A RISK AVERSE EVALUATION OF VALUE ADDED TRAITS IN ELITE SOYBEAN GERMPLASM AND CONFIRMATION OF LINKAGE GROUP (LG) B2 AND LG E SOYBEAN INSECT RESISTANCE QUANTITATIVE TRAIT LOCI

by

MICHELLE SUSAN SAMUEL-FOO

(Under the Direction of J. N. All and H. R. Boerma)

ABSTRACT

Pest vulnerability of value added characteristics in 'Benning' soybean [*Glycine max* (L.)] was evaluated through field studies and greenhouse and growth chamber insect bioassays as part of a reduced risk assessment of these lines. Benning is a soybean cultivar that was developed by the Georgia Agricultural Experiment Station and it is noted for its strong agronomic properties. This elite cultivar served as the genetic background for the value added traits (VAT) that were introgressed and evaluated for their pest susceptibility attributes. The traits evaluated included lines with improved nutritional characteristics such as low palmitic acid and low linolenic acid. These VAT are becoming increasingly important as consumers demand improved soybean for food use. Benning M and Benning MGH, near isogenic lines which contain soybean insect resistance quantitative trait locus (SIR QTL), served as resistant checks in this study and were evaluated here under natural pest populations for their effectiveness. We were interested in seeing how these VAT lines would perform in terms of vulnerability when confronted with a host of common soybean insect pests.

Ina separate study, simple sequence repeat (SSR) DNA markers, together with field experiments, and insect bioassays were utilized in an effort to confirm the existence of previously reported SIR QTLs. SIR QTLs had previously been reported on linkage group (LG) B2 and LG E of the soybean genome. At least 27 SSR markers closely associated with loci along LG B2 were genotyped to see what portion of the resistance alleles from the donor parent (PI 227687) had been successfully introgressed into the progeny. To confirm the SIR QTL on LG E, antixenosis (non preference) and antibiosis (toxicological effects) assays were employed to elucidate the effects of sharp vs blunt pubescence tip in a near-isogenic line population differing in pubescence morphology.

INDEX WORDS: Antibiosis, Antixenosis, *Euschistus servus*, *Helicoverpa zea*, Insect Resistance, *Nezara viridula Pseudoplusia includens*, Quantitative Trait Loci, Simple Sequence Repeat, Soybean

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DEDICATION

For my family

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CHAPTER I

INTRODUCTION

Learn from yesterday, live for today, hope for tomorrow.
The important thing is to not stop questioning.
~ Albert Einstein

Demands on our limited agricultural resources are at an unprecedented level as the world's population continues to expand. According to the United Nations, the twentieth century saw the world's population increase from 1.65 billion to more than 6 billion with even more growth expected in this decade. Reliance upon agriculture to efficiently feed this increased population is at an all time high. Farmers are now charged with this critical task and thankfully modern agricultural practices and technological developments, not to mention support from entomologists, agronomists, and other scientists have them well equipped to deal with this seemingly formidable task.

As a science, entomology is an integral part of any agricultural system. Insect species (both beneficial and pests) are ubiquitous, making them the most numerous animal life form on earth. In fact, Daly et. al., (1998) report that about half of the described species of living things and almost three-quarters of all animals are insects. Insects are known to inhabit all land surfaces in the earth save for the extreme polar regions and highest mountains summits. Due to their unique ability to adapt to their environment, they easily dominate the other small fauna in most habitats.

