

INHERITANCE OF RESISTANCE TO THE *DECTES* STEM BORER, *Dectes texanus*
LECONTE (COLEOPTERA: CERAMBYCIDAE), IN SOYBEAN PLANT INTRODUCTION
PI165673

by

LINA MARIA AGUIRRE-ROJAS

B.Sc., Universidad del Valle, Colombia, 2010

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Entomology
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2013

Approved by:

Major Professor
C. Michael Smith

Copyright

LINA MARIA AGUIRRE-ROJAS

2013

Abstract

The *Dectes* stem borer, *Dectes texanus* LeConte, is a pest of soybean, *Glycine max* (L.) Merrill, in North America. Larval feeding weakens plant stems, triggering lodging of the infested plants and causing significant yield losses. *D. texanus* infestations in soybean fields are increasing across Kansas and other states, necessitating the development of effective tactics to control this pest. The use of *D. texanus* -resistant soybean cultivars is a desirable strategy to control this pest since cultural and chemical control options are lacking. In previous studies, the soybean plant introduction PI165673 was identified to be resistant to *D. texanus*. The objective of this research was to determine the inheritance of resistance to *D. texanus* in PI165673. F₂ progeny plants from crosses between the *D. texanus* susceptible genotypes KS5004N and K07-1544, and the resistant genotype PI165673 were tested in the field for resistance to *D. texanus* in 2011. Seeds from the cross K07-1544/PI165673 were advanced to the F₃ generation, and F_{2:3} families were tested in the field for resistance to *D. texanus* in 2012. At 20 d after infestation with adults, the numbers of oviposition punctures and larvae on each plant were counted to estimate the oviposition puncture per larvae resistance ratio. Segregation for resistance to *D. texanus* and heritability estimates in the F₂ and F_{2:3} populations indicated that resistance is controlled by more than one gene. Thirteen F_{2:3} families had a higher (more resistant) resistance ratio than the susceptible parent K07-1544. Mean head capsule widths of larvae collected from K07-1544 and PI165673 plants in 2012 were similar, as was the percentage of larvae per larval instar. According to heritability estimates for each phenotypic trait, progress in breeding for *D. texanus* resistance using PI165673 will benefit from marker assisted selection. Identification of additional sources of *D. texanus* resistance besides that in PI165673 is needed to slow larval growth in the stem.

Table of Contents

List of Figures	vi
List of Tables	viii
Acknowledgements	ix
Dedication	x
Chapter 1 - Literature Review.....	1
Soybean	1
<i>Dectes</i> stem borer description and life cycle	2
Integrated management of <i>Dectes</i> stem borer in soybean.....	4
Genetics of soybean resistance to insect pests.	8
Chapter 2 - Resistance in Soybean PI165673 to <i>Dectes</i> Stem Borer is Polygenic	12
Introduction.....	12
Materials and Methods.....	15
Plant population development.....	15
Insect collection	15
Screening for resistance to <i>Dectes</i> stem borer	16
Evaluation of F ₂ populations	16
Evaluation of K07-1544/PI165673 F _{2:3} families.....	17
Statistical analysis	18
Results.....	20
F ₂ populations	20
K07-1544/PI165673 F _{2:3} families.....	20

Discussion	22
Conclusions and Perspectives	25
Cited Literature	50
Appendix A - Pattern of damage distribution inside 10 x 10 ft cages.	57

List of Figures

Figure 2.1. Frequency distributions of resistance ratio per plant in F ₂ soybean populations KS5004N/PI165673 (a) and K07-1544/PI165673 (b), infested with <i>D. texanus</i> in 2011. Arrows indicate parent means.....	43
Figure 2.2. Frequency distributions of number of oviposition punctures per plant in F ₂ soybean populations KS5004N/PI165673 (a) and K07-1544/PI165673 (b), infested with <i>D. texanus</i> in 2011. Arrows and stars indicate parent and F ₂ means, respectively.....	44
Figure 2.3. Frequency distributions of number of larvae per plant in F ₂ soybean populations from KS5004N/PI165673 (a) and K07-1544/PI165673 (b), infested with <i>D. texanus</i> in 2011. Arrows and stars indicate parent and F ₂ means, respectively.....	45
Figure 2.4. Percentage of total <i>D. texanus</i> larvae per instar collected from plants in soybean genotypes K07-1544 and PI165673 in 2012.....	46
Figure 2.5. Frequency distribution of the resistance ratio in plants from 108 F _{2:3} families from the cross K07-1544/PI165673 infested with <i>D. texanus</i> in 2012. Arrows and stars indicate parent and F ₂ means, respectively.....	47
Figure 2.6. Frequency distribution of number of oviposition punctures per plant in plants from 108 F _{2:3} families from the cross K07-1544/PI165673 infested with <i>D. texanus</i> in 2012. Arrows and stars indicate parent and F ₂ means, respectively.....	48
Figure 2.7. Frequency distribution of number of larvae per plant in plants from 108 F _{2:3} families from the cross K07-1544/PI165673 infested with <i>D. texanus</i> in 2012. Arrows and stars indicate parent and F ₂ means, respectively.....	49
Figure A.1. Pattern of damage distribution using the mean of each plant position across 2 cages for numbers of oviposition punctures (a), larvae (b), and Resistance Ratio (c). Plants were	

infested with *D. texanus* in 2011. Bubble size per plant position changes proportionally with smaller or larger means. 59

Figure A.2. Pattern of damage distribution using the mean of each plant position across 2 cages for numbers of oviposition punctures (a), larvae (b), and Resistance Ratio (c). Plants were infested with *D. texanus* in 2011. Bubble size per plant position changes proportionally with smaller or larger means. 60

Figure A.3. Pattern of damage distribution using the mean of each plant position across 14 cages for numbers of oviposition punctures (a), larvae (b), and Resistance Ratio (c). Plants were infested with *D. texanus* in 2012. Bubble size per plant position changes proportionally with smaller or larger means. 61

List of Tables

Table 2.1. Mean \pm SEM resistance ratio, number of oviposition punctures, and number of larvae per plant in F ₂ soybean populations KS5004N/PI165673 and K07-1544/PI165673 infested with <i>D. texanus</i> in 2011.	27
Table 2.2. Broad sense heritability percentages using the σ^2_{cage} and $\sigma^2_{\text{cage*genotype}}$ variance components from the parental plants infested with <i>D. texanus</i> in 2011	28
Table 2.3. Mean \pm SEM resistance ratio, number of oviposition punctures, and numbers of larvae per plant in soybean genotypes K07-1544 and PI165673 infested with <i>D. texanus</i> in 2012.	29
Table 2.4. Mean \pm SEM of larval head capsule width and body length per larvae per plant in soybean genotypes K07-1544 and PI165673 infested with <i>D. texanus</i> in 2012.....	30
Table 2.5. Broad sense heritability percentages using the σ^2_{cage} and $\sigma^2_{\text{cage*genotype}}$ variance components from the parental plants infested with <i>D. texanus</i> in 2012.	31
Table 2.6. Mean \pm SEM resistance ratio, numbers of oviposition punctures, and numbers of larvae per plant in 108 F _{2:3} families from the cross between K07-1544 and PI165673 infested with <i>D. texanus</i> in 2012.....	32

Acknowledgements

I would like to thank my advisor Dr. Michael Smith and my committee members, Dr. William Schapaugh, Dr. Brian McCornack, and Dr. Lawrent Buschman for their assistance and guidance through the development of this research project. Their mentoring and advice have contributed to my formation as a researcher.

I would like to thank all the student workers, friends and my family members who helped me to keep my experiments going in the field and in the lab.

I would like to thank my family for their support in my education, and I would like to thank Dr. Cesar Cardona for teaching me the importance of insects in agriculture and his advice to improve my knowledge and skills in plant resistance to insects.

Dedication

My Grandmother

Chapter 1 - Literature Review

Soybean

Soybean, *Glycine max* (L.) Merrill (Fabales: Fabaceae), is a widely cultivated crop around the world. Products derived from soybean seed are important in the food, vegetable oil and livestock industries. Soybean is grown mainly because of its high protein and oil seed content which are around 40 and 20%, respectively (Nielsen 1996, Wang 2002). Processing of the seed to obtain high protein meal and oil has added value to the soybean as a crop since oils can be used to make other products, such as biodiesel, cooking oil, meat and dairy product substitutes, and soyfeed for livestock (Panthee 2010, Qiu and Chang 2010). The demand for biodiesel and food with high protein content has accelerated the growth of soybean production worldwide and the development of high yielding cultivars (Wilson 2008). Most soybeans are produced in the U. S. A. (33%), followed by Brazil (29%), Argentina (19%) and China (5%), respectively (SOYSTATS 2012).

Although soybean was introduced to the U. S. A. as a forage crop in the 18th century, soybean yield has increased in this country from 16,899 hg/ha in 1961 to 27,910 hg/ha in 2011 (Orf 2010, FAO 2012). This increase in yield is the result of multiple breeding programs that are interested in improving yields, seed composition, pest resistance and tolerance to abiotic stresses (Panthee 2010). Approximately 26% of the world soybean production was lost due to pests between 2001 and 2003, and ~8.8% was attributed to damage caused by animal pests, including insects (Oerke 2006). In 2012, yield losses due to insects were about 5.6% on ~4 million ha of soybean planted in seven U. S. states (Musser et al. 2013). The *Dectes* stem borer, *Dectes texanus* LeConte (Coleoptera: Cerambycidae), is included among the insect pests of soybeans,

and has been recognized as a potential economic pest since 1968 in Missouri (Daugherty and Jackson 1969).

***Dectes* stem borer description and life cycle**

The *Dectes* stem borer, *D. texanus*, is a long-horned beetle and belongs to the order Coleoptera, family Cerambycidae (Dillon 1956). It is commonly known as the *Dectes* stem borer, the soybean stem borer, the sunflower stem borer, and the sunflower stem girdler (Buschman and Sloderbeck 2010). This insect is a native species of North America and is widely distributed from east of the Rocky Mountains through Northern Mexico (Bezark 2010). It has been recorded to inhabit common ragweed (*Ambrosia artemisiifolia* L.), cocklebur (*Xanthium pennsylvanicum* Vallr.), and giant ragweed (*Ambrosia trifida* L.) (Campbell 1980).

D. texanus has one generation per year, complete metamorphosis (Campbell 1980), and an adult activity period ranging between June and August. However, the beginning and end of this period varies between states. In Missouri, activity occurs from late June until mid August, in North Carolina from mid-July until mid-August, and in Kansas from late June until late August (Hatchett et al. 1975, Campbell 1980, Kaczmarek et al. 2001). Adult emergence peaks in mid-June in Tennessee, and in early July in Kansas (Patrick 1973, Kaczmarek et al. 2001).

The *D. texanus* adult is dark brown to black with short gray pubescence and has a body with an elongated and narrow shape that ranges from 6.0-11.0 mm long and 1.6-4.3 mm wide. There are prominent lateral spines near the base of the pronotum and the elytra have erect black setae projecting above the pubescence. The female has a larger body size and shorter antennae than the male. In the pupal stage, only the female has a pair of genital lobes located on the last abdominal sternite. The sex proportion is about a 1:1 ratio, and adults feed for 2 d before mating (Hatchett et al. 1975). However, Patrick (1973) observed mating 5 d after emergence in

Tennessee. Adults mate more than once in their lifetime, but females mate only with the same partner (Patrick 1973). The females lay their eggs 3 d after mating, and each female lays an average of 53 eggs in her lifetime. The female places one egg with her ovipositor in the pith after chewing a hole in the petiole. Successful oviposition depends on presence of the pith and whether or not it can be reached with the ovipositor (Patrick 1973, Hatchett et al. 1975).

Elongate shaped eggs, averaging 1.5 mm in length, are laid mainly in petioles and soft stems, and are shiny-yellow to amber before hatching. The incubation period in the field lasts from 6 to 10 d in Tennessee (Patrick 1973, Hatchett et al. 1975). The first instar larva is yellowish white and averages 1.7 mm long. Mature larvae are yellow to dark brown, slender, slightly curved and average 12 – 15 mm long. In the field, the larva goes through four instars that last 9 to 10 mo, but larvae reared in artificial diet undergo six stages (Hatchett et al. 1973). The larvae are legless, but they have strong protuberant dorsoventral ampullae on the first seven abdominal segments (Hatchett et al. 1973).

The first instar larva feeds on the pith and the interfascicular parenchyma of the petiole for 14 to 21 d. When the pith is depleted, the larva chews into the main stem. As a result of the feeding damage, the petiole wilts, turns black, drops to the ground, and scar tissue is formed around the entrance hole into the stem (Hatchett et al. 1975, Campbell 1980). The larva bores through the stem toward the lower portion of the plant. When the fourth instar larva reaches the base of the plant, it girdles the stem and overwinters in the stubble below ground (Campbell 1980). The larva closes the tunnel in the stem with a frass plug as protection from winter and possible enemies (Campbell and Van Duyn 1977). Although, many eggs are laid in the petioles, only one larva survives in the stubble since *D. texanus* larvae are cannibalistic (Patrick 1973, Hatchett et al. 1975). In mid-June, the overwintered larva becomes active, feeds on woody

stubble tissue, cuts an exit hole for adult emergence, and transforms into a pre-pupa (Hatchett et al. 1975). The pupae are yellow brown, and resemble the size and shape of the adult. The pupal stage lasts 10-15 d followed by an immature adult stage which stays inside the stubble for 1 - 2 d (Patrick 1973). Adults exit the stubble when the integument hardens (Hatchett et al. 1975, Campbell 1980).

Integrated management of *Dectes* stem borer in soybean

Chemical control. Timing and placement of insecticide applications are important factors for their success in decreasing *Dectes* larvae and adult infestations in soybean. In North Carolina, Campbell and Van Duyn (1977) evaluated diazinon, chlorpyrifos, carbofuran, ethoprop, phorate, and fonofos to control overwintering larvae, but these insecticides did not reach the larvae through the stubble, frass plug or stem. The same authors reported that spray formulations of carbaryl, malathion, methomyl, and methyl parathion were capable of controlling adults in field cages. However, the authors considered that the use of insecticides in the field would be limited by the lack of knowledge of annual adult emergence, and by the requirement for multiple insecticide applications.

In Mississippi, Laster et al. (1981) reported that soybean plants treated with eight weekly applications of methyl parathion had lower numbers of *D. texanus* adults than untreated plants, but yields were not different between both treatments. The authors attributed this lack of difference to larval damage since the insecticide did not reach the larvae feeding inside the stem. However, Andrews and Williams (1988) observed yield differences between untreated soybean plants and plants treated with this insecticide, and there was no significant differences in numbers of larvae between treatments. Tindall et al. (2010) reported that the use of insecticides to control other soybean pests reduced *D. texanus* infestations in Mississippi.

In Kansas, Kaczmarek et al. (2002) reported *D. texanus* mortality 24 h after application of low concentrations of the pyrethroid insecticides lambda-cyhalothrin and permethrin in laboratory conditions. When lambda-cyhalothrin was tested in the field, it reduced adult *D. texanus* populations between 67 to 89%, approximately (Sloderbeck and Buschman 2011). However, timed spray applications matching adult emergence and multiple applications may be required to reduce infestations (Sloderbeck et al. 2004, Sloderbeck and Buschman 2011).

Several studies (Buschman et al. 2006, 2007, Davis et al. 2008) have shown that fipronil, a systemic phenyl pyrazole insecticide applied as a soil or seed treatment, reduces *D. texanus* infestations up to 100% and that protected plants yielded 10% more than untreated control plants. Fipronil also controls larvae that have previously tunneled into and reached the main stem before treatment (Buschman et al. 2007, Niide et al. 2008). However, fipronil remains unregistered for use in soybeans (Buschman et al. 2007, Buschman and Sloderbeck 2010). Therefore, soybean resistance to *Dectes* larvae can be another strategy for management of this pest.

