.	
22	.		.		.	
21	0.0 ± 0.0 g	2	.		0.0 ± . d	1
20	0.0 ± 0.0 g	5	.		0.0 ± 0.0 d	3
19	0.0 ± 0.0 g	7	.		0.0 ± 0.0 d	8
18	0.1 ± 0.1 fg	10	.		0.4 ± 0.4 cd	8
17	0.8 ± 0.4 efg	13	.		0.8 ± 0.4 bcd	12
16	1.4 ± 0.4 defg	15	.		0.7 ± 0.2 cd	15
15	2.5 ± 0.6 bcdef	15	1.2 ± 0.6 cde	5	1.1 ± 0.4 bcd	15
14	2.3 ± 0.7 bcdefg	15	1.6 ± 0.3 cd	11	1.5 ± 0.3 abcd	15
13	3.5 ± 0.7 abcd	15	2.1 ± 0.5 bcd	14	2.3 ± 0.5 a	15
12	5.1 ± 1.1 a	15	2.6 ± 0.3 b	14	1.5 ± 0.4 abcd	15
11	4.5 ± 0.9 ab	15	2.7 ± 0.4 ab	15	1.5 ± 0.4 abcd	15
10	3.8 ± 1.1 abc	15	2.7 ± 0.3 ab	15	2.3 ± 0.6 ab	15
9	3.7 ± 1.0 abc	15	3.6 ± 0.4 a	15	1.7 ± 0.5 abc	15
8	3.8 ± 0.8 abc	15	2.1 ± 0.5 bcd	15	2.3 ± 0.5 ab	15
7	3.1 ± 0.8 abcde	15	2.1 ± 0.4 bc	15	1.3 ± 0.4 abcd	15
6	2.1 ± 0.6 cdefg	15	1.1 ± 0.3 de	15	0.6 ± 0.3 cd	15
5	1.3 ± 0.4 defg	15	0.5 ± 0.2 ef	15	0.3 ± 0.1 cd	15
4	0.9 ± 0.4 efg	15	0.1 ± 0.1 f	15	0.4 ± 0.2 cd	15
3 (bottom)	0.3 ± 0.2 fg	15	0.1 ± 0.1 f	15	0.1 ± 0.1 cd	15
Developmental stage (number of nodes):						
[2]Prelim.Obs.	9.7		9.7		9.3	
[3]1-install.	11.2		10.6		10.8	
[4]2-install.	13.5		12.1		13.1	
[5]Removal	16.5		14.0		16.0	
[6]Dissection	17.3		14.5		17.0	
[7]#days/node	3.4		5.4		3.4	
Statistical data:						
F	8.04		14.28		5.44	
df	25, 266		19, 204		25, 261	
p	< 0.0001		< 0.0001		< 0.0001	

Table 4.1 Vertical distribution of the mean number of *D. texanus* oviposition punctures on soybean varieties X3727NRS, KS4704, and KS4404, 2006

Plant node	Variety					
	X3727NRS	[1]n	KS4704	n	KS4404	n
25 (top)	.		.		.	
24	.		.		.	
23	0.0 ± 0.0 e	2	.		.	
22	0.0 ± 0.0 e	2	.		.	
21	0.0 ± 0.0 e	4	0.0 ± 0.0 d	2	0.0 ± 0.0 c	3
20	0.1 ± 0.1 e	8	0.0 ± 0.0 d	4	0.0 ± 0.0 c	5
19	0.1 ± 0.1 e	10	0.0 ± 0.0 d	5	0.0 ± 0.0 c	6
18	0.1 ± 0.1 e	13	0.0 ± 0.0 d	9	0.4 ± 0.3 c	8
17	0.4 ± 0.3 de	14	0.1 ± 0.1 d	15	0.1 ± 0.1 c	12
16	0.9 ± 0.3 cde	15	0.4 ± 0.3 cd	15	0.3 ± 0.2 c	14
15	2.1 ± 0.5 abc	15	0.3 ± 0.2 cd	15	1.0 ± 0.4 c	15
14	1.7 ± 0.3 bcd	15	1.7 ± 0.4 ab	15	2.6 ± 0.6 b	15
13	2.7 ± 0.6 ab	15	2.1 ± 0.3 ab	15	2.8 ± 0.4 ab	15
12	2.1 ± 0.3 abc	15	1.7 ± 0.3 ab	15	4.2 ± 0.9 a	15
11	3.4 ± 0.6 a	15	2.3 ± 0.4 ab	15	3.7 ± 0.5 ab	15
10	2.1 ± 0.5 abc	15	2.4 ± 0.4 a	15	3.9 ± 0.5 ab	15
9	2.1 ± 0.5 abc	15	2.4 ± 0.6 a	15	3.9 ± 0.7 ab	15
8	2.2 ± 0.5 abc	15	2.3 ± 0.5 ab	15	2.8 ± 0.6 ab	15
7	1.7 ± 0.5 bcd	15	1.7 ± 0.5 ab	15	2.9 ± 0.3 ab	15
6	1.0 ± 0.3 cde	15	1.1 ± 0.3 bcd	15	2.7 ± 0.5 ab	15
5	0.8 ± 0.2 cde	15	1.4 ± 0.3 abc	15	0.7 ± 0.3 c	15
4	0.1 ± 0.1 e	15	0.3 ± 0.2 cd	15	0.3 ± 0.2 c	15
3 (bottom)	0.0 ± 0.0 e	15	0.0 ± 0.0 d	15	0.1 ± 0.1 c	15
Developmental stage (number of nodes):						
[2]Prelim.Obs.	9.6		8.4		8.1	
[3]1-install.	11.3		10.0		9.7	
[4]2-install.	13.9		12.6		12.2	
[5]Removal	17.3		15.8		15.3	
[6]Dissection	18.3		16.7		16.3	
[7]#days/node	3.0		3.1		3.2	
Statistical data:						
F	7.27		8.44		12.64	
df	27, 280		25, 264		25, 262	
p	< 0.0001		< 0.0001		< 0.0001	

Table 4.1: (Footnotes)

[1]n : number of plants that had each node by entry.

[2]Prelim.obs.: number of nodes counted during preliminary (pre-infestation) observations on July 12 & 13, 2006.

[3]1-install.: estimated number of nodes at first cage installation (July 17, 2006).

[4]2-install. : estimated number of nodes at second cage installation (July 25, 2006).

[5]Removal: estimated number of nodes when the cage was removed (August 4, 2006).

[6]Dissection: number of nodes counted when the first replication of plants was dissected.

[7]#days/node: mean number of days required to add a new node.

Mean ± SEM followed by the same letter in a column are not significnatly different ($P < 0.05$), Duncan's Multiple Range test.

Plant node with underlined variable in each entry is the estimated apex node at first cage installation (58 d after seeding).

Oviposition punctures on the nodes of lower third and upper twenty-fifth nodes counted from base of the plant were not in table.

Beetles were released and confined in the field cages between July 17 and August 4, 2006.

Table 4.2 Vertical distribution of the mean number of *D. texanus* oviposition punctures on soybean plant introductions 165673, 165676, and 171451, 2007

Plant node	Plant Introduction					
	PI 165673	[1]n	PI 165676	n	PI 171451	n
25 (top)	0.0 ± 0.0 d	7	.		.	
24	0.0 ± 0.0 d	10	0.0 ± 0.0 d	3	.	
23	0.0 ± 0.0 d	16	0.0 ± 0.0 d	4	0.0 ± 0.0 d	4
22	0.0 ± 0.0 d	20	0.0 ± 0.0 d	5	0.0 ± 0.0 d	7
21	0.2 ± 0.2 d	24	0.0 ± 0.0 d	8	0.0 ± 0.0 d	14
20	0.2 ± 0.2 d	26	0.1 ± 0.1 d	16	0.0 ± 0.0 d	21
19	0.2 ± 0.1 d	28	0.0 ± 0.0 d	20	0.0 ± 0.0 d	27
18	0.3 ± 0.2 d	30	0.1 ± 0.1 d	25	0.0 ± 0.0 d	30
17	0.7 ± 0.2 cd	30	0.0 ± 0.0 d	27	0.3 ± 0.2 d	30
16	1.0 ± 0.3 bcd	30	0.1 ± 0.1 d	27	0.3 ± 0.3 d	30
15	1.2 ± 0.4 bcd	30	0.3 ± 0.1 d	27	0.4 ± 0.2 d	30
14	2.2 ± 0.5 abc	30	0.6 ± 0.3 cd	27	0.6 ± 0.3 cd	30
13	2.4 ± 0.5 ab	30	1.0 ± 0.4 cd	27	1.4 ± 0.4 bc	30
12	3.6 ± 0.7 a	30	3.0 ± 1.2 a	27	2.9 ± 0.5 a	30
11	2.4 ± 0.6 ab	30	2.6 ± 0.5 ab	27	2.2 ± 0.4 ab	30
10	3.6 ± 0.8 a	30	1.7 ± 0.4 bc	27	2.1 ± 0.5 ab	30
9	0.9 ± 0.2 bcd	30	0.7 ± 0.2 cd	27	0.9 ± 0.2 cd	30
8	1.1 ± 0.3 bcd	30	0.5 ± 0.1 cd	27	0.6 ± 0.2 cd	30
7	0.7 ± 0.2 cd	30	0.6 ± 0.2 cd	27	0.2 ± 0.1 d	30
6	0.2 ± 0.1 d	30	0.5 ± 0.1 cd	27	0.5 ± 0.2 d	30
5	0.1 ± 0.1 d	30	0.4 ± 0.1 cd	27	0.7 ± 0.2 cd	30
4	0.4 ± 0.1 d	30	0.3 ± 01 d	27	0.5 ± 0.2 d	30
3 (bottom)	0.1 ± 0.1 d	30	0.2 ± 0.1 d	27	0.2 ± 0.1 d	30
Developmental stage (number of nodes):						
[2]Installation	14.3		12.6		13.6	
[3]Removal	18.1		16.0		17.2	
[4]Dissection	20.6		18.3		19.4	
[5]#days/node	3.7		4.2		3.9	
Statistical data:						
F	8.74		5.67		9.73	
df	34, 682		29, 537		28, 614	
p	< 0.0001		< 0.0001		< 0.0001	

Table 4.2 Vertical distribution of the mean number of *D. texanus* oviposition punctures on fipronil treated- and untreated soybean variety 93M50, 2007

Plant node	Variety (treatment)			
	93M50 (fipronil untreated)	[1]n	93M50 (fipronil treated)	n
25 (top)	.		.	
24	.		.	
23	0.0 ± . e	1	0.0 ± . f	1
22	0.0 ± 0.0 e	4	0.0 ± 0.0 f	2
21	0.0 ± 0.0 e	9	0.0 ± 0.0 f	6
20	0.0 ± 0.0 e	20	0.0 ± 0.0 f	12
19	0.0 ± 0.0 e	24	0.0 ± 0.0 f	20
18	0.1 ± 0.1 e	25	0.1 ± 0.1 f	26
17	0.0 ± 0.0 e	25	0.2 ± 0.2 ef	27
16	0.1 ± 0.1 e	25	0.6 ± 0.3 def	28
15	0.1 ± 0.1 e	25	0.9 ± 0.3 def	29
14	0.5 ± 0.2 e	25	1.6 ± 0.4 cdef	30
13	1.9 ± 0.4 cd	25	2.1 ± 0.4 bcd	30
12	2.9 ± 0.4 abc	25	3.4 ± 0.5 ab	30
11	4.0 ± 0.5 a	25	3.9 ± 0.5 a	30
10	3.5 ± 0.5 ab	25	3.8 ± 0.5 a	30
9	2.6 ± 0.6 bcd	25	3.4 ± 0.6 ab	30
8	2.0 ± 0.6 cd	25	2.7 ± 0.4 abc	30
7	1.3 ± 0.4 de	25	1.9 ± 0.4 bcde	30
6	0.4 ± 0.1 e	25	0.6 ± 0.2 def	30
5	0.3 ± 0.2 e	25	0.5 ± 0.2 def	30
4	0.3 ± 0.1 e	25	0.2 ± 0.1 f	30
3 (bottom)	0.1 ± 0.1 e	25	0.1 ± 0.1 f	30
Developmental stage (number of nodes):				
[2]Installation	12.6		13.3	
[3]Removal	16.0		16.8	
[4]Dissection	18.2		19.0	
[5]#days/ node	4.2		4.0	
Statistical data:				
F	15.31		16.45	
df	27, 505		28, 572	
p	< 0.0001		< 0.0001	

Table 4.2: (Footnotes)

[1]n : number of plants that had each node by entry.