Foliage feeding lepidopteran pests are responsible for significant annual yield losses in soybean [*Glycine max* (L.) Merr] planted in the south eastern United States. For

instance, McPherson et al, (1996) reported that damage from caterpillar pests accounted for 51% of the damage to growers (actual damage and cost of pest control) in 1996 with an estimated 47% in damage occurring the following year. Having insect resistant cultivars would be financially beneficial to growers as less money would have to be spent on chemical control, the traditional method of pest management. Additionally, utilizing host plant resistance (HPR) has the added benefit of reduced chemical inputs in the environment. In the early 1970s the promise of cultivars with native insect resistance was elevated when Van Duyn et al., (1971b) reported the identification of Japanese Plant Introductions (PIs) with Mexican bean beetle [*Epilachna varivestis* (Mulsant)] resistance. PI 171451, PI 227687and PI229358 were later found to also have resistance to some coleopteran and lepidopteran pests, but the poor agronomic performance of these cultivars made them unpopular with growers who were unwilling to spare agronomic qualities for insect protection. Bollworm (formerly corn earworm) [*Helicoverpa zea* (Boddie)] and soybean looper [*Pseudoplusia includens* (Walker)] were among the lepidopteran pests that the PIs demonstrated resistance to in subsequent studies (Clark et al. 1972, Lambert and Klein 1984a, 1984b, 1988a, Arumuganathan and Earle 1991). Today these species, now joined by the stink bug complex remain among the top insect pests of soybean in the southeastern US.

Soybean is known to be tolerant of defoliation to a certain extent, and the plant has developed the ability to delay leaf senescence in response to injury (Haile et al. 1998). Tolerance levels are dependent on a number of factors including: defoliation intensity, plant phenology, and environmental factors such as precipitation and soil fertility (Pedigo et al. 1986). Despite soybean's ability to withstand certain levels of

foliage feeding, the crop is especially vulnerable to economically damaging levels in the southeastern USA due to the region's long growing season which allows for multiple pest generations to develop. Additionally, being in close proximity to the tropics also influences this phenomenon, since several migratory pests select this region as overwintering sites.

Interest in developing soybean cultivars that possess value added characteristics such as insect resistance and improved nutritional characteristics are on the rise. In today's health conscious society, consumers are making wiser nutritional choices as the benefits of a soy-rich diet is being promoted. In response to the FDA mandated regulation that soybean oil for human consumption must include on its label the amount of saturated fatty that it contains, several value added cultivars with reduced levels of polyunsaturated fatty acids (e.g. low palmitic acid and low stearic acid) have been developed. A search of the available literature suggested that the pest vulnerability of these value added cultivars has not been investigated thoroughly. One aim of this study was to examine what effect, if any, these value added traits had on the feeding preferences of common soybean pests.

In a separate study, based on work that had been done in our lab, we set out to confirm previously reported soybean insect resistant quantitative trait loci (QTL) on linkage groups B2 and E of the soybean genome. Recent advances in molecular biology, together with the development of genetic linkage maps, have made it possible to establish approximate numbers and locations of genes associated with insect resistance. Using restriction length fragment polymorphisms, Rector et. al., (1998, 1999, 2000) identified both major and minor quantitative trait loci (QTLs) in three mapping populations. Later Hulbert (2001) combined simple sequence repeat DNA markers with the RFLP data and

subsequently reported additional insect resistance QTLs, different from those described by Rector. Hulburt's QTLs were on linkage group (LG) B2 and LG E of the soybean genome. The QTL on LG B2 conditioned both antibiosis and antixenosis insect resistance. The QTL on LG E was of particular interest because in addition to conditioning both antibiosis and antixenosis resistance, it mapped near to the classical pubescence marker, *Pb*, on the integrated soybean linkage map (Cregan et al. 1999). Due to the fact, that these QTLs were originally mapped in a relatively small population (< 100 lines) their validity have been uncertain. There are many factors that influence the detection of QTLs segregating in a population; the size of the original mapping population used in the QTL detection study is a particularly important factor (Collard et al. 2005). In order to verify the existence of a QTL, QTL mapping studies need to be independently confirmed (Lander and Kruglyak 1995). This can be done in a number of ways including using independent populations from the same genotypes or closely related genotypes, or using larger populations or near isogenic lines (NILs). NILs are created by crossing a donor parent to a recurrent parent and then repeatedly backcrossing the F_1 hybrid to the recurrent parent while simultaneously selecting for your region of interest. Evaluating the NILs for the introgressed trait provides evidence of the existence of a reported QTL. In this research project, SSR marker data was used together with NILs of an independent population to verify the existence of the SIR QTLs reported on LG B2 and LG E. These NILs were created by using 'Benning' as the recurrent parent and PI227687 as the donor parent.