Cultural control. Harvesting before lodging occurs has been more practical and effective than the use of insecticides (Hatchett et al. 1975, Campbell 1980). However, constant field monitoring for *D. texanus* infestations is important for this strategy to be effective, and it is even more important when plants are close to maturity (Buschman and Sloderbeck 2010). Burial of soybean stubble, at least 5 cm deep, was also suggested to reduce larval survival, since soil creates a physical barrier for adult emergence (Campbell and Van Duyn 1977). Soil type is a key factor in the success of stubble burial, since hard crust soils and dry conditions reduce adult emergence (Campbell and Van Duyn 1977). However, stubble burial is incompatible with soil conservation and erosion prevention efforts (Buschman and Sloderbeck 2010). The effectiveness

of crop rotation remains uncertain because large areas of cropland planted with soybeans are easy to find by *D. texanus* (Campbell 1980, Buschman and Sloderbeck 2010). However, there is evidence that second crop soybeans have lower *D. texanus* than full season soybeans (Tindall et al. 2010). Another potential cultural control is the use of cultivated sunflowers as a trap crop for *D. texanus* oviposition. Michaud et al. (2007) reported 5% *D. texanus* infestation in a soybean field that was surrounded by cultivated sunflower plants which were 95.8% infested, but the sunflower trap crop was ineffective beyond 200 m of the soybean field.

Biological control. Several hymenopteran and one dipteran parasitoids infest *D. texanus* larvae. Hatchett et al. (1975) reported parasites sampled from larvae collected from giant ragweed which includes hymenopteran insects from the families Braconidae, Pteromalidae and Ichneumonidae. Tindall and Fothergill (2010, 2012) reported *Dolichomitus irritator* (F.) (Hymenoptera: Ichneumonidae) and *Zelia tricolor* Coquillett (Diptera: Tachinidae) parasitizing larvae in soybean stubble. However, there is no information about their efficiency as parasitoids for the development of biological control strategies. Also, no parasitoids have been reported to infest *Dectes* adults.

Host plant resistance. The soybean defense system can play an important role in controlling soybean pests. The identification of resistant cultivars would help to minimize *D. texanus* yield losses. Richardson (1975) screened 618 soybean genotypes for *D. texanus* resistance in North Carolina and found possible resistant sources. However, through 3 yr of consecutive screening, there was no consistency in the percent of infestation of putatively resistant plants. Nevertheless, the author found that *D. texanus* infestation and girdling declined in later maturity cultivars (maturity group V to VII) and in plants with higher lignin content. But

resistance assessment may have been biased since girdling and lodging are affected by plant maturity (senescence).

Laster et al. (1981) sampled plants of six soybean cultivars in the field for *D. texanus* adults and larvae in Mississippi. The authors reported that the cultivar “Tracy” had lower numbers of larvae in the stem than plants of the cultivar “Bragg”, but the same response was not found when adults were collected from the same cultivars. They suggested that “Tracy” could have an antibiotic effect on *D. texanus* larvae while “Bragg” could have antixenosis resistance to the adult. Screening for *D. texanus* resistance in Delaware was unsuccessful in identifying sources of resistance, and recent results indicate that the percentage of infested stems was >50% in screened soybean cultivars in maturity groups 4.7-4.8 (Whalen et al. 2010).

Kaczmarek (2003) evaluated *D. texanus* larval infestation among commercial soybean cultivars in Kansas and detected infestations ranging from 50-68% in irrigated fields and 17-75% in dryland fields. However, there was no consistency in resistance response variables (lodging and girdling) since growing conditions and external factors likely affected the lodging response. There was also no consistent resistance response among cultivars between different localities and environmental conditions (irrigated versus dryland fields).

Niide (2009) also evaluated different Kansas soybean cultivars including plant introductions identified by Richardson (1975) for *D. texanus* resistance. In contrast to previous attempts to assess *D. texanus* larval resistance based on the percentage of larval tunneling, stem girdling, infestation and plant lodging, Niide (2009) used the number of oviposition punctures and the number of live larvae to calculate the ratio: number of oviposition punctures/ number of live larvae (OP/Lv). The OP/Lv resistance ratio was used to explain the number of oviposition

punctures needed to produce live larva in plants of different cultivars and as a correction factor, since cultivars received different numbers of oviposition punctures.

Through four consecutive years of screening, Niide et al. (2012) found a consistent resistance response in PI165673, based on low numbers of live larvae per plant, high numbers of oviposition punctures, and a high OP/Lv ratio compared to the susceptible checks 93M50 and 93M92. The resistance response in PI165673 was similar to the positive antibiosis control which was 93M50-susceptible plants protected with fipronil systemic insecticide. Therefore, the author concluded that PI165673 could be used as a resistant parent in the development of *D. texanus* resistant soybean cultivars.

Genetics of soybean resistance to insect pests.

In. order to develop resistant soybean cultivars efficiently, we need to understand the genetics of the resistance in the soybean plant introduction PI165673. Information about the heritability and the number of genes involved in plant resistance facilitates the design of efficient and accurate breeding strategies to develop resistant cultivars (Fehr 1987, Langridge and Chalmers 2005, Smith 2005). Genetic studies and genetic mapping have been valuable in the incorporation of resistance traits into new soybean cultivars (Komatsu et al. 2010, Oki et al. 2012). Nevertheless, it has been difficult to combine traits for high yield and insect resistance into progeny because most soybean resistance to insect pests has been found in plant introductions that have poor agronomic qualities. Often, resistance genes are linked to genes that do not favor yield performance in the new cultivar compared with the donor parent, or in some cases, the level of resistance in the progeny is less than in the parent (Warrington et al. 2008). The incorporation of multiple insect resistance genes is also difficult to accomplish by classical breeding approaches. However, breeding efforts have been improved with the development of

molecular markers linked to resistance genes and genomic sequencing of crop plants (Boerma and Walker 2005, Parrott et al. 2008).

Genetic studies related to the mode of inheritance, dominance, and the localization of the resistance genes in soybean have been conducted for several soybean insect pests but not for *D. texanus* (Niide 2009). Insect resistance in soybean is controlled with either single or multiple genes in different cultivars. For example, resistance to the soybean aphid, *Aphis glycines* Matsumura, has been reported in the soybean cultivars Dowling, Jackson, PI567543C, PI243540, K1621 and P746, and their resistance is controlled by a single dominant gene (Hill et al. 2006a, b, Kang et al. 2008, Rouf Mian et al. 2008, Meng 2010, Zhang et al. 2010, Xiao et al. 2012). Resistance genes in Dowling and Jackson were mapped to linkage group (LG) M (Li et al. 2007), genes in PI567543C mapped to LG J (Zhang et al. 2010), and genes in PI243540 and K1621 mapped to LG F (Kang et al. 2008, Rouf Mian et al. 2008, Meng 2010). *A. glycines* resistance controlled by two genes has been reported in the cultivars PI567541B, PI567598B, Zhongdou27 and PI567301B (Mensah et al. 2008, Meng et al. 2011, Jun et al. 2012). Two quantitative trait loci (QTLs) in PI567541B were mapped on LGs F and M (Zhang et al. 2009), and QTLs in Zhongdou27 and PI567301B were mapped on different regions in LGs F and A2 (Meng et al. 2011, Jun et al. 2012).

Resistance to the bihar hairy caterpillar, *Spilosoma obliqua* Walker, is controlled by a single incompletely dominant gene in soybean cultivars Ankur, Bragg, Kalitur, and PK-72 (Bhattacharyya and Ram 1995). Also, resistance to the noctuid Egyptian cotton leafworm, *Spodoptera littoralis* Boisduval was controlled by a single incompletely dominant gene in PI171444 (Ojo and Ariyo 1999). Antibiosis resistance to common cutworm, *Spodoptera litura* Fabricius, is controlled by two recessive genes in the soybean cultivar Himeshirazu. Both QTL's

are located on LG M, and both are presumed to contribute to the resistance (Komatsu et al. 2005). QTLs explaining antixenosis resistance to *S. litura* were mapped on LG M in Himeshirazu and on LG H in Fukuyutaka (Oki et al. 2012). Multiple QTLs were reported to control resistance to corn earworm, *Helicoverpa zea* Boddie, in PI227687, PI229358, PI171451, and Cobb, and those contributing the most to resistance were mapped on LGs M, E, B2, G and H (Rector et al. 1998, 2000, Boerma and Walker 2005). Kenty et al. (1996) reported that the resistance to soybean looper, *Pseudoplusia includens* Walker, in D86-3429 was controlled by two genes.

Wang and Gai (2001) reported that resistance to the agromyzid beanfly, *Melanagromyza sojae* Zehntner, is controlled by one major gene along with minor genes in the soybean cultivars JNCWD, WXCJGJ, and 1138-2. Xu et al. (2010) reported that resistance to the whitefly, *Bemisia tabaci* Gennadius, in the cultivar Huapidou is controlled by two major genes and several minor genes. Resistance to *B. tabaci* in the populations Williams79/Cajene and Williams79/Corsoy79 is also quantitative, and QTLs have been mapped in LGs O, H, J, D2, G, L and D1a (Perez-Sackett et al. 2011). Multiple QTLs with additive effects that confer resistance to the bean pyralid, *Lamprosema indicata* Fabricius, in the cultivars NN1138-2 and TSBPHDJ are located on LGs D1a, D1b, C2, H, O and I (Xing et al. 2012).

Most soybean resistance to pests from the order Coleoptera is quantitative i.e. polygenic. Mebrahtu et al. (1990) reported that resistance to Mexican bean beetle, *Epilachna varivestis* Mulsant, in the moderately resistant line MBB 80-115 is controlled by several genes, although exact numbers were not specified. Two or three major genes may be involved in *E. varivestis* resistance in PI229321, PI227687, and PI220358 (Sisson et al. 1976). Rufener et al. (1989) reported that *E. varivestis* resistance in L76-0049, L78-608, and L76-0328 is controlled by more

than two genes and may be additive or partially dominant. Nine QTLs have been reported for resistance to the Japanese beetle, *Popillia japonica* Newman, in an Essex/Forrest population. Seven QTLs from the cultivar Forrest were mapped on LGs A2, N, E, A1, I, F and D2 (Yesudas et al. 2010).

Genetic studies of the number and localization of *D. texanus* resistance genes have not been conducted, as mentioned previously. However, the identification of *D. texanus* resistance in PI165673 (Niide et al. 2012) provides a good candidate for breeding *D. texanus* resistance to improve soybean cultivars adapted to Kansas and other areas affected by this pest. Since soybean resistance to other coleopteran pests is quantitative, we suspect that multiple genes in PI165673 are also involved in *D. texanus* resistance. If this is the case, incorporation of these genes into new genotypes or cultivars may require marker-assisted selection or multiple selection steps to recover high yield and resistance qualities in the same cultivar. Thus, studies to determine the inheritance mode and map of the resistance gene(s) in PI165673 are important for the development of effective and efficient breeding strategies for resistance to *D. texanus*.

Chapter 2 - Resistance in Soybean PI165673 to *Dectes* Stem Borer is Polygenic

Introduction

Soybean, *Glycine max* (L.) Merrill, is an important agricultural crop because of its high protein and oil seed content (Nielsen 1996, Wang 2002). The U. S. A. leads soybean production in the world, and it has increased from 18.5 billion kg in 1961 to 83.2 billion kg in 2011 (FAO 2012). Soybean production and market demand have increased in the last century in response to the need for alternative fuel sources and the need to feed a growing population (Wilson 2008, Qiu and Chang 2010). Approximately 26% of world soybean production was reduced by pests between 2001 and 2003 (Oerke 2006), and soybean yield losses attributed to insects was estimated to be 5.6 % in seven U.S. states in 2012 (Musser et al. 2013).

The *Dectes* stem borer, *Dectes texanus* LeConte (Coleoptera: Cerambycidae), is an insect native to North America (Bezark 2010), and was first reported to infest soybeans in Missouri in 1968 (Daugherty and Jackson 1969). Since then, *D. texanus* has been reported as a pest of soybean and sunflower, *Helianthus annuus* L., in Arkansas, Kansas, Louisiana, Mississippi, North Carolina, Tennessee, Texas and Virginia (Falter 1969, Patrick 1973, Rogers 1977, Buschman and Sloderbeck 2010, Tindall et al. 2010, Musser et al. 2013). *Dectes* infestations up to 45% were reported in ~4 million ha of soybean that were scouted in seven U.S. states in 2012 (Musser et al. 2013).

D. texanus infestations in soybean were isolated cases in Kansas, Mississippi and Missouri in 1985. However, the incidence of the *D. texanus* in previously unreported counties and states may indicate that the infestation distribution is spreading since 1985 to 2008

(Buschman and Sloderbeck 2010, Tindall et al. 2010). The increasing incidence of *D. texanus* could be associated with the expansion of soybean production, reduction of native wild hosts (Campbell 1980), and changes from tillage to non-tillage farming cultivation practices (Buschman and Sloderbeck 2010). These changes in the natural landscape and shifts in agricultural practices may have promoted the increased acceptance of soybean as a host by *D. texanus* (Campbell 1980).

Damage caused by *D. texanus* to the soybean plant occurs when the larva enters the main stem, tunnels to the base of the plant and girdles the stem (Campbell 1980). Crop losses from larval feeding occur when high wind or heavy rain causes the plant to break (lodge) at the girdle point prior to harvest (Hatchett et al. 1973, Patrick 1973, Campbell and Van Duyn 1977, Campbell 1980). Larval feeding causes an estimated seed weight and physiological yield loss of about 10% (Campbell 1980, Buschman et al. 2009). Although adults feed on foliage and petiole tissues, this damage is small compared to the larval feeding damage, and it may not be directly related to yield loss (Hatchett et al. 1975, Campbell 1980).

Crop management and protection against *D. texanus* is important since infestations can reduce yields between 15 to 33% and are increasing in U. S. soybean production areas (Buschman and Sloderbeck 2010). However, few options are available to control *D. texanus* since commercially registered insecticides do not control larval feeding damage. Bifenthrin-zeta-cypermethrin and lambda-cyhalothrin pesticides reduce *D. texanus* adult infestations, but they require multiple applications (FMC 2009, Sloderbeck and Buschman 2011). Early harvesting, field monitoring, and sunflower-trap crop are currently cultural controls recommended to avoid significant borer-related yield losses (Buschman et al. 2006, 2007, Michaud et al. 2007, Davis et al. 2008, Buschman and Sloderbeck 2010). *D. irritator*, *Z. tricolor*, and other hymenopteran

insects parasitize *D. texanus* larvae (Hatchett et al. 1975, Tindall and Fothergill 2010, 2012), but there are no biological control programs currently available with this parasitoids.

Soybean resistance is another alternative to control *D. texanus*, either by repelling adults and larvae, or by reducing larval growth and survival. Soybean plant introduction PI165673 is resistant to *D. texanus* because plants of this genotype sustain significantly fewer surviving larvae than plants of susceptible cultivars (Niide et al. 2012). However, PI165673 is in maturity group VIII, and unsuitable for Kansas growing conditions where soybeans in maturity group IV are more appropriate (Sleper and Poehlman 2006). Nevertheless, PI165673 was used as a parent to transfer resistance into progeny segregating for this resistance to develop Kansas soybean cultivars of high agronomic quality with *D. texanus* resistance.

In order to develop resistant genotypes, information about the inheritance of the resistance in PI165673, and the number of genes controlling the resistance trait(s) is needed to establish appropriate breeding strategies. Therefore, the objective of this research was to determine the inheritance of *D. texanus* resistance in soybean PI165673. Since soybean resistance to other coleopteran pests is quantitative, we suspect that multiple genes in PI165673 are also involved in *D. texanus* resistance. Information provided from this research will benefit soybean breeding programs by identifying *D. texanus* resistant progeny lines that can be improved for agronomic qualities. These lines can also be used to locate resistance gene(s) on the chromosome, and to identify molecular markers linked to the resistance. Ultimately, this research can contribute in the development of new genotypes that can be used to increase yields in areas affected by *D. texanus*.

Materials and Methods

Plant population development

Two populations were created using two soybean *D. texanus*-susceptible genotypes, KS5004N and K07-1544, that were crossed with the *D. texanus* - resistant genotype PI165673 in a winter nursery in Costa Rica. Pollen from PI165673 was transferred to the stigma of emasculated flowers of both susceptible genotypes. F₁ seeds were harvested and selfed to produce the F₂ generation for each cross. Hypocotyl color of F₂ plants fit a 3 purple: 1 green segregation ratio which confirmed that they came from a cross pollinated female plant flower. Plants from this filial generation were evaluated for resistance to the *D. texanus* in summer 2011. Remnant F₂ seed was further advanced to the F₃ generation, but only F_{2:3} families from the cross K07-1544/PI165673 were screened for resistance in summer 2012 because of limited numbers of cages and logistic constrains that did not allow for the evaluation of two different F_{2:3} populations at the same time.