[2]Installation.: estimated number of nodes when the cage was installed (July 23, 2007).

[3]Removal: estimated number of nodes when the cage was removed (August 7, 2007).

[4]Dissection: number of nodes counted when the first replication of plants was dissected.

[5]#days/node: mean number of days required to add a new node.

Mean ± SEM followed by the same letter in a column are not significnatly different ($P < 0.05$), Duncan's Multiple Range test.

Plant node with underlined variable in each entry is the estimated apex node at cage installation (54 d after seeding).

Oviposition punctures on the nodes of lower third and upper twenty-fifth nodes counted from base of the plant were not in table.

Beetles were released and confined in the field cages between July 23 and August 7, 2007.

CHAPTER 5 - Morphological Evaluation of Petioles in Soybean Plant Introductions Resistant to *Dectes texanus* LeConte

5.1. Abstract

The arrangement and thickness of various plant tissues can be critical to the resistance of crop plants to pest arthropods. The morphological characteristics of the petioles of three soybean plant introductions (PIs) were examined as possible mechanisms of resistance against oviposition by the Dectes stem borer, *Dectes texanus* LeConte. Histochemical measurements were made of the vascular tissues in cross-sections of plant petioles. Petiole diameter, petiole pith diameter, mean thickness of vascular bundles, mean thickness of sclerenchyma fibers, total width of the vascular bundles surrounding the petiole pith area, and total width of inter-vascular regions were measured, and the proportion of the perimeter occupied by vascular bundles was calculated. Differences in the mean thickness of vascular bundles and the proportion of the perimeter occupied by vascular bundles were significant among soybean genotypes tested. However, the values for the PI showing highest *D. texanus* antibiosis were no different form those for the susceptible control. Differences in the proportion of the petiole perimeter occupied by vascular bundles also showed a similar trend. Soybean leaf petiole morphology may be related to reduced *D. texanus* oviposition on petioles of PI171451 and PI165676, but results suggested that resistance identified in PI165673 is related to leaf petiole morphology. Even if the oviposition was successful, antibiosis in PI165673 somehow reduced successful egg hatch and the number of live *D. texanus* larvae.

Key words:

D. texanus oviposition, soybean petiole morphology, vascular histochemistry

5.2. Introduction

The arrangement and or thickness of plant tissues can affect the resistance of cultivated crop plants against their arthropod pests (Smith 2005). For example, Lundgren et al. (2008) reported that female *Orius insidiosus* (Say) selected plants for oviposition based on the thickness of tissues like the epidermis and scherencyma layers. The condition of these tissues had direct implications for the nutrition of the female, as well as for her offspring's ability to use the plant as food. This insect is zoophytophagous, in that it is predaceous, and also phytophagous to some degree, especially during its immature stages. Brewer et al. (1986) found that resistance to the potato leafhopper, *Empoasca fabae* (Harris), in alfalfa was based on the structure of the vascular bundles in the stem. Highly lignified xylem elements and phloem fibers, as well as the size of the intervascular cylinder surrounding the pith, were all linked to reduced *E. fabae* oviposition. The lignified tissue of the vascular cylinder appeared to contribute to *E. fabae* resistance by mechanically or chemically blocking or deterring feeding and oviposition.

Hatchett et al. (1975) reported that female Dectes stem borers, *Dectes texanus* LeConte, oviposited the majority of their eggs in the pith in the center of the petiole, and that egg deposition appeared to depend on the presence of pith and on whether the female could reach the pith with her ovipositor. Not all oviposition punctures contained eggs. Campbell (1980) obtained field observations demonstrating that *D. texanus* eggs were laid in well-developed petioles, primarily in the upper half of the plant. Richardson (1975) examined various morphological characteristics in petioles and stems of numerous soybean varieties and plant introductions. After detecting no relationship between *D. texanus* infestation and the arrangement or number of vascular bundles, Richardson (1975) concluded that soybean petiole morphology had no relationship to *D. texanus* resistance.

Attempts to use greenhouse-grown soybean plants to identify *D. texanus* resistance revealed ample oviposition on plants but little larval survival (Niide et al. unpubl., Chapter 3). Thus, we hypothesized that spindly morphology (and reduced petiole pith) of greenhouse-grown plants prevented survival of *D. texanus* eggs. The purpose of this study was to quantify the morphological stem features in soybean PIs identified in Chapter 2 as resistant to *D. texanus* in order to determine if differences in petiole vascular tissues were related to the numbers of *D. texanus* oviposition punctures on resistant soybean genotypes.

5.3. Materials and Methods

Measurements of petiole vascular tissues were taken on soybean plant introductions (PIs) PI165673, PI171451 and PI165676, identified during 2005 and 2006 evaluations as resistant to *D. texanus* (Chapter 2) and the susceptible soybean variety Pioneer 93M50. Pioneer 93M50 seed was obtained from Pioneer Hybrid Seed Co. and seed of the PIs were obtained from Dr. R. L. Nelson, USDA, ARS Soybean Germplasm Collection, Urbana, IL. Fourteen seeds from each genotype were planted at the Kansas State Univeristy North Central Kansas Experiment Field near Scandia, Kansas, 6.4 cm apart in 76.2 cm rows on May 31, 2007. The five genotypes were spaced to fit inside the footprint of a commercial patio field cage that would be installed over the plants later in the season. The arrangement of the entries within the cages was a randomized block design. There were two replications within each cage and there were six total replications in three field cages. The procumbent growth of PI plants was tied to a wire between two 1 m high posts to ensure their exposure to *D. texanus* adults. When beetles were present in the field, large 3.7 m x 3.7 m field cages (First-up Outdoor Shelters, Screen House, North Pole USA, Washington, MO) were installed over the experimental plots. Adult *D. texanus* were collected from surrounding soybeans using heavy-duty sweep nets. A total of 315 beetles were released into each field cage and confined for 15 d. Cages were removed to promote better plant growth and to allow adult beetles to leave plants and limit additional oviposition, because finding eggs is more difficult than finding larvae. Plants were caged again for ca. 7 d to allow deposited eggs to hatch.

Petiole tissue samples were collected from a single soybean plant of each of the five entries when the cages were opened to release beetles, on August 8, 2007. Petioles from the first, third, fifth, seventh and ninth fully-opened trifoliates (from the plant apex) were removed from each plant and a 2 cm-long section was cut ca. 2 cm from the petiole base. Petiole sections were placed individually into small centrifuge tubes, filled with 80% ethanol, and stored at 5.6 °C for histochemical analyses. Preserved petioles were hand-sectioned and prepared as a wet mount on a microscope slide. Sections were stained with safranin- (cell nuclei) fast green (protein/ cytoplasm) and phloroglucinol-HCl (lignin) (Peterson et al. 2008, Zimmermann 1983). Prepared sections were digitally photographed using a stereoscopic zoom microscope, (Nikon SMZ 1500, Nikon Instruments Inc., 1300 Walt Whitman Rd., Melville, NY).

Captured cross section images of the dissected petioles were analyzed using ImageJ, a public domain Java image processing program (Research Services Branch, National Institute of Mental Health, Bethesda, MD). Measurements were made of petiole diameter; petiole pith diameter; mean number and width of vascular bundles; mean width of sclerenchyma fiber layer; total vascular bundle width; total intervascular area; and proportion of total petiole area occupied by vascular bundles.

The numbers of oviposition punctures for each petiole and internode of PI165673, PI171451, PI165676, and the susceptible soybean variety Pioneer 93M50 were obtained using methods described in Chapter 2. Five plants from each replicate of each genotype were collected and examined. Plant developmental stage was recorded based on the number of fully expanded leaves (Pedersen 2004), and an estimate of the developmental stage when different events occurred was made, based on the number of days required to develop one leaf stage for each variety. These data were analyzed using the SAS GLM procedure (Colette and Robinson 2000) and means were compared using LS Means ($\alpha = 0.05$).

5.4. Results and Discussion

There were no significant differences in the diameter of the petiole or the petiole pith, the number of vascular bundles, or the thickness of the sclerenchyma layers in the petioles of the soybean plant introductions (Table 5.1). However, plant introductions 165676 and 171451 had larger vascular bundles than did the susceptible 93M50 control ($F = 5.59$; df = 3,4; $P = 0.0123$), and a significantly greater amount of the area of the pith occupied with vascular bundles ($F = 5.93$; df = 3,4; $P = 0.0101$). However, the proportion of the pith area of plant introduction 165673, which had the lowest mean number of oviposition punctures, was not significantly different from the susceptible control (Table 5.1). The petiole diameter of leaves at lower levels of the main stem increased, reaching a maximum at the third or fifth nodes below the plant apex (Table 5.2, Figures 5.1-.4, 5.6-.9). However, the petiole diameter of PI165676 did not follow this pattern, in some cases decreasing or remaining constant.

Just as differences in numbers of oviposition punctures varied significantly among different leaf positions (Chapter 4, Tables 4.1, 4.2), the number of oviposition punctures increased with decreasing leaf position from the plant apex, and in most cases reached a

maximum at position 5 or 7 (Table 5.2, Figures 5.1-.4). However, the number of petioles changed with plant growth during *D. texanus* infestation and, older leaves were exposured to oviposition for a longer period of time. The number of oviposition punctures was probably more closely related to the numbers of petioles present on infested plants rather than to petiole morphology.

In most cases, the proportion of the total width of vascular bundles to the periphery of pith reached a maximum for petioles on the fifth or seventh node (Table 5.2 , Figure 5.5). Oviposition scars are usually found on the side or bottom of petioles between the vascular ridges (personal observations, Figure 5.10). It is interesting to note that intervascular spaces appear to be larger in these areas and the vascular bundles appear to be larger under the five well-developed primary vascular bundles spaced around the petiole periphery.