Project objectives: The overall objective of this research was to:

(i) Assess the pest vulnerability of value added soybean lines that were developed in an elite soybean genetic background. Benning, a maturity group VII cultivar with desirable agronomic properties (Boerma et al. 1997b) served as the genetic background for these lines. The major insect pests surveyed in this study included the bollworm, soybean looper, green cloverworm, velvet-bean caterpillar and the stink bug complex.

(ii) Use NILs to verify the existence of the SIR QTLs on LG B2 and LG E using SSR marker data and a combination of field, greenhouse and growth chamber bioassays.

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CHAPTER II

LITERATURE REVIEW

Soybean [*Glycine max* (L.) Merr] is an important agricultural crop, commanding nearly 87% of all the oilseed meals used by the feed industry. The crop was planted to over 63 million acres (25 million hectares) in 2007 with Iowa and Illinois being the leading U.S. states in terms of hectarage dedicated to soybean. The total crop harvested in 2007 was worth in excess of 26.8 billion U.S. dollars. This was a significant increase in the value of the crop from the previous years' value of approximately 20 billion U. S. dollars despite a decrease in hectarage due to a conversion to corn production (SoyStats 2008).

Today's soybean crop is attacked by a number of insect pests including hemipteran, coleopteran and lepidopteran species though the importance of specific insect pests varies by year and region (Higley and Boethel 1994). Most recently, Boethel (2004) reported the soybean looper (*Pseudoplusia includens* Walker) and the velvetbean caterpillar (*Anticarsia gemmatalis* Hübner) as the most damaging defoliating insect pests in the southern US—[a position once reserved for the bollworm (*Helicoverpa zea* Boddie; BW)]-- due to their annual migrations which contribute to sporadic infestations and rapid population increases. In general, southern states face heightened insect pressure because of the warm climate which facilitates multiple annual generations and increased pest survival. The proximity of this region to the tropics, which is a prime overwintering

location for defoliators, is another factor that contributes to this phenomenon (Higley and Boethel 1994).

Soybean belongs to the family Leguminosae and is related taxonomically to alfalfa, peas, and clover. It is generally considered a temperate zone, short season crop, although it can be successfully cultivated in climates with hot summers and longer growing seasons such as is found in the southern USA.

Having cultivars that possess insect resistance has long been an objective of many soybean improvement programs, but thus far this achievement has been elusive. Van Duyn et al. (1971, 1972) discovered three Japanese plant introductions (PIs) when his team screened the majority of the USDA soybean germplasm accessions of Maturity Groups VII and VIII, for resistance to the Mexican bean beetle [*Epilachna varivestis* (Mulsant)]. Today these three PIs serve as the source of insect resistance for introgression into elite cultivars (Boethel 1999, Lambert and Tyler 1999). The three PIs: PI 171451 ('Kosamame'), PI 227687 ('Miyako White') and PI 229358 ('Soden-daizu') all exhibit both antibiosis and antixenosis resistance to a number of soybean insect pest species including soybean looper (*Pseudoplusia includens;* SBL), bollworm (*Helicoverpa zea;* BW), velvetbean caterpillar (*Anticarsia gemmatalis;* VBC), cabbage looper [(*Trichoplusia ni* (Hübner)], bean leaf beetle [*Cerotoma trifurcate* (Forster)], striped blister beetle [*Epicauta vittata* (Fabricius)], and the beet armyworm [*Spodoptera exigua* (Hübner)], (Clark et al. 1972; Hatchett et. al. 1976; Kilen et al. 1977; Luedders and Dickerson, 1977; Lambert and Kilen, 1984a, 1984b).