Insect collection

D. texanus adults were collected in Scandia, Abilene, and Ashland Township in Riley County, Kansas from common ragweed patches and from soybean fields. Beetles were collected with sweep nets, bagged in plastic bags, and stored in plastic chests while in transit to Manhattan, Kansas. Samples were kept cool using refrigerant packs. Beetles were counted and released in each cage upon arrival to Manhattan, Kansas. A sample of *D. texanus* adults was deposited in the Kansas State University Museum of Entomological and Prairie Arthropod Research as voucher specimen No. 227.

Screening for resistance to *Dectes* stem borer

Evaluation of F₂ populations

F₁ and F₂ plants from both populations, KS5004N/PI165673 and K07-1544/PI165673, were evaluated for *D. texanus* resistance in a field choice test in 2011. Seeds were hand planted, about 2.5 cm deep, in two 3 x 3 m plots per cross, at the Kansas State University North Agronomy Farm in Manhattan, Kansas. Two plots per population were planted; each plot consisted of four rows, 2.3 m long each, and seed was spaced 5 cm apart. Five F₁, 155 F₂ and 10 seeds of each parent were planted in a completely randomized design per plot. Only 104 and 117 F₂ plants emerged from the KS5004N/PI165673 and K07-1544/PI165673 plots, respectively, due to poor germination, drought, or seedling damage by other arthropods. K07-1544 seeds were sown in the border rows around the plots. Plots were irrigated using sprinkler cans due to lack of rainfall. Plants were caged 5 wk after planting in 3 x 3 m canopy tents (Columbia®, Columbia Sportswear). Canopy roofs and cage side mesh panels were sealed with duct tape; the bottom of the mesh was staked to the ground and buried with soil to prevent beetle escape. *D. texanus* adults were evenly distributed in each cage at a rate of one pair of beetles per plant, and the top petiole on each plant was marked on the plant stem. Plants were cut at soil level 20 d after infestation and stored in a 4°C cold room. Oviposition punctures and larvae on each plant were counted on the five petioles below the infestation mark following recommendations of Niide (2009). With this information, an oviposition puncture/larvae resistance ratio (Niide et al. 2012) was calculated for each plant to evaluate *D. texanus* antibiosis resistance. Resistance ratio can be ≥ 1 , and plants with a ratio of 1 are considered susceptible. To date, plants with ratios greater than 100 have not been reported (Niide 2009). Plants with a resistance ratio of zero were considered missing data because there could have been plants that escaped infestation.

Evaluation of K07-1544/PI165673 F_{2:3} families

In 2012, a field choice test was conducted to evaluate 108 F_{2:3} families and parental checks for *D. texanus* resistance in a randomized complete block design with 14 replicates. Each block (replicate) consisted of a 3 x 3 m plot with four rows, 2.3 m long, and 7.6 cm spacing between seeds. One seed per family and six seeds per parent were planted by hand in each plot about 2.5 cm deep. Seed spacing was 2.6 cm larger than 2011 (5.0 cm) because of the larger number of plants to be sampled in the F_{2:3} families experiment. Plots were located at the Kansas State University North Agronomy Farm in Manhattan, Kansas. Plants in border rows and between plots were K07-1544. Plots were irrigated with sprinklers due to lack of rainfall. Test plants were caged 5 wk after planting in 3 x 3 m canopy tents (Columbia® and Quest®, Quest Outdoors). Canopy roof and mesh were sealed with adhesive and duct tape; the bottom of the mesh was staked and buried with soil to prevent beetle escape. *D. texanus* adults were evenly distributed in each cage at a rate of one pair of beetles per plant at 7 wk after planting. Infestation was delayed because strong winds damaged nine cages, necessitating repair or replacement. Plants were cut at the soil level 20 d after infestation and stored in -20°C and -80°C freezers. Numbers of oviposition punctures, larvae and resistance ratios were calculated for each plant. Larval head capsule width and body length were measured from undamaged larvae collected from K07-1544 and PI165673 plants that were preserved in Pampel's solution, BioQuip Products Inc., (Water 55%, glacial acetic acid 7%, formalin 11%: 37% formaldehyde (4.4%), water (6.6%); and Anhydrol 27%: Ethyl alcohol (21.5%), methyl isobutyl ketone (0.2%), methanol (1.1%), isopropanol (2.4%), water (1.8%)) (BioQuip 2008). Head capsule and body length measurements were made using a Leica® MZ APO and a Nikon® SMZ645 stereomicroscope, respectively. Head capsules were measured across their widest point using the software Leica®

Application Suite V.3.4.0 at 60X. Larval instar was determined based on the head capsule width range described for each *D. texanus* instar by Hatchett et al. (1975).

Statistical analysis

Descriptive statistics were calculated for each variable evaluated in the F_2 generation and the $F_{2:3}$ family experiments. Analyses of variances were performed for each variable that was evaluated on the parent and F_1 plants of each cross in 2011. Analyses of variance were conducted using a generalized mixed model with the $F_{2:3}$ families and parental checks as fixed effects and blocks as random effects for numbers of oviposition punctures, numbers of larvae and the resistance ratio. Statistical analyses were conducted using the PROC GLIMMIX procedure (SAS Institute 2009) with a gamma distribution and a log link function since data were positively skewed and did not follow homogeneity of variances. Degrees of freedom were estimated using the Satterthwaite method (Littell et al. 1996). When the F- test was significant at $P < 0.05$, pairwise comparisons were conducted with a Fisher's protected least significant difference test (LSD) at $\alpha = 0.05$ significance level since the number of possible comparison combinations was large (Milliken and Johnson 2009).

The broad sense heritability was estimated for the variables evaluated among each F_2 plant population and the $F_{2:3}$ families K07-1544/PI165673. The broad sense heritability was estimated as follows: $H^2 = ((\sigma^2_F - \sigma^2_e) / \sigma^2_F) * 100$ where σ^2_F is the phenotypic variance of the F_2 or $F_{2:3}$ plant populations, and σ^2_e is the variance of environmental origin (Allard 1960, Acquaaah 2012). The non-segregant (parental) genotypes were used to calculate the σ^2_e variance among the F_2 and $F_{2:3}$ populations, respectively. Their phenotypic variance was partitioned into their respective variance components ($\sigma^2_{\text{genotype}}$, σ^2_{cage} , $\sigma^2_{\text{cage*genotype}}$, and σ^2_{error}) since there were replicates for each parental genotypes per cage in the experiments (Littell et al. 1996). Only, the

σ^2_{cage} and $\sigma^2_{\text{cage*genotype}}$ were used to calculate the environmental variance. The variances components were calculated using a PROC MIXED procedure where parental genotype and cage were considered as fixed and random effects, respectively (SAS Institute 2009).

Analyses of variances using PROC GLIMMIX with a normal distribution were calculated for mean larval head capsule width and mean body length from larvae collected from K07-1544 and PI165673. A Pearson's chi-square test was calculated to compare the numbers of larvae per instar from K07-1544 and PI165673 plants using the PROC FREQ procedure (SAS Institute 2010).

Results

F₂ populations

Differences in the mean numbers of oviposition punctures, numbers of larvae and the resistance ratios on the susceptible and resistant parents in 2011 were small and non-significant (Table 2.1). The F₁ plants from the cross K07-1544/PI165673 had mean numbers of oviposition punctures significantly higher than either parent (Table 2.1). The frequency distributions of the resistance ratio, oviposition and larval data were continuous, skewed to the right, and extended beyond most of the phenotypic ranges of the parents for both F₂ populations (Fig. 2.1, 2.2 and 2.3, Table 2.2), except for the number of oviposition punctures in KS5004N plants. The broad sense heritability varied from 73.9 to 99.9% and from 96.8 to 99.9% in the KS5004N/PI165673 and K07-1544/PI165673 F₂ populations, respectively. The mean resistance ratio per plant had the lowest percent heritability, and the number of larvae per plant had the highest percent heritability in both F₂ populations (Table 2.2).

K07-1544/PI165673 F_{2:3} families

The differences between K07-1544 and PI165673 for mean number of oviposition punctures, mean number of larvae, mean larval head capsule width and mean larval body length were non-significant (Tables 2.3 and 2.4). There were no significant differences in the percentage of *D. texanus* larvae per instar between these parents (Pearson's χ^2 test $P > 0.05$). Approximately 50% of larvae from plants of both genotypes were in the third instar at the time of sampling (Fig. 2.4). However, the mean resistance ratio was significantly greater in PI165673 plants than in K07-1544 plants (Table 2.3).

The frequency distribution of the $F_{2:3}$ families was continuous for all the phenotypic traits evaluated (Fig. 2.5, 2.6 and 2.7). The broad sense heritability among the families was 68.2% for the resistance ratio, 99.9% for numbers of oviposition punctures, and 99.6% for numbers of larvae (Table 2.5). There were differences between the $F_{2:3}$ families and the parental genotypes for the resistance ratio and for numbers of oviposition punctures ($F = 1.31$, $df = 109$, 967.4 , $P < 0.05$; $F = 1.30$, $df = 109$, 969.5 , $P < 0.05$, respectively). Thirteen $F_{2:3}$ families had higher resistance ratios than the susceptible genotype, K07-1544, and two of these 13 families had higher ratios than the resistant PI165673 genotype (Table 2.6). Eight families had a lower numbers of oviposition punctures than either parental genotype, and one family had a lower number of oviposition punctures than the resistant parent (Table 2.6). Family 146 was the only family with a higher resistance ratio than K07-1544 and with a lower number of oviposition punctures than PI165673. There was no evidence of differences between families and parental genotypes for numbers of larvae per plant ($F = 1.16$, $df = 109$, 972.5 , $P > 0.05$).

Discussion

A significant difference between the resistance ratio of *D. texanus*-susceptible (K07-1544) and resistant (PI165673) parents was detected after the number of plants sampled per genotype was increased in 2012. However, this difference was small, which made it difficult to estimate the numbers of resistance genes and mode of inheritance in the F_2 and $F_{2:3}$ generations using Mendelian phenotypic ratios. Nevertheless, the continuous bell-shaped frequency distribution of the numbers of oviposition punctures, larvae and the resistance ratio data in the F_2 and $F_{2:3}$ generation indicated that more than one gene may be involved in *D. texanus* resistance (Allard 1960). A similar trend was observed in the KS5004N/PI165673 F_2 population. Given these results, QTL mapping will be important for the detection and location of genes contributing to the *D. texanus* resistance in PI165673. However, extreme phenotypic differences between parental genotypes are desirable to locate the QTLs in the genome and quantify their contribution to the phenotype (Alonso-Blanco et al. 2006).

The 68% heritability for the resistance ratio among the $F_{2:3}$ K07-1544/PI165673 population could indicate that progress in breeding for resistance to *D. texanus*, using PI165673, can be achieved by selecting for high resistance ratios. Therefore, the phenotypic differences observed between the $F_{2:3}$ families and the parents may be attributed to contributions from the genetic backgrounds of the parents (Allard 1960). However, the environmental variation (σ^2_e) may have been underestimated since this was calculated using the parental variation (Sleper and Poehlman 2006). The contribution from the F_2 and $F_{2:3}$ family plants to the environmental variation was not included in the σ^2_{cage} and $\sigma^2_{\text{cage*genotype}}$ variance components because there was only one plant per F_2 genotype and $F_{2:3}$ family in each cage. Also, the plant populations were tested in one location. Partitioning of the parental genotypes-variance components could be used

as an approximation of environmental variance in future studies where plants are evaluated in one location or in one environment.

Broad sense heritability estimates were high for the $F_{2:3}$ families, and differences in the resistance ratio were significant between some families and the parents. The statistically higher ratios in thirteen $F_{2:3}$ families can be explained by a combination of complimentary genes or by transgressive segregation (Rieseberg et al. 1999). These families are valuable genetic resources for breeding *D. texanus* because they constitute a new genetic pool for the development of *D. texanus* resistant cultivars (deVicente and Tanksley 1993). Nevertheless, more data are needed to confirm the resistant phenotype since it is possible that the genes contributing to the resistance are affected by as yet unknown environmental factors.

There were no differences in larval head capsule width, body length and proportion of larvae per instars between larvae collected from PI165673 plants or from K07-1544 plants. This lack of difference in growth and development in larvae on the two parents indicates that PI165673 resistance factors that contribute to a reduction in numbers of larvae do not affect larval growth after they initiate feeding in the plant. Thus, more data is needed to know if the PI165673 resistance factors affect: development of the embryo in the egg, initiation of feeding by first instar larvae or altered female oviposition behavior which may result in the absence of an egg inside the oviposition puncture.

Since the larvae surviving in the PI165673 are growing at a normal rate, it is possible that one larva can reach the plant base and weaken the stem. Therefore, future evaluations of soybean resistance to *D. texanus* should include records of larval size, weight, and development rate. It will also be desirable to include resistance genes or factors that slow larval development or negatively affect female oviposition behavior. The combination of these resistance factors in a

cultivar could provide durable and long-term *D. texanus* resistance. Therefore, it will be useful to screen other soybean genotypes to increase genetic resources for *D. texanus* resistance.

Conclusions and Perspectives

The objective of this research was to determine the number of genes and the inheritance of the *D. texanus* resistance in soybean PI165673. Based on the results of experiments conducted in this research, *D. texanus* resistance exhibited by PI165673 is polygenic and may be greatly influenced by the environment. Hence, future breeding efforts will benefit from marker assisted selection to screen and evaluate *D. texanus* resistance in genotypes developed from PI165673. Markers closely linked to genes contributing the most to phenotypic resistance will be useful in transferring only these genes without other genes that may reduce yield performance (Acquaah 2012). Fine QTL mapping will be necessary to locate the resistance genes linked to molecular markers (Collard et al. 2005).

Thirteen F_{2:3} families from the cross K07-1544/PI165673 exhibited antibiosis resistance to *D. texanus*, which shows that antibiosis was inherited in progeny from PI165673. Again, QTL mapping will be necessary to detect alleles or genomic regions from K07-1544 that contribute to resistance in families with greater resistance than the resistant parent (deVicente and Tanksley 1993). These families can be further advanced to confirm and select for *D. texanus* resistance, and tested for improved agronomic qualities, so new cultivars can be deployed in Kansas growing areas affected by this pest.

The resistant parent, PI165673, had lower numbers of *D. texanus* larvae than the K07-1544 based on the resistance ratio, but surviving larvae in PI165673 plants may develop and damage stems before harvest. Thus, the PI165673 resistance factor may need to be accompanied by another resistance factor(s) that inhibits or reduces larval development to provide effective control of this pest. However, information is needed about the factor(s) responsible for reducing larval numbers and when these factors are expressed in PI165673 plants. PI165673 resistance

may affect *D. texanus* embryos, delay the initiation of feeding by first instar larvae, or have no effect on some larvae that overcome these resistance factors. More information about *D. texanus* development in PI165673 and other soybean genotypes is also needed to determine if larval, pupal or adult development is delayed on these genotypes, or if adult population emerging from this plants are adversely effected on the in the next season.

Table 2.1. Mean \pm SEM resistance ratio, number of oviposition punctures, and number of larvae per plant in F₂ soybean populations KS5004N/PI165673 and K07-1544/PI165673 infested with *D. texanus* in 2011.

F ₂ population	Genoytpe	n	Resistance Ratio		No. of Oviposition Punctures		No. of Larvae	
			Mean \pm SEM	Range	Mean \pm SEM	Range	Mean \pm SEM	Range
KS5004N/PI165673 ^a	KS5004N	18	2.1 \pm 0.3 a	1.0 - 4.4	7.7 \pm 1.4 a	1.0 - 22.0	3.3 \pm 0.4 a	1.0 - 5.0
	PI165673	6	2.6 \pm 0.4 a	1.3 - 4.0	8.5 \pm 1.4 a	4.0 - 11.0	3.7 \pm 0.7 a	1.0 - 6.0
	F ₁	2	2.0 \pm 0.1 a	1.9 - 2.0	9.5 \pm 3.5 a	6.0 - 13.0	5.0 \pm 2.0 a	3.0 - 7.0
K07-1544/PI165673 ^b	K07-1544	8	1.2 \pm 0.3 a	1.0 - 3.0	3.4 \pm 0.9 a	1.0 - 7.0	2.5 \pm 0.7 a	1.0 - 5.0
	PI165673	6	1.2 \pm 0.1 a	1.0 - 1.5	3.7 \pm 0.7 a	1.0 - 6.0	3.0 \pm 0.6 a	1.0 - 5.0
	F ₁	5	1.7 \pm 0.1 a	1.4 - 2.0	6.8 \pm 1.2 b	4.0 - 11.0	4.0 \pm 0.7 a	2.0 - 6.0

n= Number of plants,

a: ANOVA on data testing for differences between genotypes, for resistance ratio: $F = 0.15$, $df = 2, 22.13$, $P > 0.05$; for numbers of oviposition punctures: $F = 0.17$, $df = 2, 22.23$, $P > 0.05$, and for numbers of larvae: $F = 0.93$, $df = 2, 23$, $P > 0.05$.

b: ANOVA on data testing for differences between genotypes, for resistance ratio: $F = 0.80$, $df = 2, 3.14$, $P > 0.05$; for numbers of oviposition punctures: $F = 3.76$, $DF = 2, 16$, $P < 0.05$, and for numbers of larvae: $F = 1.21$, $df = 2, 16$, $P > 0.05$.