We hypothesized that resistant plant introductions should have a high ratio of the proportion of the petiole perimeter occupied by vascular bundles. Our hypothesis was based on the assumption that plants with lower ratios (or a higher proportion of intervascular space) would allow beetles to penetrate pith during oviposition more easily. This hypothesis is not supported by our results, as the highest proportion of intervascular space occurred in both the susceptible 93M50 control and in PI165673 (Table 5.1). Nevertheless, PI165673 exhibited the most antibiosis (highest OP/ Lv ratio) even through it had the highest number of oviposition punctures (Table 2.3).

In conclusion, although soybean leaf petiole morphology may be related to *D. texanus* oviposition, our data do not support the contention that *D. texanus* resistance is related to petiole morphology. However, Hatchett et al. (1975) reported that not all oviposition punctures contain eggs, and it is possible that many of the oviposition punctures we recorded may have been unsuccessful oviposition attempts, since it was difficult to determine egg deposition after larval tunneling through petioles. PI165673, a highly antibiotic genotype, had comparatively thin vascular bundles, suggesting that antibiosis is a likely cause of reduced numbers of live *D. texanus* larvae in this soybean plant introduction.

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Table 5.1 Mean ± SEM of morphological measurements in petioles of three soybean plant introductions and the variety 93M50

Soybean genotype	Petiole diameter (mm)	Petiole pith diameter (mm)	Number of vascular bundles	Mean thickness of vascular bundles (mm)
PI 165673	3.9499 ± 0.3085 a	3.1386 ± 0.2267 a	12.0 ± 0.4 a	0.5051 ± 0.0481 a
PI 165676	3.8799 ± 0.1439 a	3.1065 ± 0.1487 a	14.0 ± 1.0 a	0.4777 ± 0.0311 ab
PI 171451	4.4391 ± 0.2999 a	3.6523 ± 0.2314 a	14.2 ± 1.2 a	0.5622 ± 0.0515 a
93M50	3.4628 ± 0.3668 a	2.8137 ± 0.2782 a	13.4 ± 0.7 a	0.4008 ± 0.0517 b
Statistical data				
F	2.98	3.38	1.02	5.59
df	3,4	3,4	3,4	3,4
p	0.0737	0.0541	0.4192	0.0123

Soybean genotype	Mean thickness of sclerenchyma fibers (mm)	Total width of vascular bundles (VB) (mm)	Total wides of inter-vascular ares (IVA) (mm)	Proportion VB / (VB + IVA)
PI 165673	0.0634 ± 0.0045 a	8.2939 ± 0.8834 ab	3.1111 ± 0.2016 a	0.7193 ± 0.0321 bc
PI 165676	0.0564 ± 0.0057 a	8.9035 ± 0.2319 a	2.2306 ± 0.1992 a	0.8002 ± 0.0155 a
PI 171451	0.0668 ± 0.0085 a	9.4597 ± 1.0123 a	2.9641 ± 0.3475 a	0.7530 ± 0.0392 ab
93M50	0.0496 ± 0.0033 a	6.6956 ± 1.0184 b	3.1697 ± 0.2247 a	0.6633 ± 0.0427 c
Statistical data				
F	2.07	4.57	2.65	5.93
df	3,4	3,4	3,4	3,4
p	0.1619	0.0235	0.0968	0.0101

Mean ± SEM followed by the same letter in a columm of the comparison items are not significantly different ($P < 0.05$),

Data were analyzed with PROC GLM and means were separated by LSM.

Table 5.2 Growth and morphological measurements and mean numbers of *D. texanus* oviposition punctures in petioles of three soybean plant introductions and the variety 93M50

Genotype	[1]V	[2]R	Height (cm)	[3]P	[4]OPs	Petiole diam (mm)	Petiole pith diam (mm)	[5]#VB	[6]VB width (mm)	[7]Sc fiber width (mm)	[8]Total VB width (mm)	[9]Total IVA width (mm)	[10]VB/ (VB+IVA)
PI165673	19	0	124.3	1	0.2	2.9306	2.2963	13	0.3318	*.	4.9051	3.2738	0.5997
				3	0.7	3.7316	3.1126	11	0.4978	0.0521	8.3027	2.9100	0.7405
				5	1.2	3.9549	3.2019	12	0.5072	0.0602	9.0814	2.6169	0.7763
				7	2.4	4.7347	3.6327	13	0.6057	0.0689	9.3229	3.8014	0.7104
				9	2.4	4.3975	3.4495	11	0.5829	0.0723	9.8575	2.9533	0.7695
PI165676	15	0	87.4	1	0.3	4.1435	3.4070	16	0.4089	0.0510	9.1935	2.3792	0.7944
				3	1.0	3.9883	3.2943	15	0.4449	0.0567	8.6135	2.5034	0.7748
				5	2.6	4.1552	3.3381	11	0.5696	0.0783	9.2166	2.6612	0.7760
				7	0.7	3.7108	2.7915	12	0.5328	0.0497	9.3637	1.5319	0.8594
				9	0.6	3.4016	2.7018	16	0.4324	0.0462	8.1300	2.0774	0.7965
PI171451	17	0	83.3	1	0.3	3.2640	2.7423	11	0.3915	0.0343	5.7571	3.3016	0.6355
				3	0.4	4.7203	3.7592	13	0.6124	0.0759	9.1053	4.1422	0.6873
				5	1.4	4.5237	3.8208	18	0.5030	0.0691	10.0247	2.6319	0.7921
				7	2.2	4.7941	3.9988	14	0.6231	0.0718	11.5541	2.1455	0.8434
				9	0.9	4.8933	3.9404	15	0.6809	0.0830	10.8575	2.5994	0.8068
93M50	18	4	103.2	1	0.1	2.2648	1.8558	12	0.2574	0.0399	3.3621	3.1438	0.5168
				3	0.1	3.0501	2.5295	12	0.3386	0.0470	5.6960	2.8705	0.6649
				5	0.5	3.6163	3.0774	16	0.3709	0.0492	7.1024	3.4476	0.6732
				7	2.9	4.2096	3.3274	14	0.5062	0.0599	8.0440	3.8418	0.6768
				9	3.5	4.1730	3.2783	13	0.5311	0.0522	9.2737	2.5448	0.7847

Table 5.2: (Footnotes)

[1]V : vegitative growth stage of plant sampled

[2]R : reproductive growth stage of plant sampled

[3]P : vertical position of the petiole from the plant apex

[4]OP : mean number of oviposition puncture

[5]# VB : total number of vascular bundles in petiole cross-section

[6]VB width : mean width of vascular bundles in petiole cross-section

[7]Sc fiber width : mean width of sclerenchyma fibers in petiole cross-section

[8]Total VB width : total width of vascular bundles in petiole cross-section

[9]Total IVA width : total width of inter-vascular regions in petiole cross-section

[10]VB/ (VB+IVA) : proportion of total width of vascular bundles to total pith area, calculated as = [total width of vascular bundles/ sum of widths of vascular bundles + inter-vascular regions]

*Captured image was not clear, thickness difficult to measure

Figure 5.1 Diameter (mm) of the first, third, fifth, seventh and ninth petioles of three soybean plant introductions and the susceptible control, 93M50, (bars) and mean numbers of *D. texanus* oviposition punctures (dots) at each vertical position of the petiole

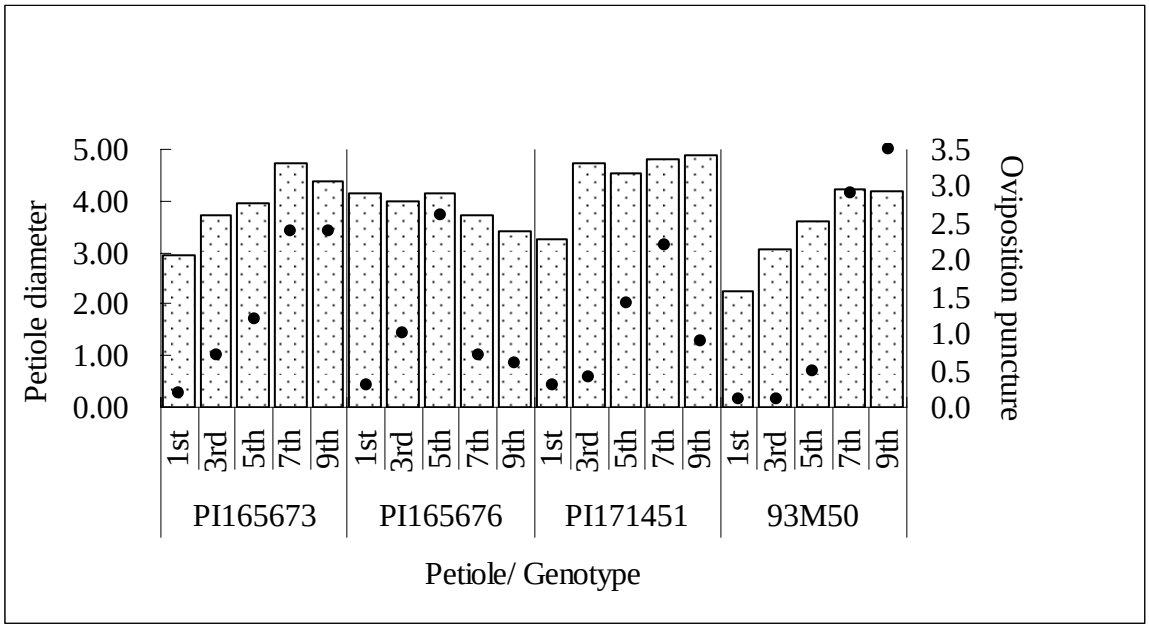


Figure 5.2 Diameter (mm) of the pith in the first, third, fifth, seventh and ninth petioles of three soybean plant introductions and the susceptible control, 93M50, (bars) and mean numbers of *D. texanus* oviposition punctures (dots) at each vertical position of the petiole