Insect pests are typically categorized by the type of damage they cause; insect pests of soybean are described in the literature as being pod feeders, stem feeders, and or

defoliators (leaf feeders) depending on which part of the plant they feed upon. Pod feeders include piercing and sucking pests such as the stink bug complex (Hemiptera: Pentatomidae) which has experienced a recent surge in prominence among soybean insect pests. These pod feeders are well equipped with mouthparts that are likened to needle-like stylets which enable them to effectively pierce through the pod and seed coat and feed upon developing seed. The combined effect of gregarious feeding by nymphs and later by single adults results in deformation of developing seed and in some cases failure of the seeds to develop in the affected pod (poor pod fill).

Stem feeders can reduce stands by weakening the stems and causing lodging or death of affected plants. In sandy soils paired with hot, dry conditions lesser cornstalk borer (*Elasmopalpus lignosellus* Zeller) can decimate established soybean plants when it tunnels into the stem of susceptible seedlings. Other stem feeders include the threecornered alfalfa hopper (*Spissistilus festinus* Say*).* Kogan and Turnipseed (1987b) reported that crops grown in lower latitudes had a greater vulnerability to attack by insect pests.

Origin of Soybean

The cultivated soybean (*Glycine max*.) belongs to the Leguminosae family and is believed to have originated in the northern and central regions of China (Probst and Judd 1973). According to reports (Hymowitz 2004), Samuel Bowen brought soybean from its native China to North America in 1765. Benjamin Franklin was also involved in this early introduction of the crop to the Americas when he sent soybean seeds to John Bartram, a botanist, who planted them in Pennsylvania (Hymowitz and Harlan 1983).

The genus *Glycine* is subdivided into *Glycine* and *Soja* (Moench) F.J. Herm. (Hymowitz and Newell 1981). *G. max* is described as a true domesticate, in that its existence would not be possible without human intervention (Fehr 1989). The crop is believed to be an ancient tetraploid that later underwent diploidization (Hadley and Hymowitz 1973). Morphologically, soybean is highly variable as is evidenced by the diversity that exists among its landraces. This variability is often tapped as a source of genetic diversity in attempts to broaden the genetic bottleneck that exists in modern propagated cultivars. In fact, modern cultivars are known to have been developed from a narrow genetic base (Gizlice et al. 1994, Carter et al. 2004). Pedigree analysis has revealed that 80% of the genes in publically available soybean cultivars that were released between 1947 and 1988 were derived from just 13 ancestral lines (Gizlice et al. 1993). These ancestors were plant introductions (PIs) that had sundry desirable agronomic qualities when grown in North America (Boerma and Walker 2005). Today, soybean is the number one oilseed crop in the world and a multibillion dollar crop in the USA (Riley 1999, SoyStats 2008).

The Soybean Genome

Soybean is one of a growing number of crop species that has a densely saturated genetic map (Cregan et al. 1999) and a wide variety of genetic tools available for research (Shoemaker 1999). Pedigree analysis has demonstrated a disparity between northern germplasm (Canada and northern USA) compared to southern germplasm (southern USA). Gizlice et al. (1993) used Restriction Fragment Length Polymorphism (RFLP) analysis to aid in making this differentiation. The soybean genome is approximately two and a half times larger than rice (*Oryza sativa* L.) and seven and a half times that of

Arabidopsis. At 1.1 Mbp/C (Arumuganathan and Earle 1991), it is less than half of corn (*Zea mays* L.) and more than 14 times smaller than bread wheat's (*Triticum aestivum* L.) genomes. Up to 60% of the soybean genome sequence is reported to be repetitive (Goldberg 1978, Gurley et al. 1979). BAC (Bacterial Artificial Chromosome) and YAC (Yeast Artificial Chromosome) libraries have been created which offer more than sufficient coverage of the crops' genome (Zhu et al. 1996, Marek and Shoemaker 1997, Danesh et al. 1998, Salimath and Bhattacharyya 1999, Tomkins et al. 1999). Due to its well-developed genetic transformation system (Zhang et al. 1999, Clemente et al. 2000, Xing et al. 2000) and the availability of its genetic map, soybean is considered a model crop system. With the exception of many disease resistance genes, most agronomically important traits are controlled by groups of several genes known as quantitative trait loci (QTL) which will be discussed in greater detail later on in this chapter.