Means followed by a different lower case letter within a column for each cross are statistically different based on a Fisher's protected LSD ($P < 0.05$) means separation test.

Table 2.2. Broad sense heritability percentages using the σ^2_{cage} and $\sigma^2_{\text{cage*genotype}}$ variance components from the parental plants infested with *D. texanus* in 2011

Phenotypic trait	σ^2 F ₂ plants	Parental genotypes		H ²
		σ^2 cage	σ^2 cage*genotype	
KS5004N/PI165673 ^a				
Resistance Ratio	1.2	0.6	0.0	73.9
No.Oviposition Punctures	25.6	10.2	0.0	80.1
No. Larvae	3.0	2.7 x 10 ⁻¹⁸	0.0	99.9
K07-1544/PI165673 ^b				
Resistance Ratio	1.6	0.1	0.0	96.8
No.Oviposition Punctures	12.1	0.1	0.0	99.6
No. Larvae	2.5	0.1	0.0	99.9

Parental genotypes: Non- segregants genotypes (Susceptible and Resistant parent, and F1 plants).

H²: Broad sense heritability, $H^2 = ((\sigma^2_{F2} - \sigma^2_e) / \sigma^2_{F2}) * 100$, where σ^2_{F2} is the phenotypic variance of the F₂ plants, and

$\sigma^2_e = (\sigma^2_{\text{cage}} + \sigma^2_{\text{cage*genotype}}) / 3$ is the variance of environmental origin.

a : F₂ population

b : F₂ population

Table 2.3. Mean \pm SEM resistance ratio, number of oviposition punctures, and numbers of larvae per plant in soybean genotypes K07-1544 and PI165673 infested with *D. texanus* in 2012.

Genotype	n	Resistance Ratio		No. of Oviposition Punctures		No. of Larvae	
		Mean \pm SEM	Range	Mean \pm SEM	Range	Mean \pm SEM	Range
K07-1544	68	1.7 \pm 0.1 a	1.0 - 3.3	10.3 \pm 1.1 a	1.0 - 52.0	6.1 \pm 0.6 a	1.0 - 28.0
PI165673	36	2.1 \pm 0.2 b	1.0 - 5.0	10.8 \pm 1.4 a	1.0 - 32.0	5.4 \pm 0.6 a	1.0 - 15.0

n: number of plants,

ANOVA on data testing for differences between genotypes, for resistance ratio $F = 4.37$, $df = 1, 103$, $P < 0.05$, for numbers of oviposition punctures: $F = 0.08$, $df = 1, 102$, $P > 0.05$, and for numbers of larvae: $F = 1.11$, $df = 1, 103$, $P > 0.05$.

Means followed by a different lower case letter within a column are statistically different based on a Fisher's protected LSD ($P < 0.05$) means separation test.

Table 2.4. Mean \pm SEM of larval head capsule width and body length per larvae per plant in soybean genotypes K07-1544 and PI165673 infested with *D. texanus* in 2012.

Genotype	Larval Head Capsule Width (mm)			Larval Body Length (mm)		
	n	Mean \pm SEM	Range	n	Mean \pm SEM	Range
K07-1544	62	0.8 \pm 0.03 a	0.4 - 1.3	60	7.0 \pm 0.5 a	2.5 - 11.3
PI165673	34	0.8 \pm 0.03 a	0.4 - 1.4	32	7.7 \pm 0.6 a	2.0 -15.0

n: number of plants,

ANOVA on data testing for differences between genotypes, for larval head capsule width: $F = 0.32$, $df = 1$, 85.1, $P > 0.05$, and for larval body length: $F = 1.34$, $df = 1$, 12.4, $P > 0.05$. Means followed by the same lower case letter within a column are not statistically different based on F test.

Table 2.5. Broad sense heritability percentages using the σ^2_{cage} and $\sigma^2_{\text{cage*genotype}}$ variance components from the parental plants infested with *D. texanus* in 2012.

Phenotypic trait	σ^2 F _{2:3} families	Parental genotypes		H ²
		σ^2_{cage}	$\sigma^2_{\text{cage*genotype}}$	
Resistance Ratio	0.1	0.07	0.0	68.2
No.Oviposition Punctures	6.0	2.5×10^{-17}	0.0	99.9
No. Larvae	1.3	0.01	0.0	99.6

Parental genotypes: Non – segregant genotypes (Susceptible and resistant parents).

H²: Broad sense heritability, $H^2 = ((\sigma^2_{F_{2:3}} - \sigma^2_e) / \sigma^2_{F_{2:3}}) * 100$, where $\sigma^2_{F_{2:3}}$ is the phenotypic variance of the F_{2:3} families, and $\sigma^2_e = (\sigma^2_{\text{cage}} + \sigma^2_{\text{cage*genotype}}) / 2$ is the variance of environmental origin.

Table 2.6. Mean \pm SEM resistance ratio, numbers of oviposition punctures, and numbers of larvae per plant in 108 F_{2:3} families from the cross between K07-1544 and PI165673 infested with *D. texanus* in 2012.

F _{2:3} family	n	Resistance Ratio			No. of Oviposition Punctures			No. of Larvae
		Comparison against			Comparison against			Mean \pm SEM
		K07-1544		PI165673	K07-1544		PI165673	
		Mean \pm SEM	<i>P</i> - value	<i>P</i> - value	Mean \pm SEM	<i>P</i> - value	<i>P</i> - value	
129	5	1.2 \pm 0.2	ns	**	5.2 \pm 1.6	*	*	4.6 \pm 1.3
157	7	1.2 \pm 0.2	ns	**	8.1 \pm 2.1	ns	ns	6.1 \pm 1.4
189	9	1.3 \pm 0.2	ns	**	8.1 \pm 1.8	ns	ns	6.7 \pm 1.4
73	7	1.4 \pm 0.2	ns	*	10.2 \pm 2.6	ns	ns	7.5 \pm 1.8
84	6	1.4 \pm 0.3	ns	*	6.7 \pm 1.9	ns	ns	4.3 \pm 1.1
166	9	1.5 \pm 0.2	ns	*	9.2 \pm 2.1	ns	ns	6.3 \pm 1.3
183	6	1.5 \pm 0.3	ns	ns	8.1 \pm 2.3	ns	ns	4.7 \pm 1.1
20	10	1.5 \pm 0.2	ns	ns	5.3 \pm 1.2	**	**	3.4 \pm 0.7
88	8	1.5 \pm 0.2	ns	ns	9.2 \pm 2.2	ns	ns	5.9 \pm 1.3
184	7	1.6 \pm 0.3	ns	ns	6.4 \pm 1.7	ns	ns	4.2 \pm 1.0

Table 2.6. Continuation

		Resistance Ratio			No. of Oviposition punctures			No. of Larvae
F _{2:3} family	n	Comparison against			Comparison against			
		K07-1544		PI165673	K07-1544		PI165673	
		Mean ± SEM	P - value	P - value	Mean ± SEM	P - value	P - value	
45	9	1.6 ± 0.2	ns	ns	8.4 ± 1.9	ns	ns	5.0 ± 1.0
72	5	1.6 ± 0.3	ns	ns	6.9 ± 2.1	ns	ns	4.3 ± 1.2
51	8	1.6 ± 0.2	ns	ns	5.1 ± 1.3	**	**	3.2 ± 0.7
46	8	1.6 ± 0.2	ns	ns	8.8 ± 2.1	ns	ns	5.7 ± 1.2
118	10	1.6 ± 0.2	ns	ns	11.7 ± 2.6	ns	ns	7.0 ± 1.4
31	9	1.6 ± 0.2	ns	ns	11.7 ± 2.7	ns	ns	6.9 ± 1.4
54	13	1.6 ± 0.2	ns	ns	10.8 ± 2.1	ns	ns	7.2 ± 1.3
11	11	1.6 ± 0.2	ns	ns	9.8 ± 2.1	ns	ns	5.9 ± 1.1
179	11	1.6 ± 0.2	ns	ns	7.4 ± 1.6	ns	ns	4.7 ± 0.9
6	9	1.6 ± 0.2	ns	ns	9.6 ± 2.2	ns	ns	5.8 ± 1.1
193	10	1.6 ± 0.2	ns	ns	9.9 ± 2.2	ns	ns	5.8 ± 1.1

Table 2.6. Continuation

		Resistance Ratio			No. of Oviposition punctures			No. of Larvae
F _{2:3} family	n	Comparison against			Comparison against			Mean ± SEM
		K07-1544	PI165673		K07-1544	PI165673		
		Mean ± SEM	<i>P</i> - value	<i>P</i> - value	Mean ± SEM	<i>P</i> - value	<i>P</i> - value	
35	8	1.7 ± 0.3	ns	ns	10.5 ± 2.5	ns	ns	7.0 ± 1.5
60	9	1.7 ± 0.2	ns	ns	7.6 ± 1.8	ns	ns	4.2 ± 0.9
124	13	1.7 ± 0.2	ns	ns	8.7 ± 1.7	ns	ns	5.4 ± 1.0
47	10	1.7 ± 0.2	ns	ns	11.1 ± 2.4	ns	ns	6.4 ± 1.2
153	10	1.7 ± 0.2	ns	ns	10.4 ± 2.3	ns	ns	6.2 ± 1.2
8	9	1.7 ± 0.2	ns	ns	6.8 ± 1.6	ns	ns	4.3 ± 0.9
145	9	1.7 ± 0.2	ns	ns	8.7 ± 2.0	ns	ns	5.6 ± 1.2
57	7	1.7 ± 0.3	ns	ns	9.1 ± 2.4	ns	ns	5.8 ± 1.4
50	11	1.7 ± 0.2	ns	ns	11.4 ± 2.3	ns	ns	7.3 ± 1.4
163	9	1.7 ± 0.2	ns	ns	11.7 ± 2.7	ns	ns	6.7 ± 1.4
78	3	1.7 ± 0.4	ns	ns	9.5 ± 3.7	ns	ns	5.3 ± 1.8

Table 2.6. Continuation

		Resistance Ratio			No. of Oviposition punctures			No. of Larvae
F _{2:3} family	n	Comparison against			Comparison against			
		K07-1544	PI165673		K07-1544	PI165673		
		Mean ± SEM	<i>P</i> - value	<i>P</i> - value	Mean ± SEM	<i>P</i> - value	<i>P</i> - value	Mean ± SEM
111	4	1.7 ± 0.4	ns	ns	5.1 ± 1.7	*	*	2.9 ± 0.9
79	6	1.8 ± 0.3	ns	ns	7.4 ± 2.1	ns	ns	4.9 ± 1.2
125	11	1.8 ± 0.2	ns	ns	7.5 ± 1.6	ns	ns	4.2 ± 0.8
92	10	1.8 ± 0.2	ns	ns	10.6 ± 2.3	ns	ns	6.1 ± 1.2
41	11	1.8 ± 0.2	ns	ns	12.0 ± 2.5	ns	ns	7.0 ± 1.3
173	12	1.8 ± 0.2	ns	ns	12.2 ± 2.5	ns	ns	7.0 ± 1.3
7	12	1.8 ± 0.2	ns	ns	9.1 ± 1.8	ns	ns	5.0 ± 0.9
130	7	1.8 ± 0.3	ns	ns	14.9 ± 3.9	ns	ns	8.4 ± 2.0
13	12	1.8 ± 0.2	ns	ns	10.2 ± 2.1	ns	ns	5.4 ± 1.0
160	9	1.8 ± 0.3	ns	ns	11.3 ± 2.6	ns	ns	6.2 ± 1.3
86	9	1.8 ± 0.3	ns	ns	6.0 ± 1.4	*	*	3.7 ± 0.8

Table 2.6. Continuation

F_{2:3} family	n	Resistance Ratio			No. of Oviposition punctures			No. of Larvae
		Comparison against			Comparison against			Mean ± SEM
		K07-1544		PI165673	K07-1544		PI165673	
		Mean ± SEM	P - value	P - value	Mean ± SEM	P - value	P - value	
182	8	1.8 ± 0.3	ns	ns	11.5 ± 2.8	ns	ns	6.5 ± 1.4
22	8	1.8 ± 0.3	ns	ns	8.2 ± 2.0	ns	ns	4.6 ± 1.0
15	14	1.8 ± 0.2	ns	ns	10.0 ± 1.9	ns	ns	5.2 ± 0.9
137	9	1.8 ± 0.3	ns	ns	11.6 ± 2.7	ns	ns	6.4 ± 1.3
139	10	1.8 ± 0.3	ns	ns	10.2 ± 2.3	ns	ns	5.9 ± 1.2
135	10	1.8 ± 0.3	ns	ns	12.2 ± 2.7	ns	ns	6.3 ± 1.2
121	8	1.8 ± 0.3	ns	ns	8.5 ± 2.0	ns	ns	5.2 ± 1.1
126	8	1.9 ± 0.3	ns	ns	10.9 ± 2.7	ns	ns	6.3 ± 1.4
1	9	1.9 ± 0.3	ns	ns	11.4 ± 2.6	ns	ns	5.5 ± 1.1
172	11	1.9 ± 0.2	ns	ns	13.3 ± 2.8	ns	ns	7.7 ± 1.5
147	9	1.9 ± 0.3	ns	ns	10.8 ± 2.5	ns	ns	5.9 ± 1.2

Table 2.6. Continuation

F_{2:3} family	n	Resistance Ratio			No. of Oviposition punctures			No. of Larvae
		Comparison against			Comparison against			
		K07-1544		PI165673	K07-1544		PI165673	
		Mean ± SEM	P - value	P - value	Mean ± SEM	P - value	P - value	
56	11	1.9 ± 0.2	ns	ns	6.7 ± 1.4	*	*	3.5 ± 0.7
176	10	1.9 ± 0.3	ns	ns	11.7 ± 2.6	ns	ns	6.2 ± 1.2
98	10	1.9 ± 0.3	ns	ns	8.7 ± 1.9	ns	ns	5.2 ± 1.0
24	7	1.9 ± 0.3	ns	ns	8.7 ± 2.2	ns	ns	4.9 ± 1.2
64	13	1.9 ± 0.2	ns	ns	11.6 ± 2.3	ns	ns	6.1 ± 1.1
104	7	1.9 ± 0.3	ns	ns	10.3 ± 2.7	ns	ns	5.0 ± 1.2
30	10	1.9 ± 0.3	ns	ns	10.4 ± 2.3	ns	ns	5.4 ± 1.1
66	8	1.9 ± 0.3	ns	ns	10.5 ± 2.6	ns	ns	5.7 ± 1.3
133	11	1.9 ± 0.3	ns	ns	6.5 ± 1.4	*	*	3.6 ± 0.7
158	9	2.0 ± 0.3	ns	ns	9.5 ± 2.2	ns	ns	5.1 ± 1.1
82	7	2.0 ± 0.3	ns	ns	7.5 ± 1.9	ns	ns	3.4 ± 0.8

Table 2.6. Continuation

F_{2:3} family	n	Resistance Ratio			No. of Oviposition punctures			No. of Larvae
		Comparison against			Comparison against			Mean ± SEM
		K07-1544		PI165673	K07-1544		PI165673	
		Mean ± SEM	P - value	P - value	Mean ± SEM	P - value	P - value	
168	8	2.0 ± 0.3	ns	ns	11.7 ± 2.8	ns	ns	5.5 ± 1.2
27	9	2.0 ± 0.3	ns	ns	6.7 ± 1.5	ns	*	4.3 ± 0.9
144	13	2.0 ± 0.2	ns	ns	12.9 ± 2.5	ns	ns	7.1 ± 1.3
108	10	2.0 ± 0.3	ns	ns	8.8 ± 1.9	ns	ns	5.2 ± 1.0
191	10	2.0 ± 0.3	ns	ns	13.2 ± 2.9	ns	ns	6.8 ± 1.4
154	13	2.0 ± 0.2	ns	ns	10.3 ± 2.0	ns	ns	5.2 ± 0.9
116	12	2.0 ± 0.3	ns	ns	12.4 ± 2.5	ns	ns	6.0 ± 1.1
28	9	2.0 ± 0.3	ns	ns	10.1 ± 2.3	ns	ns	4.8 ± 1.0
162	11	2.0 ± 0.3	ns	ns	10.1 ± 2.1	ns	ns	5.2 ± 1.0
4	10	2.0 ± 0.3	ns	ns	13.0 ± 2.9	ns	ns	6.3 ± 1.3
32	8	2.0 ± 0.3	ns	ns	10.6 ± 2.6	ns	ns	6.2 ± 1.4