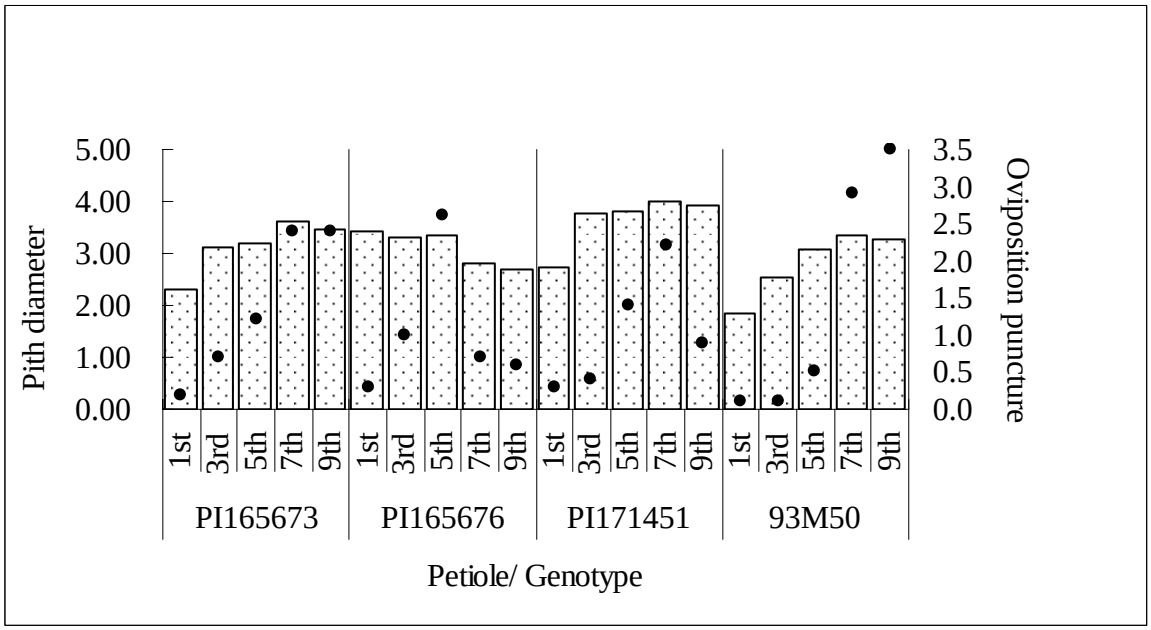


Figure 5.3 Mean thickness (mm) of vascular bundles in the pith of the first, third, fifth, seventh and ninth petioles of three soybean plant introductions and the susceptible control, 93M50, (bars) and mean numbers of *D. texanus* oviposition punctures (dots) at each vertical position of the petiole

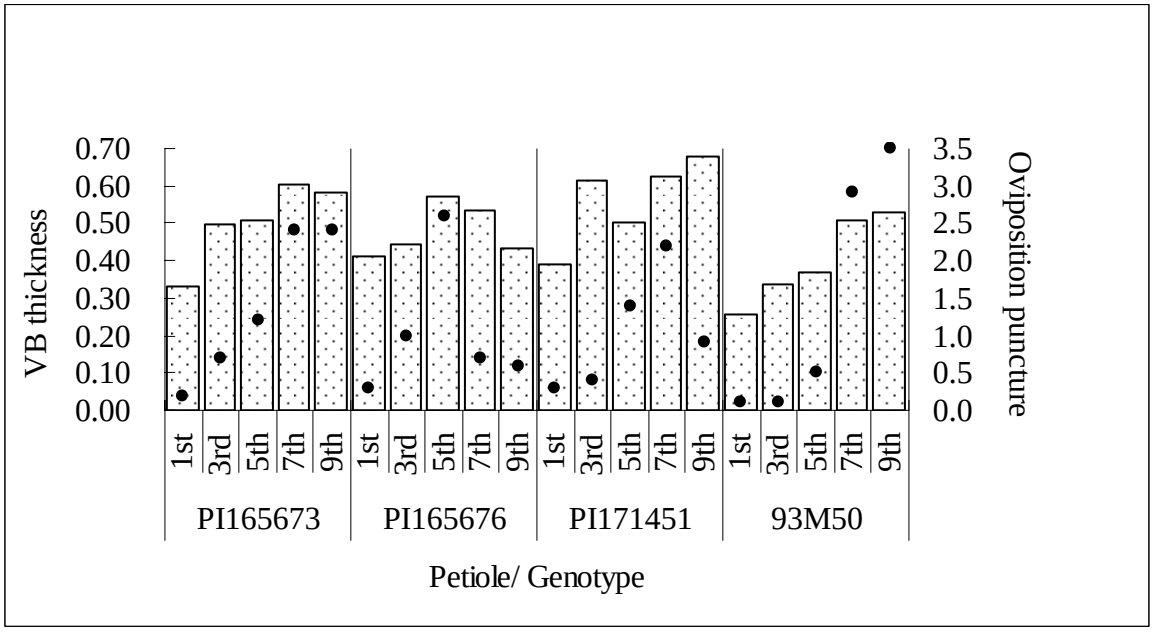


Figure 5.4 Mean thickness (mm) of sclerenchyma fibers in the pith of the first, third, fifth, seventh and ninth petioles of three soybean plant introductions and the susceptible control, 93M50, (bars) and mean numbers of *D. texanus* oviposition punctures (dots) at each vertical position of the petiole

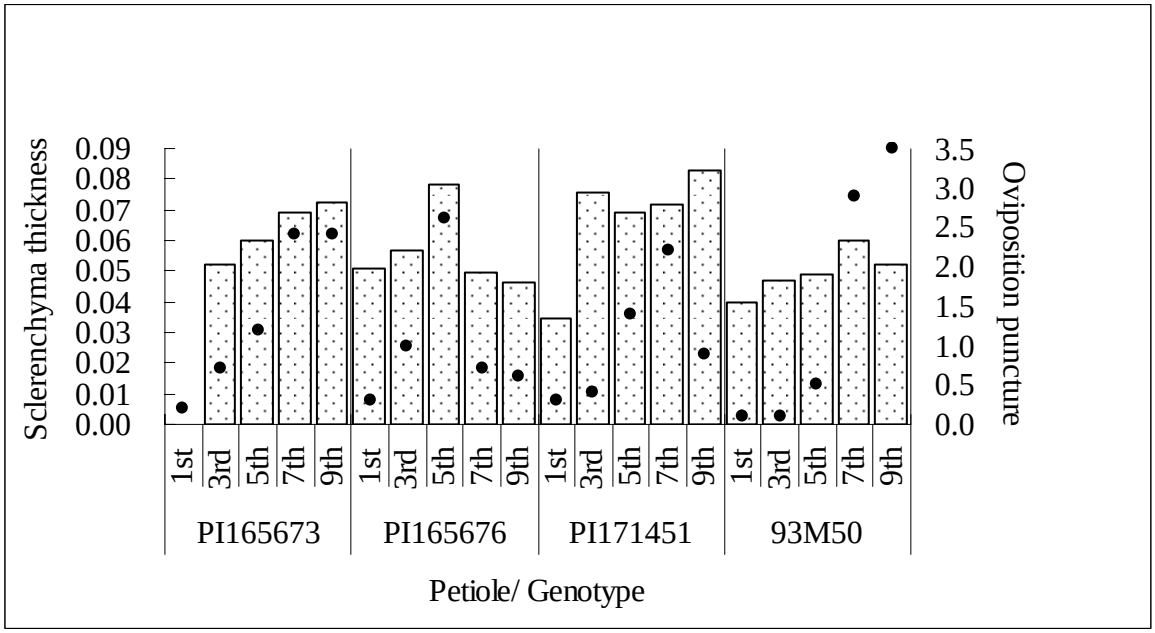


Figure 5.5 Proportion of the total petiole pith area of three soybean plant introductions and the susceptible control, 93M50, included within the total width of vascular bundle (bars) and mean numbers of *D. texanus* oviposition punctures (dots) at each vertical position of the petiole