Soybean Entomology

Soybean serves as a host for 36 insect species from three insect orders in North America, eight of which are known as economically important species (Lambert and Tyler 1999). Of these eight species, five are exclusive foliage feeders; two are exclusive fruit feeders with the remaining one able to feed on both foliage and fruit. Pod feeding damage caused by chewing and piercing/sucking insects often results in deformation of seeds and poor pod fill. Damage caused by defoliators is often minimized with the plant compensating for this loss by producing more leaves. (Lambert and Tyler 1999) report that soybean can recover from up to 40% defoliation prior to flowering and up to 30% after flowering has begun, with little or no yield losses.

BW and SBL remain among the most consistent soybean defoliators in the southeastern USA. Most recently the stink bug complex (Hemiptera: Pentatomidae) has joined the ranks of major pests. A brief overview of the predominant soybean pests will provide some background to the current studies.

Major Lepidopteran Soybean Pests

Bollworm [*Helicoverpa zea* (Boddie)]

The bollworm (Lepidoptera: Noctuidae; BW) is widely distributed. It is a highly dispersive, polyphagous species that in the mid 80's was labeled as being the number one pest of soybean in the USA (Kogan and Turnipseed 1987a). Typically, four generations occur per year in Georgia and neighboring southeastern states (Van Duyn et al. 1994). Mature larvae move to the soil surface where they burrow about 5-10 cm into the soil and pupate to later emerge once the soils warm up in spring. Larvae are typically variable in color and upon hatching they search for an appropriate feeding site on their host plant. Although newly hatched larvae may often be observed feeding together, older larvae are noted for being aggressive and cannibalistic. Second generation moths usually emerge between mid-July to early August when they typically colonize blooming soybean flowers.

Adult BW are sexually dimorphic and have a 25-38 mm wingspan (Van Duyn et al. 1994). Females have yellowish-brown to pinkish-brown forewings, while males typically have light yellow-olive forewings. The forewings have a dark spot near the center and the hind wings are typically broad and light colored. BW moths have a dark brown outer band and a narrow intermarginal band. They are known to be strong fliers and can migrate long distances (Funderburk 1994a). Larvae hatch from cream colored

eggs that are deposited along the entire plant, though they are usually concentrated at the plant terminals. They wander the plant until they find a suitable feeding site. Fecundity ranges from 500 to 3000 eggs per female. At eclosion, larvae are 1.5 mm and develop through 5-6 instars over 20-21 days to approximately 42 mm. The larvae are characterized by having an orange head and longitudinal cream bands on a green, yellowish or black body. Larvae consume leaves, stems, flowers, pods and seeds within pods on both full season and double-cropped soybean (Van Duyn et al. 1994). Early instars cause limited damage to newly emerging leaves and flowers but the last two instars are responsible for approximately 96% of the total damage. Severe infestations are usually observed when peak moth populations are synchronized with peak soybean flowering.

Disrupting this phenological synchrony in the southeastern US can help reduce infestation levels and can be achieved by early plantings of soybeans from maturity groups (MGs) V and VI to avoid peak emergence of second generation moths (Kogan and Turnipseed 1987a, Van Duyn et al. 1994). In the early 1970s however, a switch to earlier maturing groups coincided with a similar switch in maize cropping systems to early maturing corn hybrids and as a result, lessened the number of attractive oviposition sites that were available in mid-July (Kogan and Turnipseed 1987b). This resulted in increased colonization of early planted, early flowering MG V cultivars with a simultaneous increase in soybean damage being observed in the southeastern Coastal Plain. There are a number of practices that can be employed to both monitor BW populations and discourage pest buildup. These include using narrow-row spacing and careful scouting with sweep nets and drop cloths (Funderburk 1994a).