Table 2.6. Continuation

F_{2:3} family	n	Resistance Ratio			No. of Oviposition punctures			No. of Larvae
		Comparison against			Comparison against			Mean ± SEM
		K07-1544		PI165673	K07-1544		PI165673	
		Mean ± SEM	P - value	P - value	Mean ± SEM	P - value	P - value	
9	9	2.1 ± 0.3	ns	ns	10.5 ± 2.4	ns	ns	5.5 ± 1.2
174	10	2.1 ± 0.3	ns	ns	12.7 ± 2.8	ns	ns	6.0 ± 1.2
114	9	2.1 ± 0.3	ns	ns	6.9 ± 1.6	ns	ns	3.4 ± 0.7
119	9	2.1 ± 0.3	ns	ns	9.8 ± 2.3	ns	ns	5.0 ± 1.0
178	8	2.1 ± 0.3	ns	ns	11.2 ± 2.7	ns	ns	6.3 ± 1.4
68	7	2.1 ± 0.3	ns	ns	15.7 ± 4.1	ns	ns	7.9 ± 1.8
192	10	2.1 ± 0.3	ns	ns	15.1 ± 3.3	ns	ns	6.4 ± 1.3
38	9	2.2 ± 0.3	ns	ns	12.9 ± 3.0	ns	ns	7.2 ± 1.5
151	10	2.2 ± 0.3	ns	ns	12.4 ± 2.7	ns	ns	5.7 ± 1.1
70	10	2.2 ± 0.3	ns	ns	11.3 ± 2.5	ns	ns	5.2 ± 1.0
152	6	2.2 ± 0.4	ns	ns	14.3 ± 4.0	ns	ns	6.6 ± 1.7

Table 2.6. Continuation

F_{2:3} family	n	Resistance Ratio			No. of Oviposition punctures			No. of Larvae
		Comparison against			Comparison against			Mean \pm SEM
		K07-1544		PI165673	K07-1544		PI165673	
		Mean \pm SEM	P - value	P - value	Mean \pm SEM	P - value	P - value	
33	11	2.2 \pm 0.3	ns	ns	9.7 \pm 2.1	ns	ns	4.4 \pm 0.8
97	7	2.3 \pm 0.4	ns	ns	9.6 \pm 2.5	ns	ns	5.1 \pm 1.2
42	10	2.3 \pm 0.3	ns	ns	7.2 \pm 1.6	ns	ns	3.6 \pm 0.7
12	10	2.3 \pm 0.3	ns	ns	12.6 \pm 2.8	ns	ns	6.0 \pm 1.2
106	9	2.3 \pm 0.3	ns	ns	12.2 \pm 2.8	ns	ns	5.0 \pm 1.1
19	7	2.3 \pm 0.4	ns	ns	13.4 \pm 3.5	ns	ns	6.5 \pm 1.5
167	7	2.3 \pm 0.4	ns	ns	7.8 \pm 2.0	ns	ns	4.0 \pm 0.9
101	12	2.3 \pm 0.3	*	ns	13.4 \pm 2.7	ns	ns	6.1 \pm 1.1
146	10	2.3 \pm 0.3	*	ns	5.8 \pm 1.3	*	**	3.4 \pm 0.7
25	12	2.3 \pm 0.3	*	ns	11.9 \pm 2.4	ns	ns	5.5 \pm 1.0
102	9	2.3 \pm 0.3	*	ns	13.5 \pm 3.1	ns	ns	6.1 \pm 1.3

Table 2.6. Continuation

F_{2:3} family	n	Resistance Ratio			No. of Oviposition punctures			No. of Larvae
		Comparison against			Comparison against			Mean ± SEM
		K07-1544		PI165673	K07-1544		PI165673	
		Mean ± SEM	P - value	P - value	Mean ± SEM	P - value	P - value	
67	5	2.4 ± 0.5	ns	ns	6.7 ± 2.0	ns	ns	3.9 ± 1.1
100	10	2.4 ± 0.3	*	ns	15.5 ± 3.4	ns	ns	6.6 ± 1.3
132	7	2.4 ± 0.4	*	ns	12.3 ± 3.2	ns	ns	5.7 ± 1.3
122	11	2.5 ± 0.3	**	ns	10.3 ± 2.2	ns	ns	4.6 ± 0.9
44	7	2.6 ± 0.4	*	ns	10.5 ± 2.7	ns	ns	4.7 ± 1.1
161	7	2.6 ± 0.4	*	ns	11.8 ± 3.1	ns	ns	5.9 ± 1.4
52	7	2.7 ± 0.4	**	ns	14.6 ± 3.8	ns	ns	5.8 ± 1.4
164	10	2.7 ± 0.4	**	ns	12.2 ± 2.7	ns	ns	5.4 ± 1.1
95	13	2.7 ± 0.3	**	*	13.8 ± 2.7	ns	ns	6.3 ± 1.1
185	9	2.9 ± 0.4	**	*	11.3 ± 2.6	ns	ns	4.7 ± 1.0

F_{2:3} family: Family identification number,

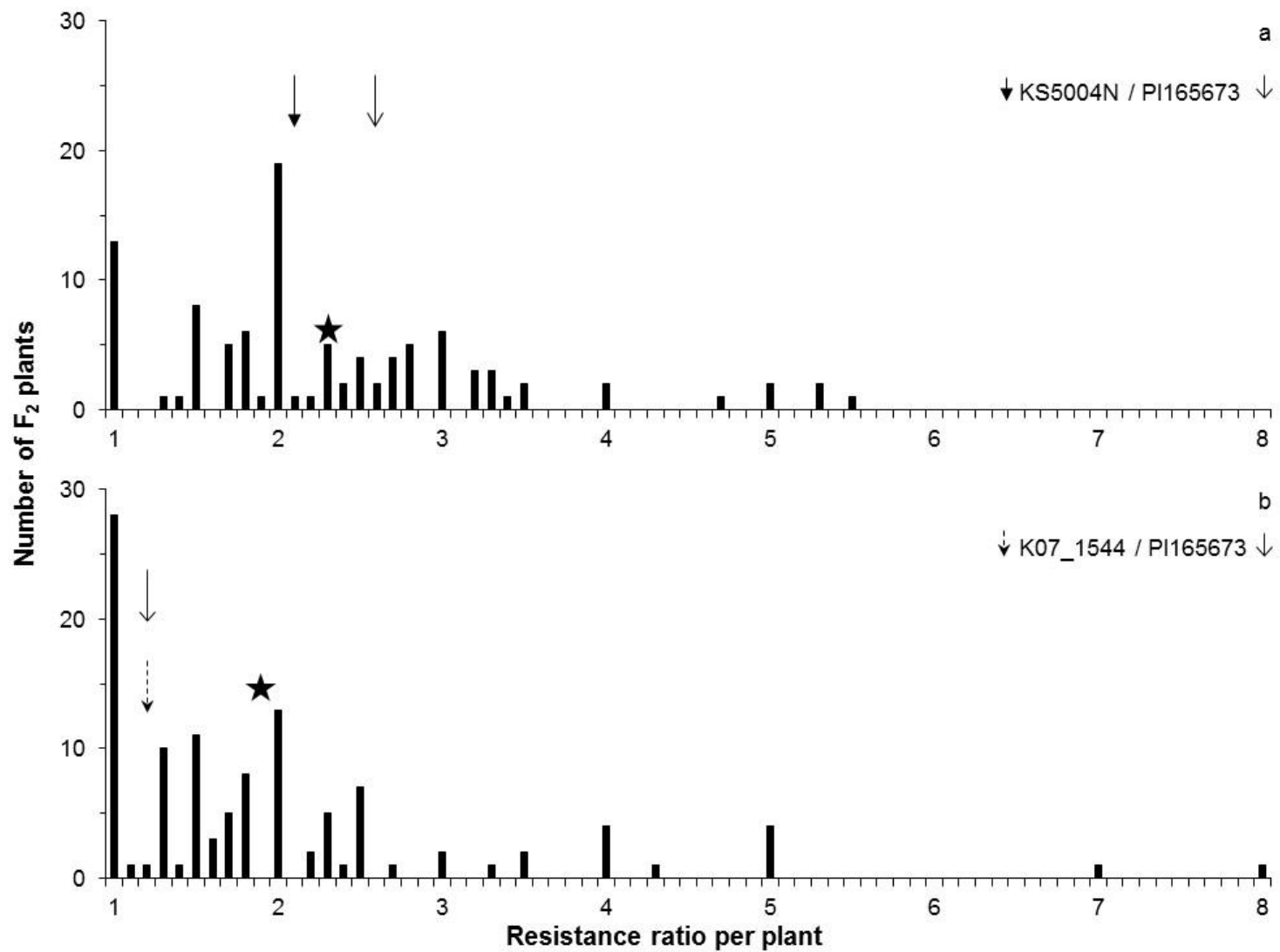
n: number of F₃ plants per family,

Table 2.6. Continuation

ns= not significant,

*= significant at 5% level,

**= significant at 1% level



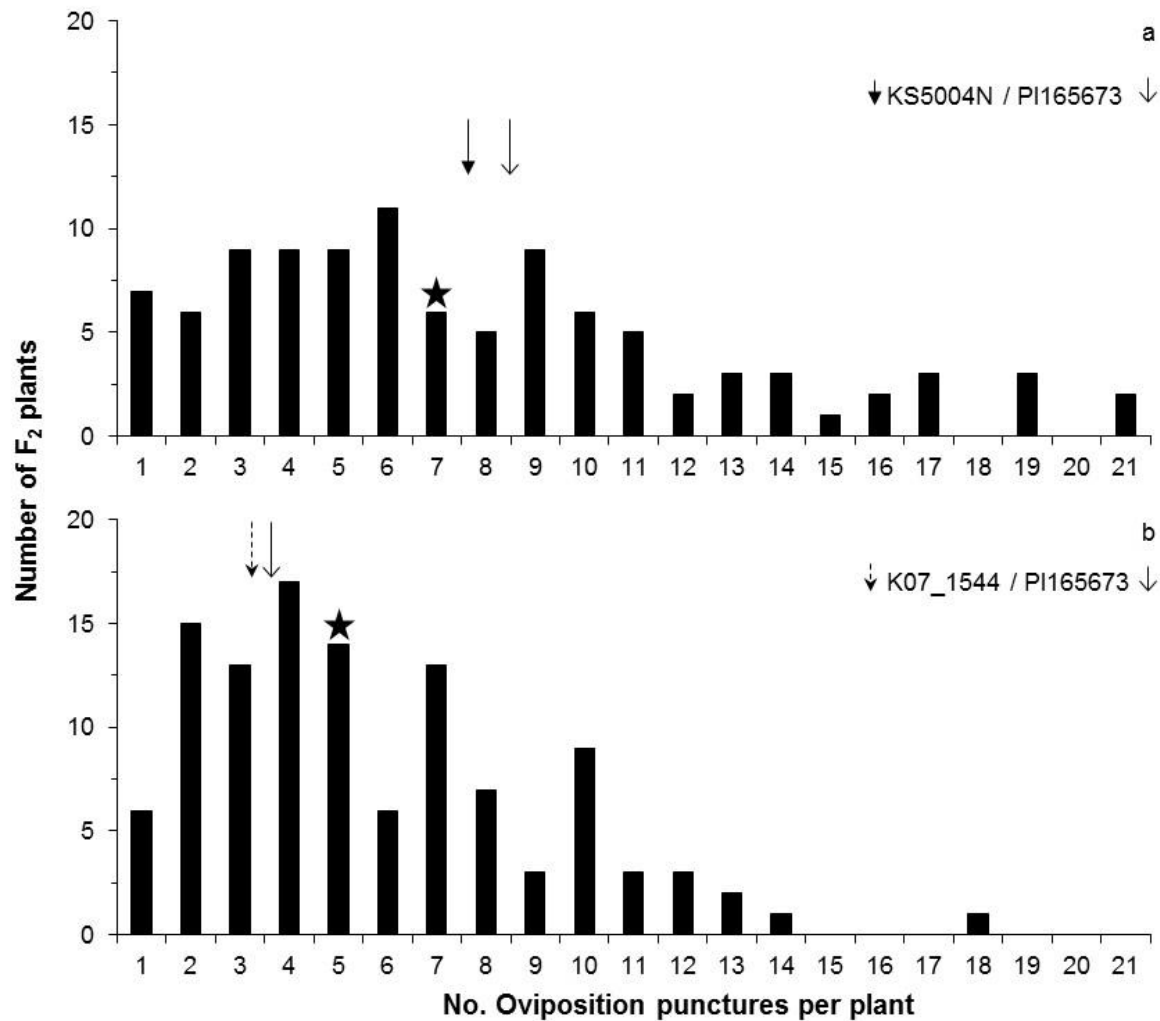


Figure 2.2. Frequency distributions of number of oviposition punctures per plant in F₂ soybean populations

KS5004N/PI165673 (a) and K07-1544/PI165673 (b), infested with *D. texanus* in 2011. Arrows and stars indicate parent and F₂ means, respectively.

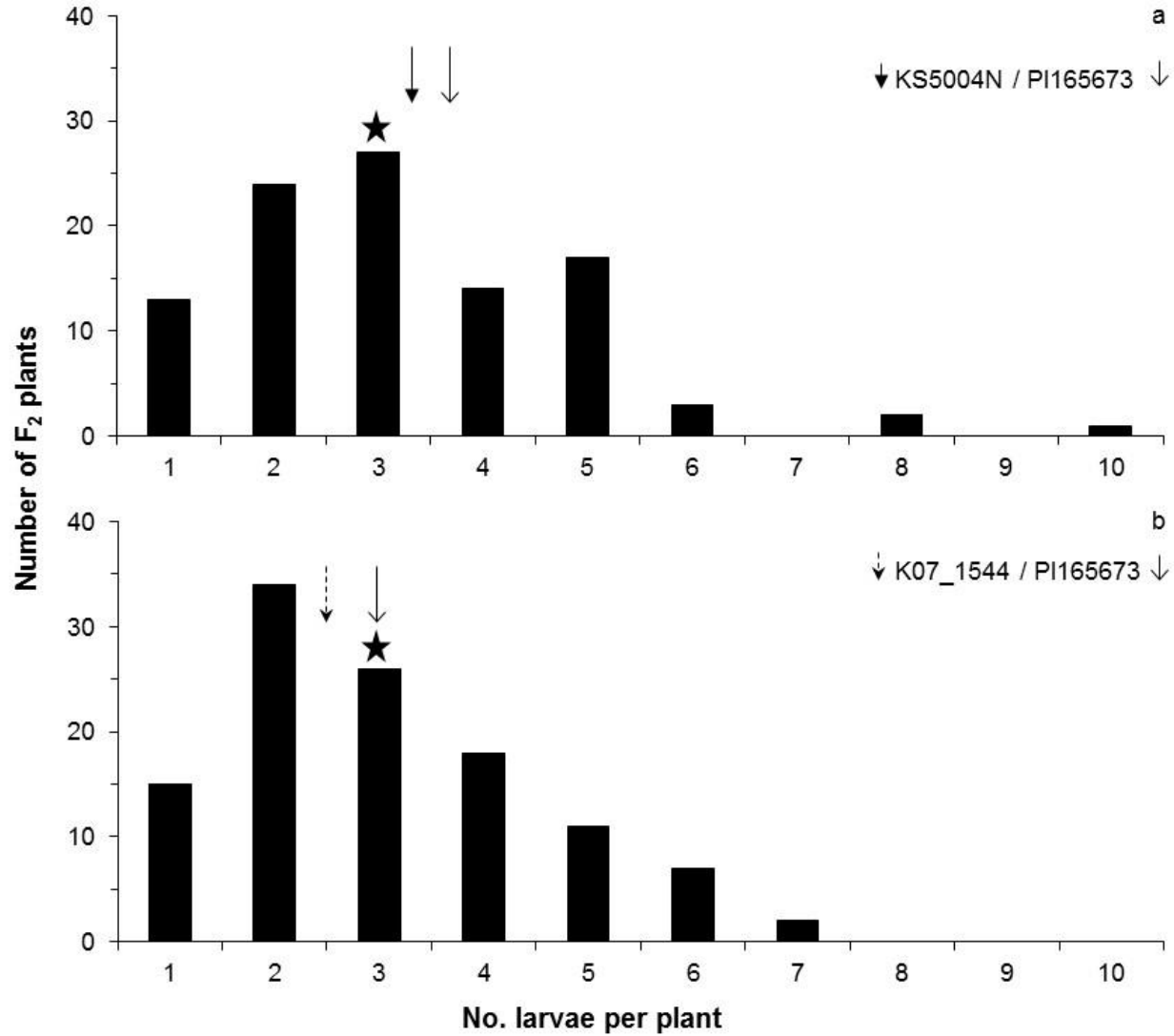


Figure 2.3. Frequency distributions of number of larvae per plant in F₂ soybean populations from KS5004N/PI165673 (a) and K07-1544/PI165673 (b), infested with *D. texanus* in 2011. Arrows and stars indicate parent and F₂ means, respectively.