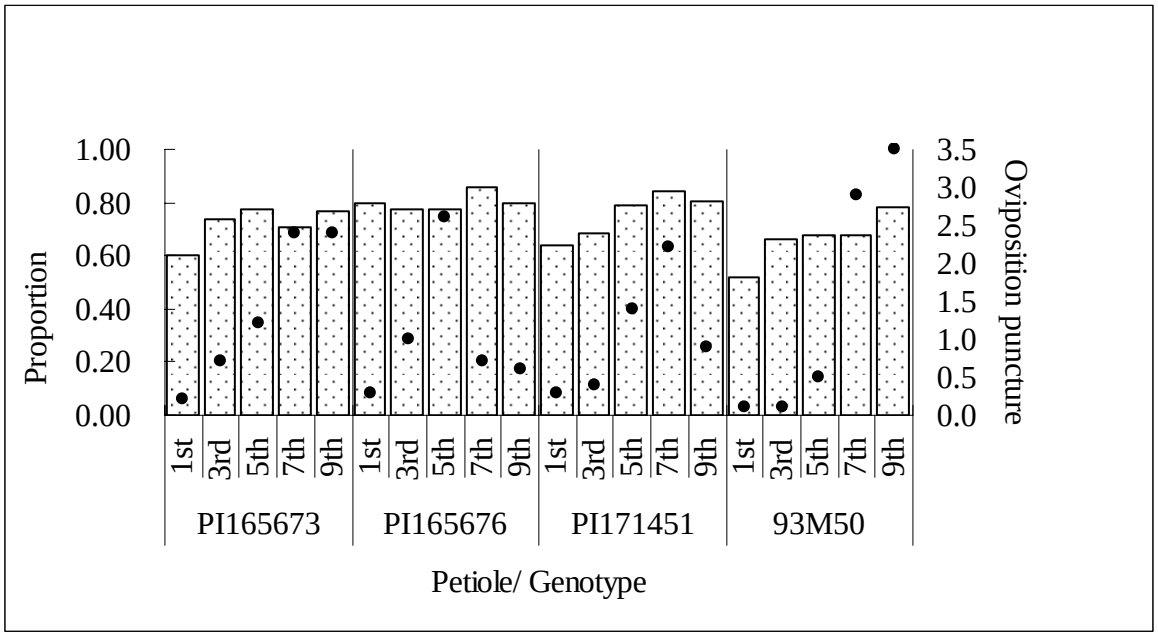


Figure 5.6 Cross-section images of petioles in PI165673

A- first petiole

B- third petiole

C- fifth petiole

D- seventh petiole

E- ninth petiole

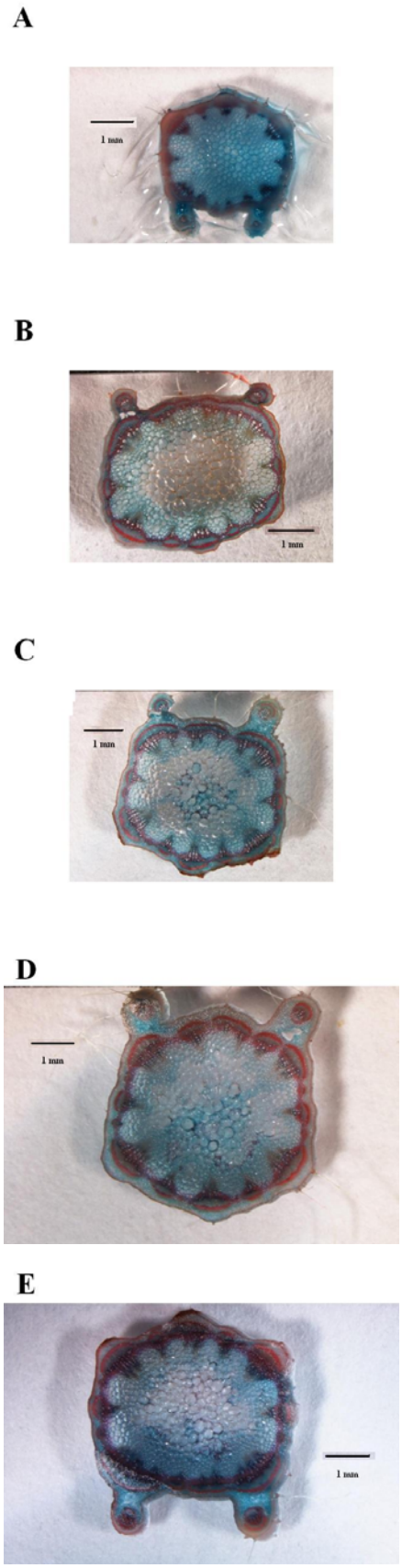


Figure 5.7 Cross-section images of petioles in PI165676

A- first petiole

B- third petiole

C- fifth petiole

D- seventh petiole

E- ninth petiole

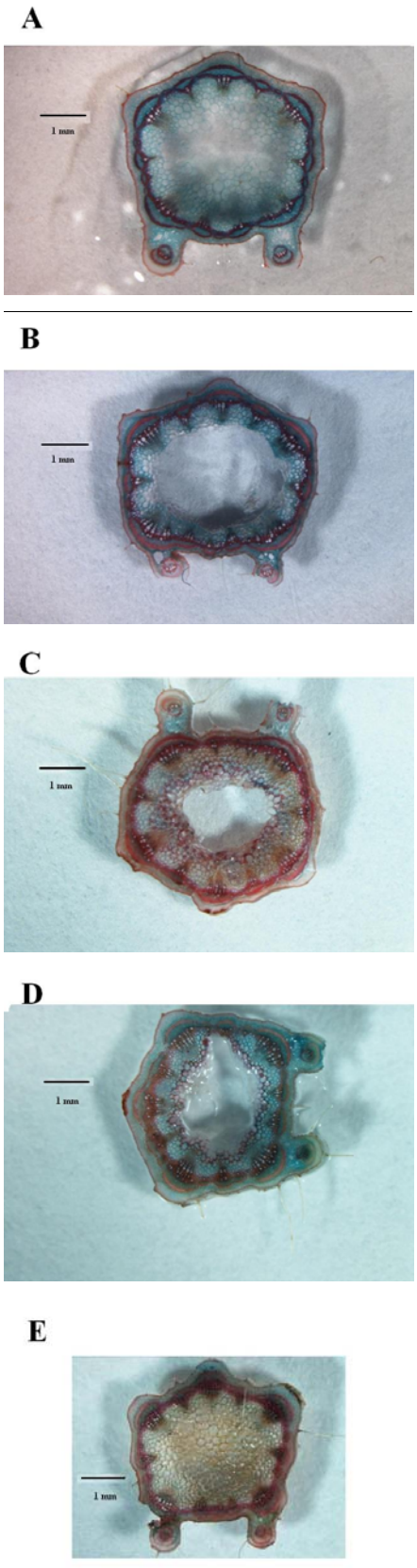
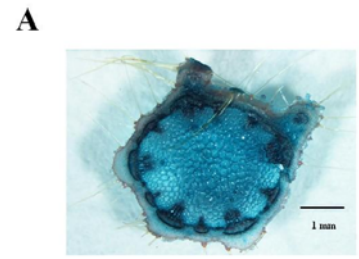
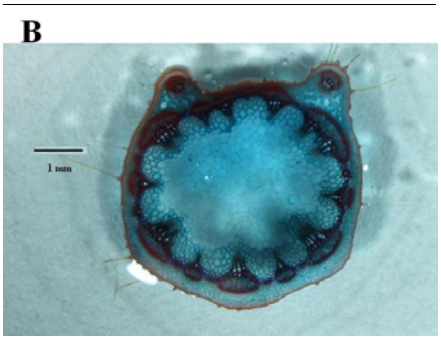


Figure 5.8 Cross-section images of petioles in PI171451

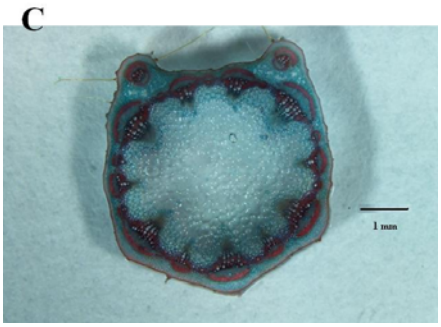
A- first petiole



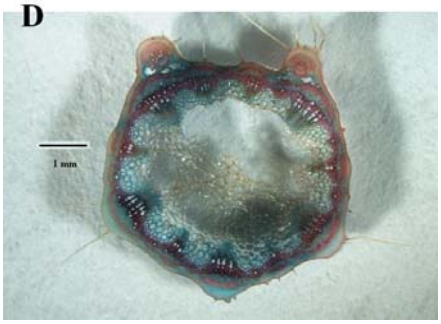
B- third petiole



C- fifth petiole



D- seventh petiole



E- ninth petiole

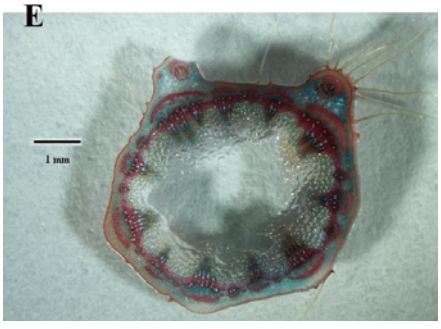
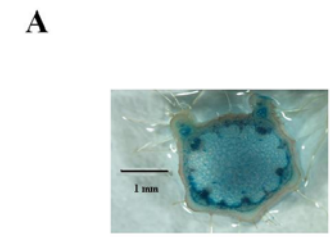
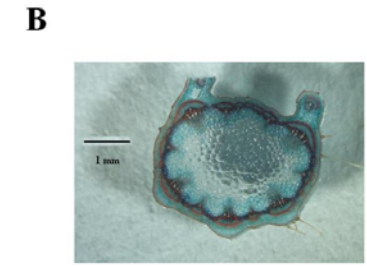


Figure 5.9 Cross-section images of petioles in 93M50

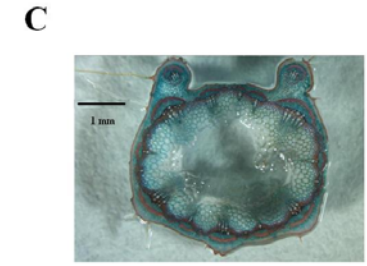
A- first petiole



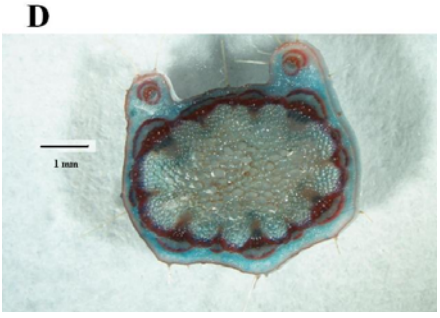
B- third petiole



C- fifth petiole



D- seventh petiole



E- ninth petiole

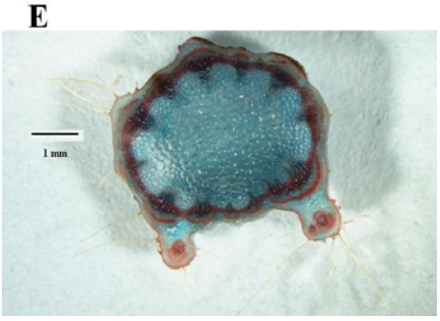


Figure 5.10 Cross-section image of a soybean petiole with *D. texanus* oviposition puncture (OP)

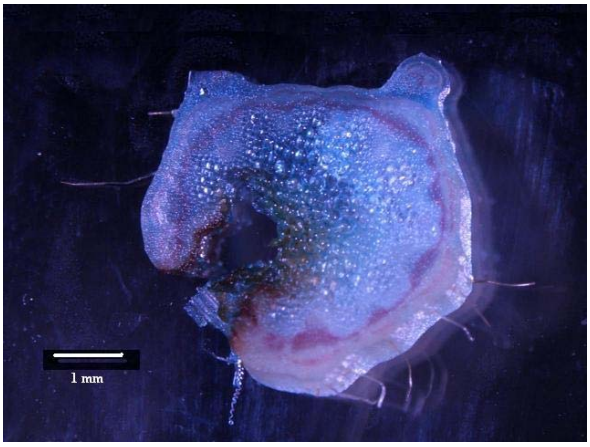
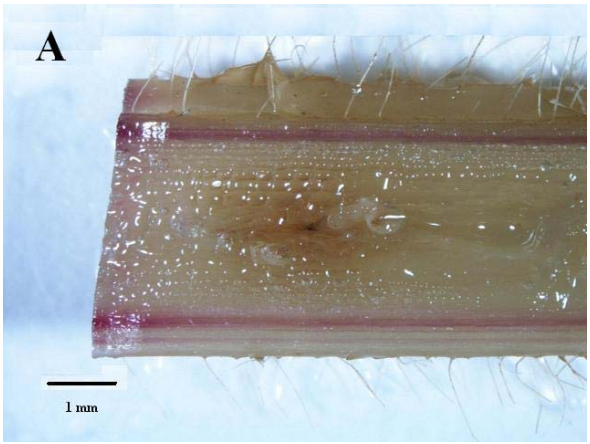
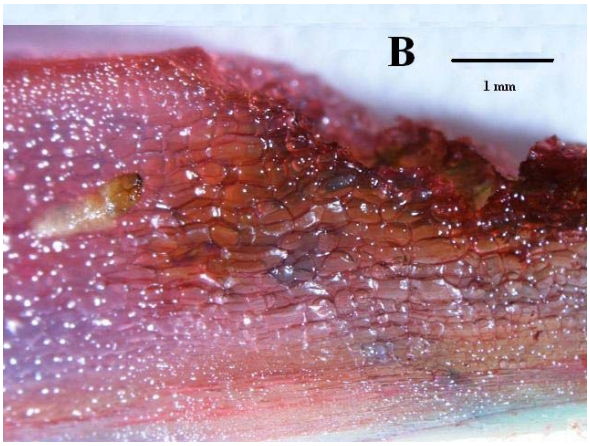


Figure 5.11 Longitudinally cross section images of soybean petiole pith with neonate *D. texanus* larva

A- 100 x maginification;

B- 150 x magnification





CHAPTER 6 - Evaluation of Soybean Tolerance of Stem Damage from Feeding by Larvae of the Dectes Stem Borer, *Dectes texanus* LeConte, Based on Differences in Yield Response to Insecticide Treated- and Untreated Soybean Plants

6.1. Abstract

Host plant resistance to the Dectes stem borer, *Dectes texanus* LeConte, has been reported to be present in some very late maturing soybean varieties but no resistance has been identified in varieties adapted in the Midwestern U.S. Previous research has focused on identification of antibiosis and antixenosis resistance to *D. texanus* in soybean varieties and plant introductions. To date, evidence of soybean tolerance of *D. texanus* larval feeding damage has not been investigated. However, there are no useful strategies for managing *D. texanus*, making it difficult to measure yield losses associated with this pest. Foliar insecticide sprays are not feasible for managing *D. texanus*, as the larval stage is spent inside the host plant stem.