Soybean Looper [*Pseudoplusia includens* (Walker)]

The soybean looper (SBL) is a noctuid, highly polyphagous moth that, like the BW, is found dispersed throughout the Western Hemisphere. It is a member of the subfamily Plusiinae which also contains the cabbage looper (Sullivan and Boethel 1994). These moths are reported to prefer cotton to soybean when the cotton crop has a densely developed canopy (Felland et al. 1992). When soybean is chosen as an oviposition host though, the SBL moths seem to prefer plants that are nearly mature and in later growth stages (Mascarenhas and Boethel 1997). Although SBL moth populations typically remain below economic injury levels, they sometimes exceed economic levels in southeastern agro ecosystems when cotton and soybean are grown concurrently.

The average life cycle of SBL moths in soybean fields during late August to September is 26.5 days (Mitchell 1967). Female moths lay approximately 640 greenish-white eggs in single file on the underside of leaves. Tiny larvae with two pairs of prologs hatch from these eggs. Larvae are various shades of green and possess four pairs of longitudinal stripes along the length of their body. Larvae mature to a length of 35mm. Larvae feed mainly on the bottom layers of soybean with older larvae preferring mature foliage (Sullivan and Boethel 1994). First and second instars feed on the underside of leaves and produce a characteristic ‘window-pane’ effect. Older instars are able to severely defoliate leaves and produce a lace-like appearance on leaves that have been fed upon with leaf veins remaining intact. It has been well documented that fifth and sixth instars account for approximately 90% of total soybean defoliation, 50% of which occurs in the last two days of larval growth and development. Occasionally SBL larvae may damage soybean flowers and developing pods.

SBL can overwinter in the southern regions of Florida and Texas in the USA. Central America and the Caribbean are the major reservoirs for moths as they immigrate northward. In Georgia, moths appear in mid summer (June-July) and typically produce two generations per growing season. Damage potential from economic injury levels (EILs) usually occurs between mid-August to September. Economic thresholds for defoliation by SBL are >35% prior to flowering and 15-20% after flowering/during pod fill, or 13-27 larvae per row-m (Sullivan and Boethel 1994). Economic damage by SBL can be reduced by employing cultural methods whereby early maturing (MG III and IV) cultivars are planted so that the plants mature before periods of heaviest moth populations. Natural enemies of SBL include bigeyed bugs, spined soldier bugs, and several parasitoid wasps which attack eggs, larvae, and/or pupae (Sullivan and Boethel 1994). Additionally, entomopathogens such as *Entomophthora gammae* and *Nomuraea rileyi* infect SBL. These natural predators and pathogens aid in keeping SBL pest populations below economic thresholds (Sullivan and Boethel 1994).

Lesser Cornstalk Borer [*Elasmopalpus lignosellus* (Zeller)]

The lesser cornstalk borer (LCB) occurs widely in the western hemisphere and is a problem in much of the southern United States as an occasional pest of over 60 crops in 14 families, with legumes and grasses being the preferred hosts (Funderburk and Mack 1994). Despite its wide distribution, damage is limited principally to crops in sandy soil (Metcalf et al. 1962). It was first discovered outside the continental USA in July 1986 infesting sugarcane in Kauai Hawaii (Chang and Ota 1987).