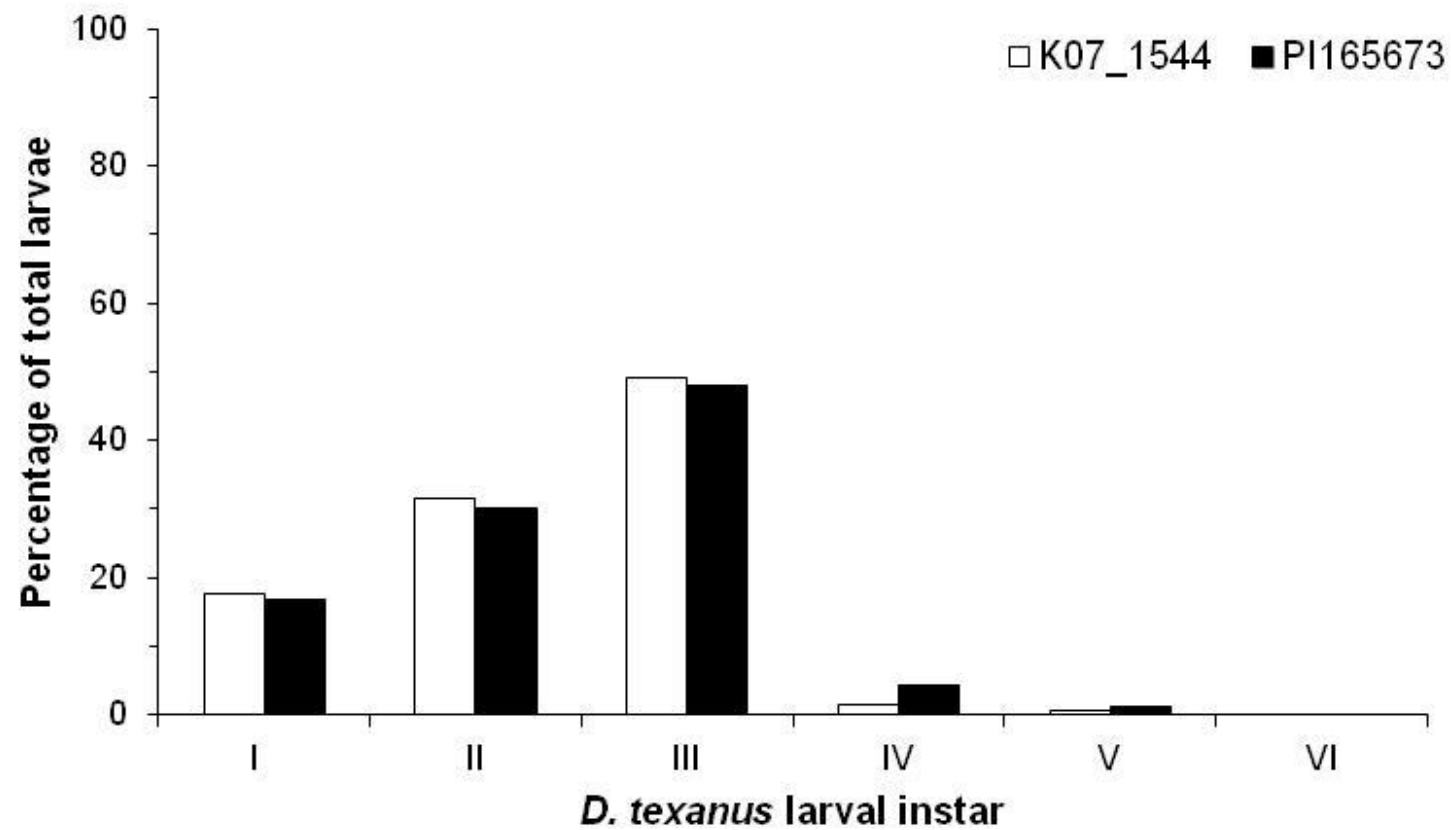


Figure 2.4. Percentage of total *D. texanus* larvae per instar collected from plants in soybean genotypes K07-1544 and PI165673 in 2012.

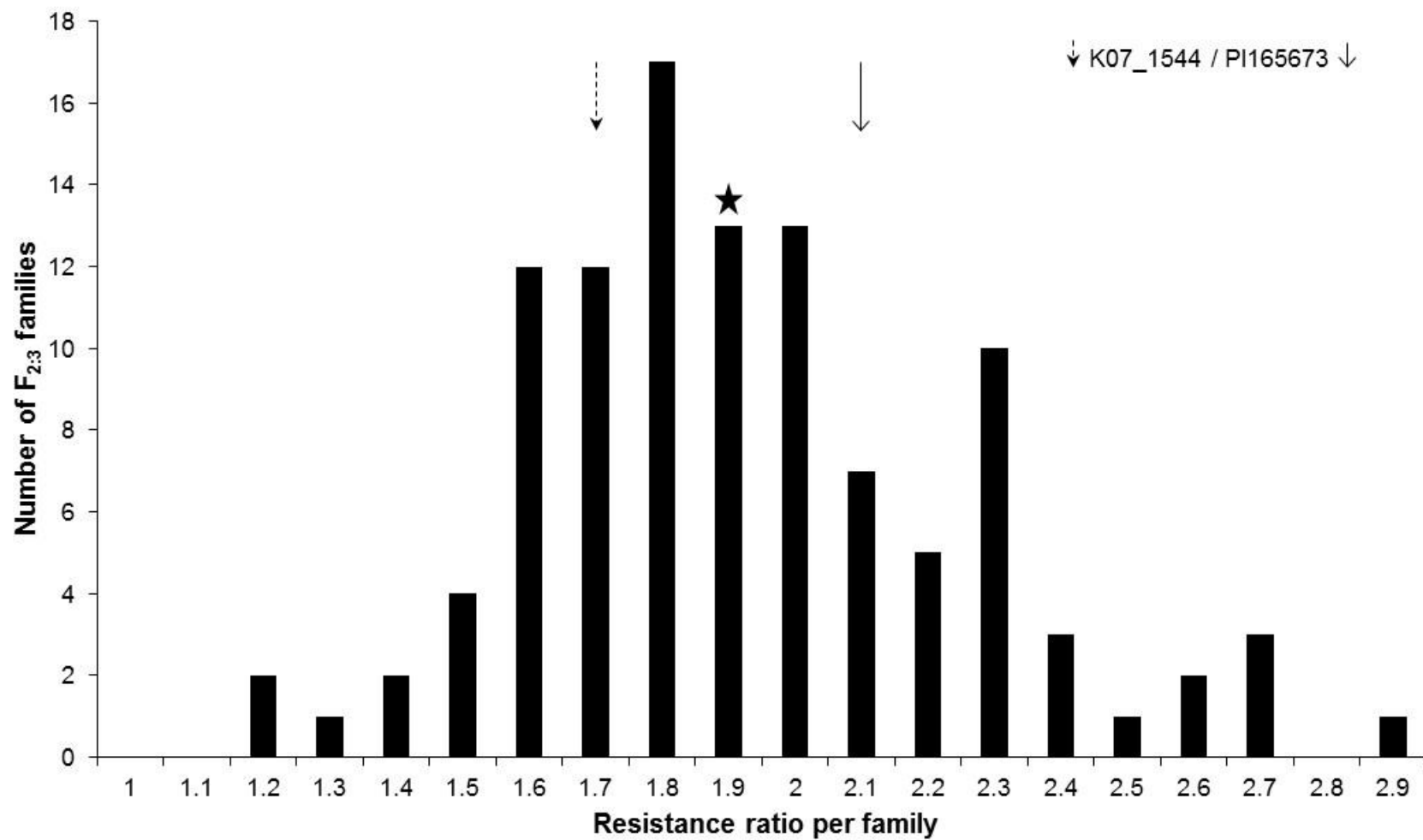


Figure 2.5. Frequency distribution of the resistance ratio in plants from 108 $F_{2:3}$ families from the cross K07-1544/PI165673 infested with *D. texanus* in 2012. Arrows and stars indicate parent and F_2 means, respectively.

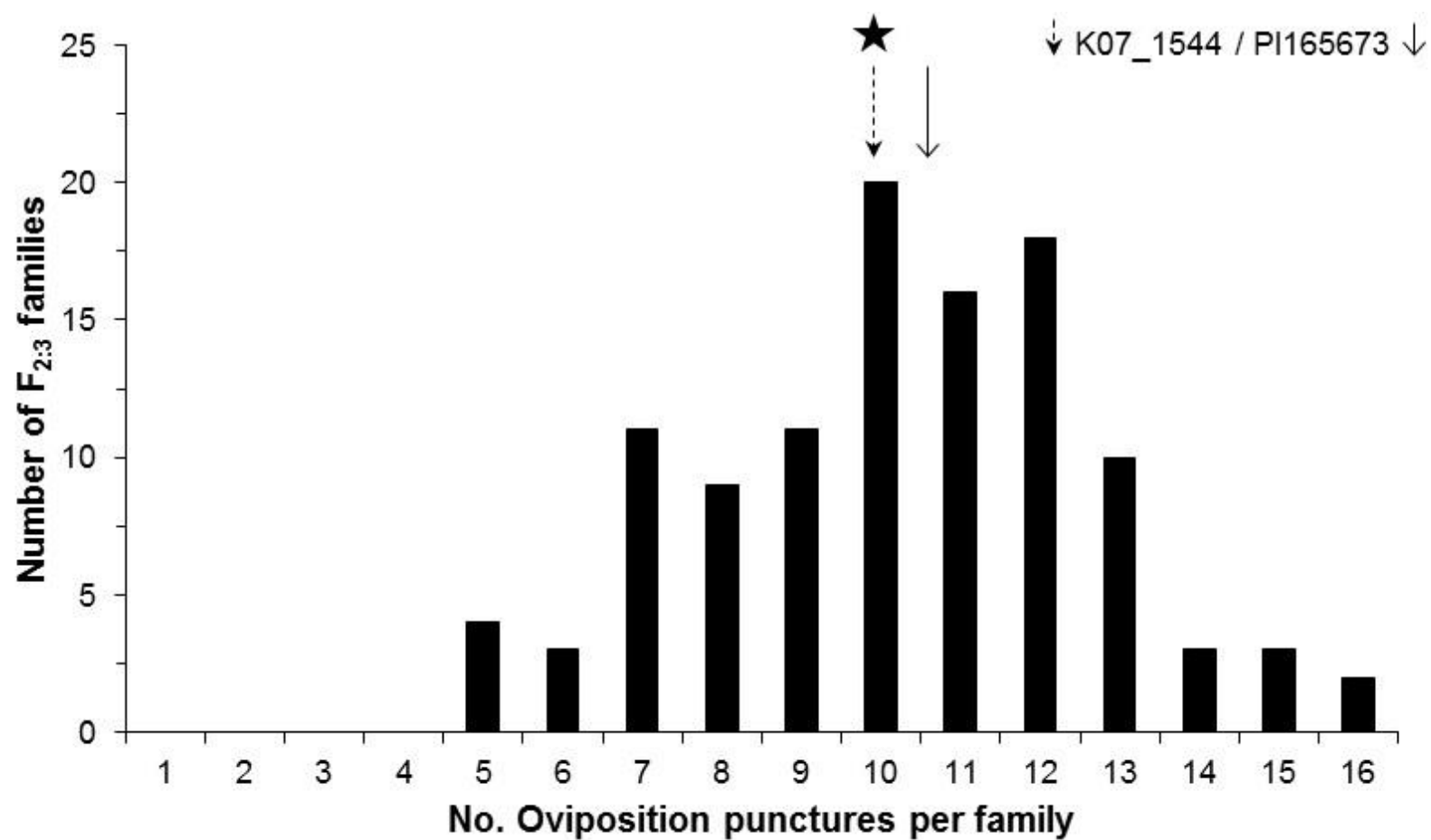


Figure 2.6. Frequency distribution of number of oviposition punctures per plant in plants from 108 $F_{2:3}$ families from the cross K07-1544/PI165673 infested with *D. texanus* in 2012. Arrows and stars indicate parent and F_2 means, respectively.

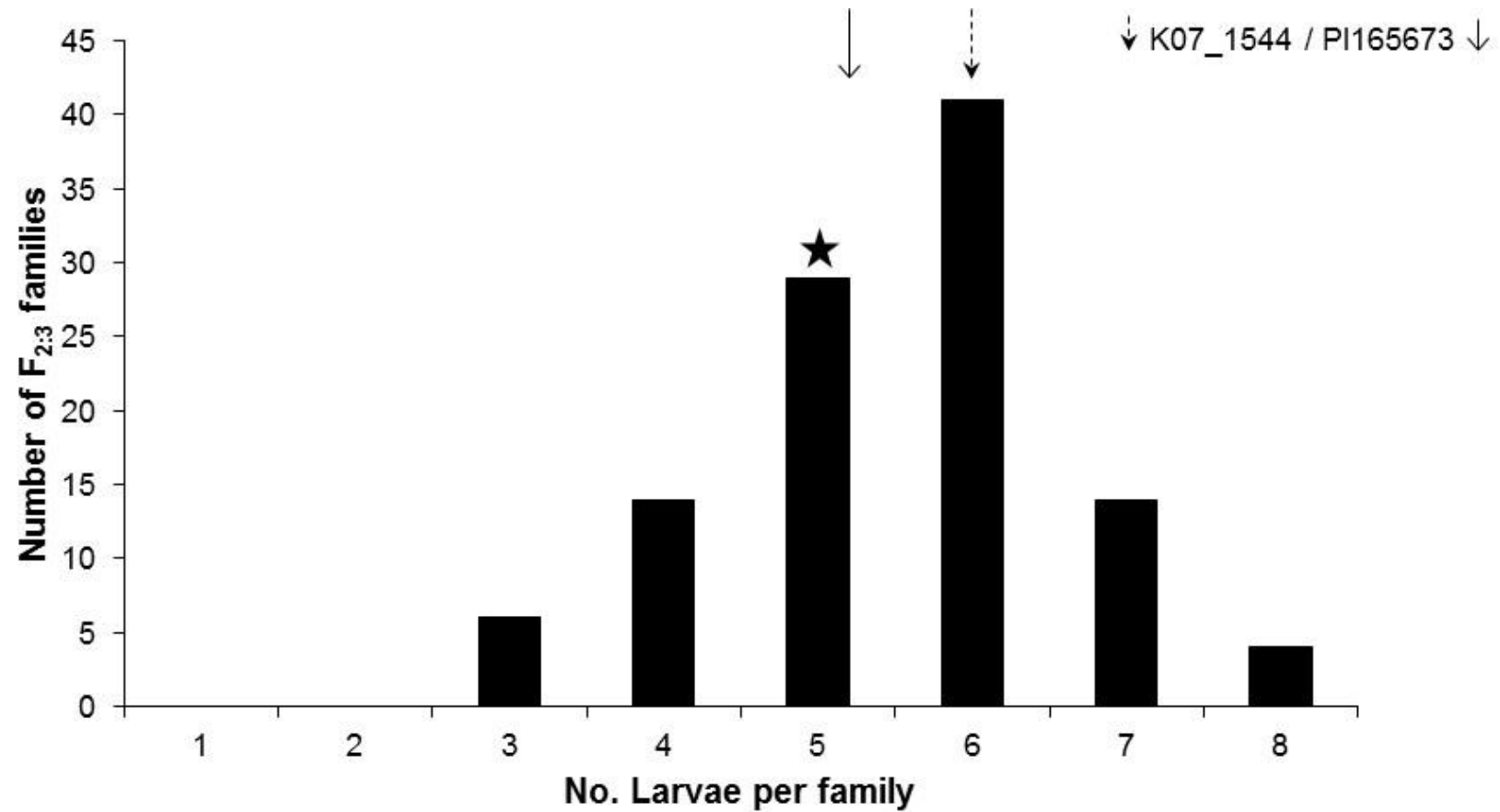


Figure 2.7. Frequency distribution of number of larvae per plant in plants from 108 F_{2:3} families from the cross K07-1544/PI165673 infested with *D. texanus* in 2012. Arrows and stars indicate parent and F₂ means, respectively.