In this study, yield responses of untreated soybean plants and plants treated with the systemic insecticide, fipronil (phenyl pyrazole) were measured to to determine the effect of *D. texanus* larval damage on plant seed yield. Fipronil foliar treatments significantly reduced *D. texanus* infestations (77% in 2006, 41% in 2007), but differences in yield between untreated and treated plants were not significant. Seed-treatment experiments in 2007 demonstrated that fipronil significantly reduced *D. texanus* infestations from 77 to 98%. However, differences in seed yield between seed treated and untreated plants were not significant. In 2008, the effects of the fipronil seed-treatment were highly significant for both *D. texanus* infestation and grain yield. A yield loss of 8.2% and a 2.9% loss due to plant lodging was associated with *D. texanus* infestations. No conclusions about soybean tolerance could be drawn from the results, as only one variety was evaluated. However, these results demonstrate that fipronil seed treatment is a useful technology for use in protecting soybean grain yields from *D. texanus* infestations.

Key words:

systemic insecticide, fipronil, soybean grain yield, physiological yield loss, plant lodging

6.2. Introduction

The importance of the Dectes stem borer, *Dectes texanus* LeConte, as a pest of soybeans and sunflowers is increasing in some areas in North America, raising producer concerns because management options are not available for this pest (Buschman and Sloderbeck 2009, Lentz 1994, and Sloderbeck et al. 2003, 2008). Older recommendations suggest that crop rotation and stubble destruction can be used to reduce *D. texanus* damage. However, such cultural practices appear to have lost their efficacy and are no longer compatible with current agronomic practices. Insecticide treatments for managing this pest have not been successful (Campbell and VanDuyn 1977). Plants are re-infested after treatment because beetles are fairly mobile, at least on a local scale.

D. texanus resistance has been reported to be present in some very late maturing soybean varieties (Richardson 1975), but no resistance has been identified in varieties adapted in the Midwest (Kaczmarek 2001b). Host plant resistance is usually associated with antibiosis and antixenosis, however tolerance is also a potential mode of action. In previous work, efforts were directed to identify antibiosis and antixenosis in commercial soybean varieties and in soybean plant introductions (Richardson 1975, Niide 2009 Chapters 2, 3, 4, and 5). There is no evidence of *D. texanus* tolerance in soybean varieties.

Recently, Sloderbeck et al. (2004) were able to demonstrate that applications of a pyrethroid insecticide, lambda-cyhalothrin (Warrior™) could reduce *D. texanus* infestations up to 80%. In addition, Buschman et al. (2005) demonstrated that a systemic insecticide, fipronil, suppressed larval populations in soybean plants. Unfortunately, this insecticide may never receive registration for use on commercial soybeans. So to date, there are no effective or usable management strategies for *D. texanus* in soybean.

D. texanus spends the larval stage inside the host plant stem, protected from most foliar insecticide treatments. Eggs are laid in the pith, usually in the leaf petioles, and newly hatched larva feed in the pith of the leaf petiole for several weeks until they reach the second to third instar (Hatchett et al. 1975). The third instar larva tunnels into the main stem where it continues to feed on pith as it tunnels up and down the plant (Patrick 1973). As the plant approaches maturity, the larva moves to the crown area of the plant where it prepares an overwintering chamber. The larva then cuts the stem off from the inside, usually 3-10 cm above ground level,

an event termed “girdling”. Campbell and VanDuyn (1977) suggested that girdling might protect the overwintering larvae from natural enemies or extreme environmental conditions. Buschman et al. (2002) have suggested that this behavior reduces competition for the only usable overwintering site in the plant. Girdled soybean stems break off and fall to the ground, resulting in plant “lodging”. Lodged plants are difficult to retrieve with harvest equipment, and large quantities of soybean seed are left unharvested. If the soybeans are harvested when plants reach maturity, limited lodging occurs. However, if harvest is delayed lodging increases and yield losses can be serious. Higgins et al. (1999) reported losses of 672-1,545 kg/ ha caused by lodging in Kansas when harvesting was delayed. Lodging soybean fields infested with *D. texanus* increases over time because not all stems are girdled simultaneously. Campbell (1980) reported that in addition to lodging losses, a 10% loss in soybean seed weight occurred in infested plants.

The only period when *D. texanus* is outside the plant and exposed to insecticide treatments is in the adult stage. Adults are present during late June through late August in Kansas. Females are present for ca. 8 weeks and males for ca. 4 weeks (Kaczmarek et al. 2001c, Sloderbeck et al. 2003). *D. texanus* appears to have two peaks of emergence (Hatchett et al. 1975, Niide et al. 2006). Use of insecticides to control *D. texanus* has previously not been feasible (Campbell and VanDuyn 1977). The prolonged presence of *D. texanus* adults during the summer means they reinfest treated areas, necessitating multiple insecticide treatments to suppress infestations.

Direct yield losses from *D. texanus* larval stem tunneling and from plant stem lodging have been difficult to quantify because there are no effective treatments, but several recent efforts have been made to explore improved methods for the chemical control of this insect pest. Kaczmarek et al. (2002) determined that residual treatments of lambda-cyhalothrin, permethrin and carbaryl were effective in controlling *D. texanus* beetles under laboratory conditions. Sloderbeck et al. (2004) conducted several trials with aerial applications of lambda-cyhalothrin and found that two applications could be used to reduce Dectes stem borer infestations to acceptable levels. Imidacloprid and clothianidin seed treatments did not provide protection from *D. texanus* larval damage (Higgins et al. 2003, Kaczmarek et al. 2001a). Buschman et al. (2005, 2006) tested a number of systemic insecticides to target the first and second instars feeding inside the plant. They found that fipronil applied either as a soil treatment or as a foliar treatment, gave significant control of larvae. These treatments were effective enough to document a 10%

increase in grain yield for fipronil-treated over untreated soybean plants (Buschman and Sloderbeck 2006).

Fipronil, a phenyl pyrazole, insecticide, is effective against a broad range of insect pests even when used at low doses. Fipronil interferes with the passage of chloride ions through the gamma-aminobutyric acid (GABA)- regulated chloride channel, thus disrupting central nerve system activity. Fipronil binds to insect GABA receptors with higher affinity than it does to the vertebrate GABA sites, which accounts for increased toxicity to insects (Gant et al. 1998, Scharf and Siegfried 1999). Fipronil has been found to have efficacy against other cerambycids (Grosman and Upton 2006) and is used to treat pests of corn and cotton (Maloney 2003, Mulrooney and Goli 1999).

The objective of this research was to determine if there was any evidence of plant tolerance to *D. texanus* in soybean varieties. To determine tolerance, we examined yield responses of soybean varieties treated- or untreated with fipronil applied as foliar or seed treatments. These evaluations were conducted as a part of a larger fipronil efficacy trial conducted at several locations in Kansas. The results from one trial are reported here.

6.3. Materials and Methods

Six Kansas varieties in each of the maturity groups II, III and IV (2006) including Nex2403RR, DB32C25, 93M50, 3727NRS, KS4404RR and KS4704RR and four varieties in maturity groups between III and IV (2007) including DB32C25, 93M92, KS4404RR and KS4704RR were assessed in preliminary trials to determine if there was evidence of tolerance in soybean. Varieties were selected to include some with the highest and lowest rates of susceptibility to *D. texanus* (Kaczmarek et al. 2001b, Khajuria et al. 2005). Seed was machine-planted at 16 seeds per 30.5 cm row. on May 17 (2006) and May 28 (2007) at the North Central Kansas Experiment Field near Scandia, Kansas. Plots were four rows wide (2.3 m) and 6.1 m (2006) and 8.5 m (2007) long. There was a 0.9 m -wide alley at each end of the plots. The experimental arrangement was a randomized block design with three (2006) and four (2007) replications. Fipronil (phenyl pyrazole) was applied as a foliar treatment on July 18 (2006) and July 26 (2007) during adult activity, and was targeted at the first two larval instars developing in leaf petioles, based on development times reported by Hatchett et al. (1975). Treatments were

applied with a backpack sprayer, using a hand-held boom with two nozzles (CornJet® VisiFlo® Hollow Corn Spray Nozzle TX-VS6, TeeJet Technologies, Wheaton, IL) directed at a single row (Figure 6.2). The nozzles were held 15.2-20.3 cm from the plants to maximize coverage of the upper canopy. The sprayer was calibrated to deliver 290.5 g of fipronil (Regent SC, BASF Corporation, Research Triangle Park, NC) / ha. A chronometer was used to measure the time spent on each row to help maintain appropriate speed (9.4 sec per 7.6 m row at 30 psi).

In 2007, Pioneer 93M50 (maturity group III) was also planted and seed treated with three different rates of fipronil with an untreated control. Treated seed were planted together in plots receiving foliar treatments. Seed was sent in early April with fipronil (Regent® 500TS, BASF Corporation, Research Triangle Park, NC) (25, 50 and 100 mg AI/ 100 kg seed) and planted with the untreated check. The seed treatments were included in the experimental design and planted with the four varieties receiving foliar treatments.

Because it was difficult to show a significant yield difference between treated- and untreated plots, objectives of 2008 experiments were focused on documenting a significant yield response to fipronil treatment. Plots were enlarged to eight rows wide (5.3 m) and 19.8 m long five replications. In these experiments, Pioneer 93M92 (maturity group III) was treated with fipronil (Regent® 500TS) at 100 mg AI/ 100 kg seed and planted with untreated seed as a control. Plots were machine planted on May 16 at 16 seeds per 30.5 cm row using a small-plot row-crop planter. The four-row plots were harvested at normal harvest, when the soybeans reached maturity (October 8), and late harvest, when soybeans lodged extensively (November 18).

D. texanus infestations were determined by dissecting 20 plants (10 plants in 2008) at the end of the season (September 22, 2006; September 21, 2007; September 30, 2008) (Figure 6.3). In 2006, five consecutive plants from each of the four rows in a plot were collected. In 2007, two sets of five consecutive plants were collected from each of the two outside rows in a plot. In 2008, five consecutive plants were collected from each of two center rows in a plot. The numbers of entry nodes, numbers of upper stem tunnels, numbers of tunnels reaching the plant base, and numbers of live larvae were recorded. The percentage of plants girdled per 91.4 cm row was recorded late in the growing season (April 15) of the 2008 efficacy trial. Grain yield data were collected by machine harvesting two middle rows in each plot on October 12, 2006 and November 2, 2007; and on October 8, 2008 for normal harvest and November 18, 2008 for late harvest. Normal harvest in 2008 occurred when plants dried enough to harvest and late

harvest occurred after many *D. texanus*-infested plants had lodged. Late harvest was ca. 6 weeks after normal harvest. Yield data was converted to bu/acre based on 13% moisture.

The SAS-ANOVA procedure (Colette and Robinson 2000) was used to analyze the data as a two-factor experiment, variety (six in 2006, four in 2007) and insecticide treatment (treated and untreated) with three or four replications. In 2007, data were analyzed as a simple randomized block design with four treatments and four replications. In 2008, data were analyzed as a simple randomized block design with two treatments and five replications. Yield data were analyzed as a two-factor experiment with two levels of insecticide seed treatment, two harvest times and five replications. Means in all experiments were compared using LSD ($\alpha = 0.05$).