Cited Literature

- Acquaah, G. 2012.** Principles of plant genetics and breeding, Second ed. Wiley-Blackwell, John Wiley & Sons, Ltd., Chichester, UK.
- Allard, R. W. 1960.** Principles of plant breeding, John Wiley & Sons, Inc., New York.
- Alonso-Blanco, C., M. Koornneef, and J. W. Ooijen. 2006.** QTL Analysis, pp. 79-99. In J. Salinas and J. J. Sanchez-Serrano (eds.), Arabidopsis Protocols vol. 323, Second ed. Humana Press Inc., Totawa, New Jersey.
- Andrews, G. L., and R. L. Williams. 1988.** An estimate of the effect of soybean stem borer on yields. Technical Bulletin (Mississippi Agricultural and Forestry Experiment Station) 153: 1-5.
- Bezark, L. G. 2010.** A photographic catalog of the Cerambycidae of the new world. California department of food and agriculture, <http://plant.cdfa.ca.gov/byciddb/results.asp>.
- Bhattacharyya, P. K., and H. H. Ram. 1995.** Inheritance and biochemical basis of resistance to *Spilosoma obliqua* Walker in interspecific crosses of soybean. Plant Breeding 114: 366-368.
- BioQuip. 2008.** Pampel's solution: Material safety data sheet. BioQuip Products, Inc., Rancho Dominguez, CA.
- Boerma, H. R., and D. Walker. 2005.** Discovery and utilization of QTLs for insect resistance in soybean. Genetica 123: 181-189.
- Buschman, L. L., and P. E. Sloderbeck. 2010.** Pest status and distribution of the stem borer, *Dectes texanus*, in Kansas. J. Insect Sci. 10: 1-12.
- Buschman, L. L., H. Davis, and P. Sloderbeck. 2006.** Efficacy of in-season applications of systemic insecticide to control *Dectes* stem borers in soybean. Field day 2006, Southwest Research Extension Center, Kansas State University. Report of Progress 961: 65-69.
- Buschman, L. L., H. Davis, R. Currie, and P. Sloderbeck. 2007.** Efficacy of systemic insecticides applied as a foliar or seed treatments to control *Dectes* stem borers in soybean at Garden City, KS, 2006. Field day 2007, Southwest Research Extension Center, Kansas State University. Report of progress 980: 65-67.
- Buschman, L. L., A. Joshi, P. Sloderbeck, and T. Niide. 2009.** Yield losses associated with *Dectes* stem borers in soybean and efficacy of fipronil seed treatments, Garden City, 2008. Field day 2009, Southwest research-extension center, Kansas State University. Report of progress 1014: 84-90.
- Campbell, W. 1980.** Sampling coleopterous stem borers in soybean, pp. 357-373. In M. Kogan and D. Herzog (eds.), Sampling methods in soybean entomology. Springer, New York.

- Campbell, W. V., and J. W. Van Duyn. 1977.** Cultural and chemical control of *Dectes texanus* on soybeans. J. Econ. Entomol. 70: 256-258.
- Collard, B. C. Y., M. Z. Z. Jahufer, J. B. Brouwer, and E. C. K. Pang. 2005.** An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. Euphytica 142: 169-196.
- Daugherty, D. M., and R. D. Jackson. 1969.** Economic damage to soybeans caused by a cerambycid beetle. Proc. North Central Branch Entomol. Soc. Am. 24: 36.
- Davis, H., L. L. Buschman, P. Sloderbeck, and A. Joshi. 2008.** Efficacy of fipronil applied as foliar and seed treatment to control *Dectes* stem borers in soybean, Garden City, KS, 2007 – South Circle. Field day 2008, Southwest Research Extension Center, Kansas State University. Report of progress 997: 49-53.
- deVicente, M. C., and S. D. Tanksley. 1993.** QTL analysis of transgressive segregation in an interspecific tomato cross. Genetics 134: 585-596.
- Dillon, L. S. 1956.** The nearctic components of the tribe Acanthocinini (Coleoptera: Cerambycidae), Part III. Ann. Entomol. Soc. Am. 49: 207-235.
- Falter, J. M. 1969.** *Dectes* sp. (Coleoptera: Cerambycidae): a unique and potentially important pest of soybeans. J Elisha Mitchell Sci Soc 85: 123.
- FAO. 2012.** FAOSTAT. Food and agriculture organization of the United Nations. Available at <http://faostat.fao.org/>.
- Fehr, W. R. 1987.** Principles of cultivar development. Volume 1. Theory and technique, Macmillan Publishing Company, New York.
- FMC. 2009.** Hero insecticide, Soybean (*Dectes*) stem borer control, pp. 1-2. FMC Corporation.
- Hatchett, J. H., R. D. Jackson, and R. M. Barry. 1973.** Rearing a weed Cerambycid, *Dectes texanus*, on an artificial medium, with notes on biology. Ann. Entomol. Soc. Am. 66: 519-522.
- Hatchett, J. H., D. M. Daugherty, J. C. Robbins, R. M. Barry, and E. C. Houser. 1975.** Biology in Missouri of *Dectes texanus*, a new pest of soybean. Ann. Entomol. Soc. Am. 68: 209-213.
- Hill, C. B., Y. Li, and G. L. Hartman. 2006a.** Soybean aphid resistance in soybean Jackson is controlled by a single dominant gene. Crop Sci. 46: 1606-1608.
- Hill, C. B., Y. Li, and G. L. Hartman. 2006b.** A single dominant gene for resistance to the soybean aphid in the soybean cultivar Dowling. Crop Sci. 46: 1601-1605.
- Jun, T.-H., M. A. Rouf Mian, and A. Michel. 2012.** Genetic mapping revealed two loci for soybean aphid resistance in PI 567301B. Theor. Appl. Genet. 124: 13-22.

- Kaczmarek, M. 2003.** A study of the soybean stem borer including life cycle, insecticidal susceptibility and possible resistance of soybean varieties. Masters of Science, Kansas State University. Manhattan, Kansas.
- Kaczmarek, M., R. A. Higgins, P. Sloderbeck, and W. T. Schapaugh. 2001.** Seasonal occurrence of soybean stem borer (*Dectes texanus texanus*) in Republic County, Kansas. Poster presented at the Annual Meeting of the Entomological Society of America. San Diego, California. <http://www.entomology.ksu.edu/DesktopDefault.aspx?tabid=717>.
- Kaczmarek, M., K. Y. Zhu, R. A. Higgins, and P. Sloderbeck. 2002.** Comparative toxicities of three insecticides to soybean stem borer, *Dectes texanus texanus* (Coleoptera, Cerambycidae) adults. Poster presented at the North Central Branch Meeting of the Entomological Society of America. East Lansing, Michigan. <http://www.entomology.ksu.edu/DesktopDefault.aspx?tabid=717>.
- Kang, S.-T., M. A. Rouf Mian, and R. B. Hammond. 2008.** Soybean aphid resistance in PI243540 is controlled by a single dominant gene. Crop Sci. 48: 1744-1748.
- Kenty, M. M., K. Hinson, K. H. Quesenberry, and D. S. Wofford. 1996.** Inheritance of resistance to the soybean looper in soybean. Crop Sci. 36: 1532-1537.
- Komatsu, K., M. Takahashi, and Y. Nakazawa. 2010.** Genetic study on resistance to the common cutworm and other leaf-eating insects in soybean [*Glycine max*]. Japan Agricultural Research Quarterly: JARQ 44: 117-125.
- Komatsu, K., S. Okuda, M. Takahashi, R. Matsunaga, and Y. Nakazawa. 2005.** QTL mapping of antibiosis resistance to common cutworm (*Spodoptera litura* Fabricius) in soybean. Crop Sci. 45: 2044-2048.
- Langridge, P., and K. Chalmers. 2005.** The principle: identification and application of molecular markers, pp. 3-22. In H. Lörz and G. Wenzel (eds.), Molecular marker systems in plant breeding and crop improvement. Springer-Verlag, Berlin, Germany.
- Laster, M. L., G. R. Tupper, E. E. Hartwig, and W. O. Thom. 1981.** Studies of the stem borer, *Dectes texanus*, on soybeans in Issaquena County, Mississippi, 1978. Mississippi Agricultural and Forestry Experiment Station. Research Report 6: 3.
- Li, Y., C. Hill, S. Carlson, B. Diers, and G. Hartman. 2007.** Soybean aphid resistance genes in the soybean cultivars Dowling and Jackson map to linkage group M. Mol. Breed. 19: 25-34.
- Littell, R. C., G. A. Milliken, W. W. Stroup, and R. D. Wolfinger. 1996.** SAS system for mixed models, SAS Institute Inc., Cary, North Carolina.
- Mebrahtu, T., W. Kenworthy, and T. Elden. 1990.** Genetic study of resistance to the Mexican bean beetle in soybean lines. J. Genet. & Breed. 44: 7-12.

- Meng, F., Y. Han, W. Teng, Y. Li, and W. Li. 2011.** QTL underlying the resistance to soybean aphid (*Aphis glycines* Matsumura) through isoflavone-mediated antibiosis in soybean cultivar ‘Zhongdou 27’. *Theor. Appl. Genet.* 123: 1459-1465.
- Meng, J. 2010.** Genetic analysis of soybean aphid resistance gene in soybean K1621. Doctor of Philosophy, Kansas State University. Manhattan, Kansas.
- Mensah, C., C. DiFonzo, and D. Wang. 2008.** Inheritance of soybean aphid resistance in PI 567541B and PI 567598B. *Crop Sci.* 48: 1759-1763.
- Michaud, J. P., J. A. Qureshi, and A. K. Grant. 2007.** Sunflowers as a trap crop for reducing soybean losses to the stalk borer *Dectes texanus* (Coleoptera: Cerambycidae). *Pest Manage. Sci.* 63: 903-909.
- Milliken, G. A., and D. E. Johnson. 2009.** Analysis of messy data: Designed experiments, vol. 1, Second ed. Chapman and Hall, CRC Press, Boca Raton, Florida.
- Musser, F. R., A. L. Catchot, J. A. Davis, D. A. Herbert, G. M. Lorenz, T. Reed, D. D. Reisig, and S. D. Stewart. 2013.** 2012 Soybean insect losses in the Southern US. *Midsouth Entomologist* 6: 12-24.
- Nielsen, N. C. 1996.** Soybean seed composition, pp. 127-163. In D. P. S. Verma and R. C. Shoemaker (eds.), *Soybean: Genetics, molecular biology and biotechnology*. CAB International, Wallingford, United Kingdom.
- Niide, T. 2009.** Development of soybean host plant resistance and other management options for the stem borer, *Dectes texanus* LeConte. Doctor of Philosophy, Kansas State University. Manhattan, Kansas.
- Niide, T., L. L. Buschman, B. Gordon, P. Sloderbeck, H. Davis, and C. Khajuria. 2008.** Efficacy of fipronil applied as foliar and seed treatment to control *Dectes* stem borers in soybean, Scandia, KS, 2007. Field day 2008, Southwest Research Extension Center, Kansas State University. Report of progress 997: 54-58.
- Niide, T., R. A. Higgins, R. J. Whitworth, W. T. Schapaugh, C. M. Smith, and L. L. Buschman. 2012.** Antibiosis Resistance in Soybean Plant Introductions to *Dectes texanus* (Coleoptera: Cerambycidae). *J. Econ. Entomol.* 105: 598-607.
- Oerke, E. C. 2006.** Crop losses to pests. *J. Agric. Sci.* 144: 31-43.
- Ojo, D., and O. Ariyo. 1999.** Inheritance of resistance to the soybean defoliator (*Spodoptera littoralis* (Boisd))[*Glycine max* (L.) Merril-Nigeria]. *J. Genet. & Breed.* 53: 25-30.
- Oki, N., K. Komatsu, T. Sayama, M. Ishimoto, M. Takahashi, and M. Takahashi. 2012.** Genetic analysis of antixenosis resistance to the common cutworm (*Spodoptera litura* Fabricius) and its relationship with pubescence characteristics in soybean (*Glycine max* (L.) Merr.). *Breeding science* 61: 608-617.

- Orf, J. 2010.** Introduction, pp. 1-18. In K. Bilyeu, M. B. Ratnaparkhe and C. Kole (eds.), Genetics, genomics, and breeding of soybeans. CRC Press, Science Publishers, Inc., Enfield, New Hampshire.
- Panthee, D. R. 2010.** Varietal improvement in soybean, pp. 92-112. In G. Singh (ed.), The soybean: Botany, production and uses. CABI, Wallingford, UK.
- Parrott, W., D. Walker, S. Zhu, H. R. Boerma, and J. All. 2008.** Genomics of Insect-Soybean Interactions, pp. 269-291. In G. Stacey (ed.), Genetics and Genomics of Soybean, vol. 2. Springer, New York.
- Patrick, C. R. 1973.** Observations on the biology of *Dectes texanus texanus* (Coleoptera Cerambycidae) in Tennessee. J Georgia Entomol Soc 8: 277-279.
- Perez-Sackett, P., S. Cianzio, P. Kara, M. Aviles, and R. Palmer. 2011.** QTL mapping of whitefly resistance in soybean. Journal of Crop Improvement 25: 134-150.
- Qiu, L., and R. Chang. 2010.** The origin and history of soybean, pp. 1-23. In G. Singh (ed.), The soybean: Botany, production and uses. CABI, Wallingford, UK.
- Rector, B. G., J. N. All, W. A. Parrott, and H. R. Boerma. 1998.** Identification of molecular markers linked to quantitative trait loci for soybean resistance to corn earworm. Theor. Appl. Genet. 96: 786-790.
- Rector, B. G., J. N. All, W. A. Parrott, and H. R. Boerma. 2000.** Quantitative trait loci for antibiosis resistance to corn earworm in soybean. Crop Sci. 40: 233-238.
- Richardson, L. G. 1975.** Resistance of soybeans to stem borer *Dectes texanus texanus* LeConte. Doctor of philosophy, North Carolina State University. Raleigh, NC.
- Rieseberg, L. H., M. A. Archer, and R. K. Wayne. 1999.** Transgressive segregation, adaptation and speciation. Heredity 83: 363-372.
- Rogers, C. E. 1977.** Cerambycid pests of sunflower: Distribution and behavior in the Southern plains. Environ. Entomol. 6: 833-838.
- Rouf Mian, M. A., S.-T. Kang, S. Beil, and R. Hammond. 2008.** Genetic linkage mapping of the soybean aphid resistance gene in PI 243540. Theor. Appl. Genet. 117: 955-962.
- Rufener, G. K., S. K. St. Martin, R. L. Cooper, and R. B. Hammond. 1989.** Genetics of antibiosis resistance to Mexican bean beetle in soybean. Crop Sci. 29: 618-622.
- SAS Institute. 2009.** The GLIMMIX procedure, SAS/STAT user's guide, version 9.2. SAS Institute, Cary, North Carolina.
- SAS Institute. 2010.** The Freq procedure, pp. 64-216. In SAS Institute (ed.), Base SAS 9.2 procedures guide: Statistical procedures, Third ed. SAS Institute Inc., Cary, NC.

- Sisson, V. A., P. A. Miller, W. V. Campbell, and J. W. Van Duyn. 1976.** Evidence of inheritance of resistance to the Mexican bean beetle in soybeans. *Crop Sci.* 16: 835-837.
- Sleper, D. A., and J. M. Poehlman. 2006.** Breeding field crops, Fifth ed. Blackwell publishing, Ames, Iowa.
- Sloderbeck, P., L. L. Buschman, and R. A. Higgins. 2004.** Soybean stem borer management trials 2001- 2003. Field day 2004, Southwest research-extension center, Kansas State University. Report of progress 927: 41-44.
- Sloderbeck, P. E., and L. L. Buschman. 2011.** Aerial insecticide treatments for management of *Dectes* stem borer, *Dectes texanus*, in soybean. *J. Insect Sci.* 11: 1-10.
- Smith, C. M. 2005.** Plant resistance to arthropods: Molecular and conventional approaches, Springer, Dordrecht, The Netherlands.
- SOYSTATS. 2012.** A reference guide to important soybean facts and figures. The American Soybean Association. Available at <http://www.soystats.com>.
- Tindall, K. V., and K. Fothergill. 2010.** *Zelia tricolor* (Diptera: Tachinidae): First host record from *Dectes texanus* (Coleoptera: Cerambycidae). *Fla. Entomol.* 93: 635-636.
- Tindall, K. V., and K. Fothergill. 2012.** *Dolichomitus irritator* (Hymenoptera: Ichneumonidae): A new parasite of *Dectes texanus* (Coleoptera: Cerambycidae) in soybeans. *Fla. Entomol.* 95: 238-240.
- Tindall, K. V., S. Stewart, F. Musser, G. Lorenz, W. Bailey, J. House, R. Henry, D. Hastings, M. Wallace, and K. Fothergill. 2010.** Distribution of the Long-Horned Beetle, *Dectes texanus*, in Soybeans of Missouri, Western Tennessee, Mississippi, and Arkansas. *J. Insect Sci.* 10: 1-12.
- Wang, J., and J. Gai. 2001.** Mixed inheritance model for resistance to agromyzid beanfly (*Melanagromyza sojae* Zehntner) in soybean. *Euphytica* 122: 9-18.
- Wang, T. 2002.** Soybean oil, pp. 18-58. In F. D. Gunstone (ed.), Vegetable oils in food technology: Composition, properties and uses. Blackwell Publishing, Oxford, UK.
- Warrington, C., S. Zhu, W. Parrott, J. All, and H. Boerma. 2008.** Seed yield of near-isogenic soybean lines with introgressed quantitative trait loci conditioning resistance to corn earworm (Lepidoptera: Noctuidae) and soybean looper (Lepidoptera: Noctuidae) from PI 229358. *J. Econ. Entomol.* 101: 1471-1477.
- Whalen, J., B. Cissel, B. Uniatowski, and J. Pesek. 2010.** Evaluate soybean varieties for management of *Dectes* stem borer in soybeans. Delaware soybean report 2010. University of Delaware, Newark, DE.
- Wilson, R. F. 2008.** Soybean: Market driven research needs, pp. 3-15. In G. Stacey (ed.), Genetics and genomics of soybean. Springer, New York.

- Xiao, L., Y. Zhong, J. Zhang, B. Wang, and T. Wu. 2012.** Inheritance of Resistance to *Aphis glycines* in Soybean P746 from China. J. Econ. Entomol. 105: 2167-2171.
- Xing, G., B. Zhou, Y. Wang, T. Zhao, D. Yu, S. Chen, and J. Gai. 2012.** Genetic components and major QTL confer resistance to bean pyralid (*Lamprosema indicata* Fabricius) under multiple environments in four RIL populations of soybean. Theor. Appl. Genet. 125: 859-875.
- Xu, R., W. LI, L.-f. ZHANG, Y.-h. LIN, B. QI, and H. XING. 2010.** A study on the inheritance of resistance to whitefly in soybean. Scientia Agricultura Sinica 1: 013.
- Yesudas, C. R., H. Sharma, and D. A. Lightfoot. 2010.** Identification of QTL in soybean underlying resistance to herbivory by Japanese beetles (*Popillia japonica*, Newman). Theor. Appl. Genet. 121: 353-362.
- Zhang, G., C. Gu, and D. Wang. 2009.** Molecular mapping of soybean aphid resistance genes in PI 567541B. Theor. Appl. Genet. 118: 473-482.
- Zhang, G., C. Gu, and D. Wang. 2010.** A novel locus for soybean aphid resistance. Theor. Appl. Genet. 120: 1183-1191.

Appendix A - Pattern of damage distribution inside 10 x 10 ft cages.

The pattern of damage distribution inside the cages was attributed visually as an indirect measurement of a random – uniform distribution of the beetles inside the cages to detect any possible edge effects. The data from experiments in 2011 and 2012 were used to globally visualize the pattern of damage distribution across the cages that were used to evaluate each F_2 population and $F_{2:3}$ families. A mean was calculated for each plant position inside the cage, and each position was averaged across all cages used in each F_2 and $F_{2:3}$ plant population. The mean of plant position was calculated for each variable, i.e. numbers of oviposition punctures and larvae, and the resistance ratio. The average for each plant position was calculated ignoring the plant genotype, i.e. KS5004N, K07-1544, PI165673, or any F_2 and $F_{2:3}$ plant. The mean for each plant position across cages was plotted using a bubble plot with the following coordinates: rows were on the X axis, and plant positions within rows were on the Y axis. The bubble size changes proportionally with smaller or larger means (Fig. B1, B2, and B3).

The bubble plots from the F_2 KS5004N/PI165673 and K07-1544/PI165673 population data indicate a random pattern of damage distribution inside the cages, in general (Fig. B1 and B2). However, there were many missing plants inside the cages used for both populations. In particular, there were more missing plants on rows 1 and 3 from the F_2 KS5004N/PI165673 cages which may indicate that plant positions in row 4 attracted more beetles than rows 1 and 3. In the case of the cages used for the $F_{2:3}$ K07-1544/PI165673 population, larger means were observed in the three plant positions closer to the North and South border within rows for numbers of oviposition punctures and larvae. The pattern of damage distribution was similar between rows for all variables. There were no evidences of clusters of plant positions with larger means for the resistance ratio across all cages (Fig. B3).

In general, any possible cage effects were moderate since there was no strong evidence for one plant position to be more preferred than another, and the resistance ratio displayed a uniform pattern of damage distribution across plants in all cages. Nevertheless, these observations were made from a visual perspective, without taking into account the plant genotype for each plant position in all cages. Also, there were missing data from plants that did not emerge, broke or were stunted in growth. A more appropriate experimental design is needed to test for possible cage edge effects or plant position preference by the beetle inside the cages. This could be achieved by using the same plant genotype across all cages and reducing the numbers of missing plants.

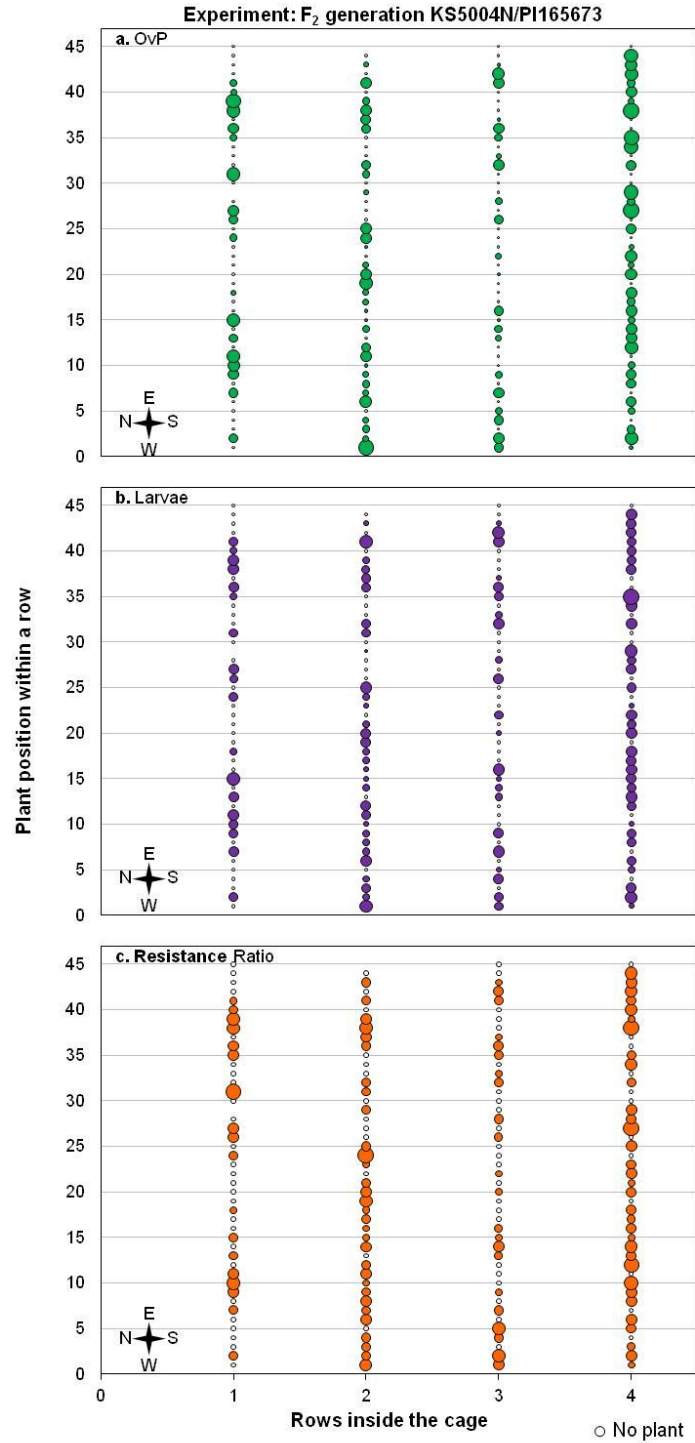


Figure A.1. Pattern of damage distribution using the mean of each plant position across 2 cages for numbers of oviposition punctures (a), larvae (b), and Resistance Ratio (c). Plants were infested with *D. texanus* in 2011. Bubble size per plant position changes proportionally with smaller or larger means.

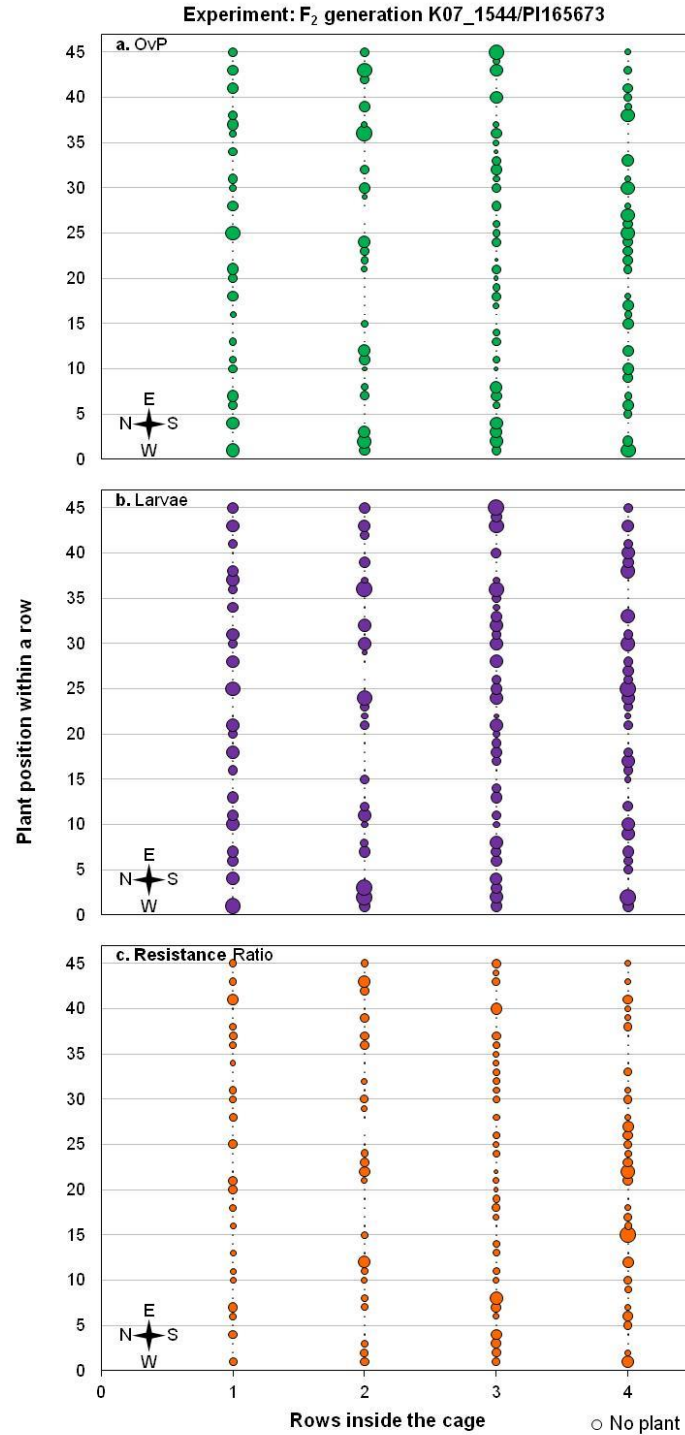


Figure A.2. Pattern of damage distribution using the mean of each plant position across 2 cages for numbers of oviposition punctures (a), larvae (b), and Resistance Ratio (c). Plants were infested with *D. texanus* in 2011. Bubble size per plant position changes proportionally with smaller or larger means.

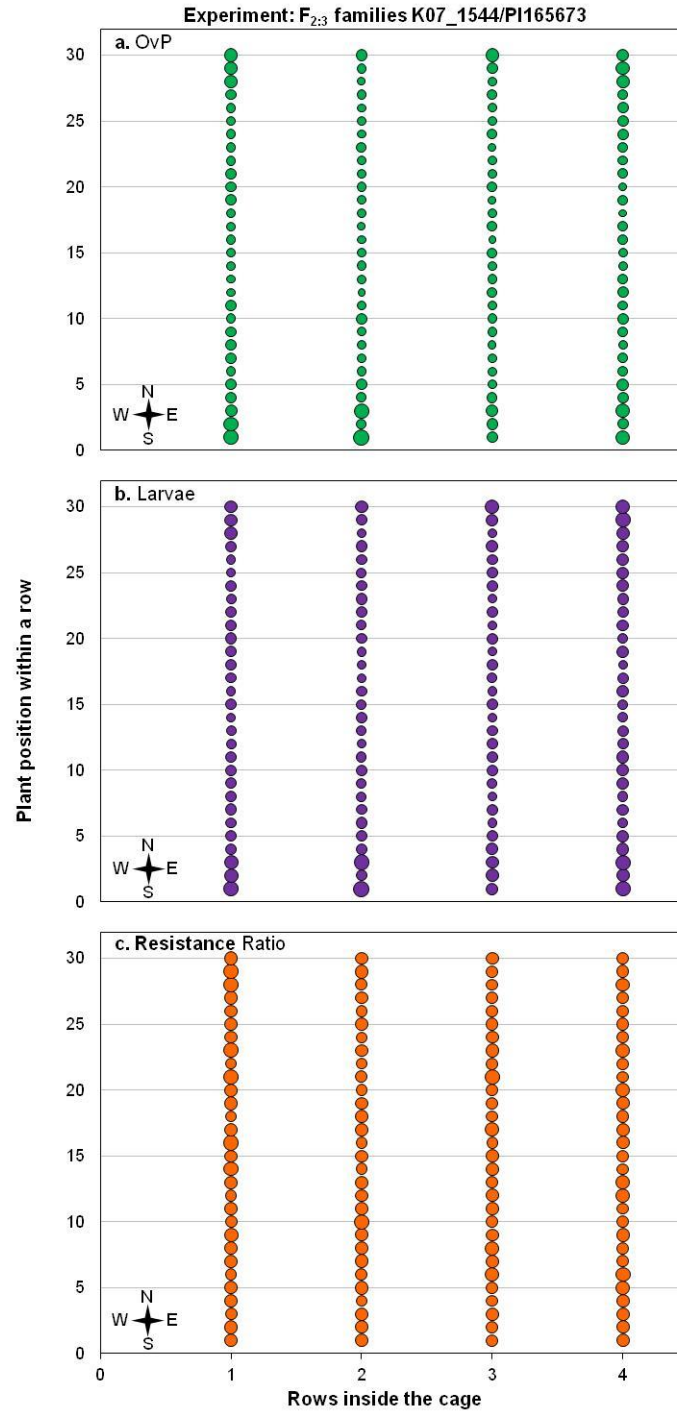


Figure A.3. Pattern of damage distribution using the mean of each plant position across 14 cages for numbers of oviposition punctures (a), larvae (b), and Resistance Ratio (c). Plants were infested with *D. texanus* in 2012. Bubble size per plant position changes proportionally with smaller or larger means.