6.4. Results and Discussion

In 2006, the average infestation in untreated plots for the six tested entries averaged 54% (37-67%), but the differences across the varieties were non-significant (P = 0.83) (Table 6.1). The average infestation for treated plots was 12% ($F = 15{,}625.00$; df = 1,2; $P < 0.0001$) (Table 1). Treated plants had significantly fewer entry nodes ($F = 135.13$; df = 1,2; $P < 0.0001$), stems tunneled ($F = 76.25$; df = 1,2; $P < 0.0001$), stems tunneled to the base ($F = 58.02$; df = 1,2; $P < 0.0001$) and live larvae ($F = 38.49$; df = 1,2; $P < 0.0001$). Fipronil foliar treatment significantly reduced *D. texanus* infestations by a range of 78% to 88%. Fipronil seed treatment also reduced infestations by 77% . Grain yield was fairly uniform across varieties and insecticide treatments, increasing grain yield by 2.9% (126.1 kg/ ha), but this difference was non - significant ($F = 32.87$; df = 1,2; $P = 0.4339$) (Table 6.1). The lack of significance was surprising because of the significantl reduction in *Dectes* infestations. However, the overall rate of infestation (54%) and the small plot size were probably insufficient to measure losses with enough precision to allow statistical significance. Thus, there was no evidence for *D. texanus* tolerance.

In 2007, the *D. texanus* infestation averaged 68% (61-76%) in untreated plots of the four tested varieties (Table 6.2). Foliar-treated plants had significantly fewer entry nodes, stem tunneling, stem tunneling to the base, live larvae and percentage plants girdled, than treated plants (Table 6.2). Although infestations ranged from 61 to 76 % of untreated plants, only 6-44 % of these plants were girdled by April of the following year. Treated plots had virtually no girdling, and average percent *D. texanus* control among the four varieties ranged from 41 to 96%

for the different infestation indicators. Although the timing of foliar spray treatments appeared to be late, dead *D. texanus* larvae that had already tunneled to the main stem were recovered. Grain yield was fairly uniform across varieties and across insecticide treatments. There was an increased grain yield of 165.9 kg/ ha (4.4%) which again was non-significant (F = 2.70; df = 1,3; P = 0.1151) (Table 6.2). This was surprising because fipronil treatment significantly reduced infestations and infestations were greater (68%) than in 2006. As in 2006 however, small plot size and small numbers of replications were probably insufficient to measure losses with enough precision to allow statistical significance (Table 6.2). Again, there was no evidence of *D. texanus* tolerance.

In 2007, all fipronil seed treatments significantly reduced *D. texanus* infestations relative to untreated plants, and efficacy increased with fipronil dose (25 mg AI/ 100 kg seed, 79-100 % reduction; 50 mg AI/ 100 kg seed, 77-100% reduction; 100 mg AI/ 100 kg seed, 99-100 % reduction). (Table 6.3). Seed-treated plants had significantly fewer entry nodes (F = 21.27; df = 3,3; P = 0.0002), stem tunneling (F = 39.31; df = 3,3; P < 0.0001), stem tunneling to the plant base (F = 41.20; df = 3,3; P < 0.0001) and live larvae (F= 37.36; df = 3,3; P < 0.0001), compared with untreated plants (Table 6.3). This trend could be important at locations where *D. texanus* infestations were typically greater than other less-infestated areas (Buschman et al. 2009). The seed-treated plots yielded 53.2 to 298.5 kg/ ha more than untreated plots, but this difference was non - significant (F = 0.81; df = 3,3; P = 0.5211). Significant physiological yield losses associated with *D. texanus* infestation were not demonstrated in these evaluations, and again, there was no evidence for *D. texanus* tolerance.

In 2008, 100 mg AI fipronil / 100 kg seed significantly reduced (100% control) the numbers of *D. texanus* entry nodes (F = 212.3; df = 1,4; P < 0.0001), larval stem tunneling (F = 154.86; df = 1,4; P <0.0001), larval stem tunneling to the plant base (F = 201.6; df = 1,4; P < 0.0001) and numbers of live larvae (F = 55.55; df = 1,4; P < 0.0001) (Table 6.4). Residual activity of seed treatments remained effective into August when larvae were actively tunneling into plant stems. The effects of the seed treatment (F = 41.06; df = 1,4; P < 0.0001) and harvest date (F = 78.67; df = 1,4; P < 0.0001) on grain yield were both highly significant, while the interaction of the two factors was not significant (F = 0.99; df = 1,1; P = 0.3388) (Table 6.4).

At normal harvest, plots planted with treated seed had 371.5 kg/ ha more grain than the untreated plots, and at late harvest the difference was 504.1 kg/ ha (Table 6.5). The yield

differences between treated- and untreated plots amounted to 8.2% in normal harvested, plots and 13.0% in late harvested plots. These physiological yield losses associated with untreated seed might be attributed to *D. texanus* larval feeding damage. *D. texanus* larval tunneling in primary or secondary stems may interfere with water and nutrient transport, and may also cause soybean plants to have reduced productivity. Losses at normal harvest might be attributed to physiological disruption, since there was very little lodging. Consequently, there was very little growth remaining in plots after harvest. However, at late harvest, yield losses were larger, 670.0 kg/ ha for untreated seed and 537.3 kg/ ha for treated seed, for losses of 14.7 and 11.8 %, respectively (Table 6.5, Figure 6.1). Losses for untreated plots can be associated with lodging losses plus harvest delay (mostly pod shattering). Therefore, *D. texanus*-related plant lodging losses were calculated as the difference between late-harvest yield losses in untreated- and treated seed as 132.7 kg/ ha or 2.9%. Although these data provide no evidence of soybean plant tolerance, they demonstrate the first significant yield increases associated with protection of soybean plants with fipronil seed treatments as a useful technology to protect soybean grain yield from *D. texanus*. Presently, fipronil is not yet registered for use on soybean. However, previous recommendations of timely soybean harvest (Campbell and VanDuyn 1977, Sloderbeck et al. 2003) remain effective as management tools to reduce grain yield losses caused by lodging and pod shattering related to *D. texanus* larval feeding damage.

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Table 6.1 Factorial analysis of the efficacy of fipronil foliar treatment on Kansas soybean varieties for control of *Dectes texanus*, 2006

Comparison factors (Genotype/ Maturity group)	Treatment	Entry nodes per 20 plants	Stem tunneling per 20 plants	Base tunneling per 20 plants	Live larvae per 20 plants	% plants infested	Grain yield kg/ ha
Variety means							
Nex2403RR Mid II	Unsprayed	8.7	7.3	4.7	4.3	36.7	3907.6
	Sprayed	2.7	2.7	1.0	0.7	13.3	4478.0
DB32C25 Early III	Unsprayed	25.0	12.7	2.3	7.7	60.0	4073.4
	Sprayed	4.0	3.0	1.0	0.7	15.0	4000.5
93M50 Mid III	Unsprayed	17.7	13.3	2.0	6.0	66.7	4577.6
	Sprayed	4.0	3.0	0.0	0.0	15.0	4292.2
3727NRS Late III	Unsprayed	13.7	10.3	8.0	6.7	51.7	4192.7
	Sprayed	1.7	1.0	1.0	0.7	5.0	4789.8
KS4404RR Early IV	Unsprayed	16.7	12.0	6.0	3.3	60.0	4623.9
	Sprayed	2.7	2.7	1.0	1.0	13.3	4464.9
KS4704RR Mid IV	Unsprayed	13.0	9.7	5.3	5.7	48.3	4438.3
	Sprayed	3.3	2.3	1.0	1.0	11.7	4550.9
Insecticide treatment means							
All entries	Unsprayed	15.8 a	10.9 a	4.7 a	5.6 a	53.9 a	4305.6
	Sprayed	3.1 b	2.4 b	0.8 b	0.7 b	12.2 b	4431.5
% control/ yield increase		81%	78%	82%	88%	77%	+ 2.9%
Factorial analysis probabilities							
Variety means		0.0027	0.3505	0.0087	0.8315	0.8315	0.3909
Insecticide treatment means		< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.4339
Interaction		0.0175	0.5685	0.0465	0.7699	0.7699	0.4876

Mean followed by the same letter in a columm of the comparison items are not significantly different ($P < 0.05$),

Data were analyzed with PROC GLM and means were separated by LSM.

Table 6.2 Factorial analysis of the efficacy of fipronil foliar treatment on Kansas soybean varieties for control of *Dectes texanus*, 2007

Comparison factors (Genotype/ Maturity group)		Treatment	Entry nodes per 20 plants	Stem tunneling per 20 plants	Base tunneling per 20 plants	Live larvae per 20 plants	% plants infested	% plants girdled	Grain yield kg/ ha
Variety means									
DB32C25	Early III	Unsprayed	19.0 a	12.5 a	8.5 a	8.8 a	63.8 a	44.0 a	4199.5a
		Sprayed	10.5 b	8.0 b	0.5 b	0.8 b	45.0 b	0.0 b	4590.7a
93M92	Late III	Unsprayed	20.5 a	14.3 a	5.0 a	8.5 a	70.0 a	20.3 a	4312.2a
		Sprayed	7.5 b	4.8 b	0.3 b	0.3 b	32.5 b	0.0 b	4703.7a
KS4404RR	Early IV	Unsprayed	25.3 a	16.8 a	5.8 a	9.5 a	76.3 a	6.3 a	4325.4a
		Sprayed	10.0 b	6.5 b	0.3 b	0.5 b	36.3 b	0.0 b	4113.2a
KS4704RR	Mid IV	Unsprayed	18.3 a	11.8 a	3.3 a	7.0 a	61.3 a	20.5 a	4270.9a
		Sprayed	8.8 b	5.3 b	0.0 b	0.3 b	35.0 b	0.0 b	4371.9a
Insecticide treatment means									
All entries		Unsprayed	20.8 a	13.8 a	5.6 a	8.4 a	67.8 a	22.8 a	4279.0a
		Sprayed	9.2 b	6.1 b	0.3 b	0.4 b	37.2 b	0.0 b	4444.9a
% control/ yield increase			50.5%	50.8%	96.1%	94.5%	41.4%	100.0%	+ 4.4%
Factorial analysis probabilities									
Variety means			0.5798	0.3855	0.0337	0.6442	0.7825	< 0.5000	0.2500
Insecticide treatment means			< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.1151
Interaction			0.6988	0.3728	0.0948	0.7939	0.5659	<0.5000	0.1258

Mean followed by the same letter in a columm of the comparison items are not significantly different ($P < 0.05$),

Data were analyzed with PROC GLM and means were separated by LSM.

Table 6.3 Efficacy of fipronil seed treatments to control *Dectes texanus* in soyban variety 93M50, 2007

Comparison factors (Genotype/ Maturity group)	Treatment	Entry nodes per 20 plants	Stem tunneling per 20 plants	Base tunneling per 20 plants	Live larvae per 20 plants	% plants infested	% plants girdled	Grain yield kg/ ha
Seed treatment means								
93M50 Mid III	100gm AI/ 100kg	0.3 b	0.0 b	0.0 b	0.0 b	1.3 b	0.0 b	4557.6a
	50gm AI/ 100kg	3.5 b	1.8 b	0.0 b	0.0 b	13.8 b	0.0 b	4312.2a
	25gm AI/ 100kg	2.3 b	1.5 b	0.3 b	0.5 b	11.3 b	0.0 b	4438.3a
	Untreated	16.0 a	11.0 a	3.5 a	6.0 a	56.3 a	24.5 a	4259.0a
% control/ yield increase (against control)								
93M50 Mid III	100gm AI/ 100kg	98.8	100.0	100.0	100.0	98.1	100.0	+ 464.4
	50gm AI/ 100kg	77.1	87.0	100.0	100.0	77.1	100.0	+ 79.5
	25gm AI/ 100kg	79.4	85.6	93.8	91.4	79.7	100.0	+ 278.5
ANOVA F-test probability								
Insecticide treatment means		< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.5211

Mean followed by the same letter in a columm of the comparison items are not significantly different ($P < 0.05$),

Data were analyzed with PROC GLM and means were separated by LSM.

Table 6.4 Factorial analysis of the efficacy of fipronil seed treatment and harvest time to control *Dectes texanus* in soybean variety 93M92, 2008

Comparison factors (Genotype/ Maturity group)		Treatment	Entry nodes per 20 plants	Stem tunneling per 20 plants	Base tunneling per 20 plants	Live larvae per 20 plants	% plants infested	Grain yield kg/ ha
Insecticide treatment means								
93M92	Late III	Untreated	14.9 a	7.7 a	6.0 a	4.3 a	75.0 a	4225.9b
		Treated	0.0 b	0.0 b	0.0 b	0.0 b	0.0 b	4657.1a
% control/ yield increase			100%	100%	100%	100%	100%	+10.6%
Harvest time means								
93M92	Late III	Normal	n/a	n/a	n/a	n/a	n/a	4743.4a
		Late	n/a	n/a	n/a	n/a	n/a	4139.8b
% yield increase			n/a	n/a	n/a	n/a	n/a	-12.6%
Factorial analysis probabilities								
Insecticide treatment			< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Harvest time			n/a	n/a	n/a	n/a	n/a	< 0.0001
Interaction			n/a	n/a	n/a	n/a	n/a	0.3388

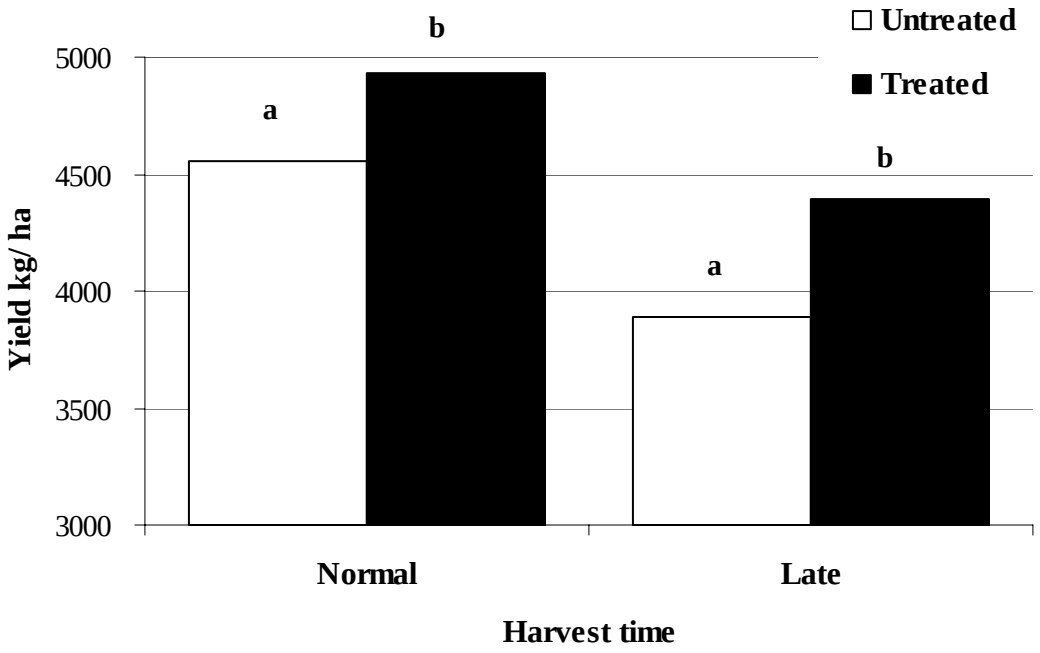
Mean followed by the same letter in a columm of the comparison items are not significantly different ($P < 0.05$),

Data were analyzed with PROC GLM and means were separated by LSM.

Table 6.5 *Dectes texanus*- related grain yield loss components in soybean variety 93M92 calculated using yield data from fipronil seed treatment efficacy evaluations, 2008

Yield loss components Calculation	kg/ ha	% loss
Physiological loss		
(Yield on treated/ normal harvest) - (Yield on untreated/ normal harvest)	371.5	8.2
(Yield on treated/ late harvest) - (Yield on untreated/ late harvest)	504.1	13.0
Harvest-delay loss		
(Yield on treated/ normal harvest) - (Yield on treated/ late harvest)	537.3	11.8
Harvest delay and lodging loss		
(Yield on untreated/ normal harvest) - (Yield on untreated/ late harvest)	670.0	14.7
Lodging loss		
(Harvest delay and lodging loss) - (Harvest delay loss)	132.7	2.9
Total losses		
(Yield on treated/ normal harvest) - (Yield on untreated/ late harvest)	1041.5	22.9

Figure 6.1 Grain yield of fipronil treated- and untreated plants of soybean variety 93M92 at normal- and late harvest dates in 2008



* Bars in the same harvest time followed by same letters are not significantly different (LSD; $P < 0.05$)

Figure 6.2 Fipronil foliar spray application

Figure 6.3 On-site *D. texanus* damaged soybean stem evaluation

CHAPTER 7 - Summary

Over the past several decades, the Dectes stem borer, *Dectes texanus* LeConte, has become an economically important pest of soybean cultivation in several parts of North America, but the seasonal biology of *D. texanus* makes it difficult to develop effective management options. *D. texanus* spends most of its lifecycle inside the host plant, so it is difficult to reach with most insecticide treatments. Adults emerge over an extended period from late June through late August in Kansas, and re-infest treated areas. Efforts have been made to identify host plant resistance against *D. texanus* in soybean germplasm, but results have been inconsistent. Plants that appear resistant one year turn susceptible the following year or in the same year in an adjacent field. Since conventional indices of plant resistance may be responsible for inconsistent results, improved criteria for resistance are needed. Consistent quantification of soybean yield losses caused by *D. texanus* damage has also difficult to determine. Plant lodging, caused by the larvae girdling the base of the plant, is obvious but difficult to quantify in the field. Physiological grain yield losses caused by *D. texanus* larval tunneling have also been difficult to quantify because no management tools exist that allow comparison of grain yields in treated and untreated plants.

The rationale for this project was to develop techniques that allow accurate identification of sources of *D. texanus* resistance in soybeans. The objectives of this study were to; 1.) develop new techniques and procedures that allow identification of antibiosis (reduced larval survival) and/or antixenosis (oviposition non-preference) effective against *D. texanus* in soybean; 2.) use the techniques to evaluate Kansas soybean varieties and soybean plant introductions from the USDA Soybean Germplasm Collection for antibiosis and antixenosis resistance to *D. texanus*; 3.) determine *D. texanus* oviposition preference and focus sampling on those regions when sampling; 4.) investigate whether morphological differences among soybean varieties *D. texanus* associated with *D. texanus* resistance to; and 5.) develop and test techniques to identify tolerance of *D. texanus* larval damage in soybean varieties based on yield responses in insecticide treated- and untreated soybean plots.

Instead of percent stem infestations or percent stem girdling, the number of oviposition scars was recorded as an indicator of *D. texanus* host preference. The number of larvae that

hatched and survived initial feeding in soybean plant petioles was recorded as an indicator of *D. texanus* larval antibiosis. However, since different varieties may receive differential oviposition in choice tests, the OP/ Lv ratio was calculated as a correction. The OP/ Lv ratio represents the number of oviposition scars needed to produce one live larva on each soybean line. The susceptible commercial varieties, treated with the systemic insecticide, fipronil, served as a positive control. The OP/ Lv ratio for the positive control was consistently higher than the ratio for any other soybean line and appeared to work well as an indicator of larval antibiosis. Recording the numbers of oviposition punctures and live larvae during the growing season were time-consuming and labor-intensive operations. However, these observations increased the sensitivity and repeatability of the results for identifying *D. texanus* resistance in soybean.

Soybean plant introduction (PI)165673 was identified as consistently having a very high OP/ Lv ratio that was not significantly different from the fipronil treated positive control. This result was duplicated in all four years of this study. In addition, the resistance in PI165673 was shown to be independent of maturity group.

No-choice greenhouse evaluations were conducted in 2005 and 2006 to demonstrate *D. texanus* resistance in soybean plant introductions, as well as in evaluated transgenic soybean plants containing the *Manduca sexta* chitinase gene. Greenhouse grown plants exposed to beetles in cages produced very few live larvae, even in susceptible lines, and appeared to be morphologically inappropriate for successful *D. texanus* oviposition and/or larval survival.

Observations revealed that *D. texanus* oviposition occurs mainly on petioles of the upper five nodes of the soybean plant canopy, in both resistant and susceptible plants. These results may be useful for developing improved *D. texanus* management tactics that more accurately detect infestations, and may also be most useful in developing more efficient sampling techniques for future research studies. Histo-morphological analyses of soybean leaf petioles indicated that petiole morphology maybe related to *D. texanus* oviposition non-preference, but the reduced numbers of live larvae in the most resistant plant introduction was more likely caused by antibiosis rather than plant morphology.

Soybean yield responses to *D. texanus* larval feeding damage were evaluated in insecticide treated- and untreated plants, using foliar and seed treatments of the systemic insecticide fipronil (phenyl pyrazole). Fipronil foliar treatments significantly reduced *D. texanus* larval infestations by 77% in 2006 and by 41% in 2007. However, differences in grain yield in

the same plots were not statistically significant. Fipronil seed treatments gave similar results of significant reductions in larval infestations but no statistically significant differences in seed yields. In 2008, analyses of fipronil seed treatments and harvest dates demonstrated a significant physiological yield loss of 8.2% and a plant lodging loss of 2.9% to be associated with *D. texanus* infestations. Since only one soyben variety was tested, no conclusions about tolerance resistance could be made. Future evaluations of additional germplasm will require larger plots and more replications. Nevertheless, results of these experiments demonstrate that fipronil seed treatments are useful in protecting soybean grain yields from *D. texanus* larval feeding damage.

In conclusion, a soybean genotype resistant to *D. texanus* larval feeding has been identified to improve soybean varieties, based on a sensitive and precise soybean antibiosis parameter, the *D. texanus* OP/ Lv ratio. In addition, fipronil was shown to be effective in protecting soybean plants from *D. texanus* larval feeding damage, and in creating positive control plants for comparative use in plant resistance screening. Further screening is necessary to identify additional sources of *D. texanus* resistance in soybean, and to determine the genetics of larval antibiosis in PI165673. The procumbent growth habit of PI165673 in Kansas climatic conditions indicates that substantial plant breeding research will be required to transfer this resistance into an agronomically acceptable background. Additional research is necessary to improve techniques for growing soybean plants and handling *D. texanus* populations so that germplasm evaluations can be conducted under improved greenhouse growing conditions.