The LCB is a stem-feeding insect that is mainly a pest in late-planted soybean on dry, sandy soils. LCB is a semi subterranean pest in the sense that larvae are active on the

soil surface but are often also found in silken tubes 5-20 cm deep in the soil. LCB is adapted to hot, dry weather conditions and therefore tend to be more abundant and damaging following unusually warm, dry weather (Turnipseed 1973). Soybean planted in weedy fields or in fields that contain burned crop residues is more likely to become infested. Weed hosts of LCB include nutsedges (*Cyperus rotundus*), watergrass (*Hydrochloa caroliniensis*), Johnsongrass (*Sorghum halepense*), crabgrass (*Digitaria sanguinalis*), wild oats (*Avena fatua*), Bermuda grass (*Cynodon dactylon*), wiregrass (*Aristida stricta*), and goose grass (*Eleusine indica*) (Gardner and All 1982, Isley and Miner 1994). Infestations have also been observed in double cropped soybean planted behind winter wheat (Funderburk and Mack 1994). Larvae cause damage when they feed upon and tunnel within the stems of plants. This typically occurs to seedlings at the soil surface. Normally, the tunneling is restricted to the lower region of the plant where girdling may occur. This is often fatal to seedlings and any plants that survive this action become susceptible to lodging. Wilting is one of the first signs of attack in susceptible plants, and infested areas of fields often have a very thin stand. Silken webbing forming a small tube in the soil at the base of the stalk is evidence of the attack of LCB. Larvae produce characteristic silken webbing as a small tube that is found attached to soil particles at the bore hole of the plant close to the soil surface and this is often useful in diagnosing a LCB infestation. A single larva can damage numerous seedlings when it bores into the stalk base near the soil surface causing damage to vascular tissues that results in a characteristic "dead hearts" symptom and also allows pathogens to enter the plant (Smith and Ota 2002).

Female moths lay eggs resembling sand grains on hosts such as maize or Johnsongrass (Turnipseed 1973). Tiny larvae hatch from these and initially begin feeding on leaves or roots and later burrow into stems. Larvae mature through six instars and are typically bluish in color with dark, transverse bands (Funderburk and Mack 1994). Adults are approximately 1.3cm in length and the wings are folded when the moths are at rest. Males are tan and possess a dark stripe running along the middle of the back while females are uniformly dark. Under ideal conditions for moth survival and reproduction, up to five generations can be produced annually in the southeastern USA.

Modified planting practices are utilized to minimize crop losses. As LCB populations tend to increase over the course of a season, interrupting the synchrony between moth emergence and soybean plantings can help avoid damage by this pest. Tillage and weed destruction are cultural practices that are also employed, as this helps to destroy larvae that might be present in soils. However, crop culture that uses conservation tillage (i.e., retention of crop residue at the soil surface) experiences less injury from LCB feeding because the larvae feed freely on crop residue and other organic matter, sparing the young crop plants (All et al. 1979).

The sporadic nature of economically devastating infestations and the covert feeding habits of LCB make this a difficult pest to observe and control. Insecticide applications are only fiscally responsible under high pest pressures, but post-planting insecticide applications are only effective prior to extensive stem damage (Funderburk and Mack 1994).

Green Cloverworm [*Hypena scrabra* (Fabricius)]

The green cloverworm (GCW) is a noctuid moth that is generally considered a minor pest in soybean. It is found from the eastern USA westward into the Great Plains states, and northward into southeastern Canada and occurs in most soybean fields during each growing season. It is a common defoliator in soybean that can cause significant yield losses throughout the southern and central regions of the USA (Stone and Pedigo 1972, Myers and Pedigo 1977, Hammond and Pedigo 1982, Ostie and Pedigo 1985). GCW feed extensively on soybean leaves. Young larvae skeletonize the underside of leaves and older larvae eat all of the leaf except the large veins. They feed initially on the top one-third of the plant, which often gives it a badly damaged appearance which may or may not be an accurate predictor of economic damage.

Adult moths are dark brown or black in color with spotted/mottled wings and are difficult to identify with an untrained eye. GCW larvae are characteristically slender and light green in color with a pair of white longitudinal stripes on either side of its body. They vary in length from 1.5 mm (first instar) to 30.5 mm (sixth instar). They possess three pairs of prologs near the middle of the body and a single pair near the end. The larvae thrash about violently when disturbed. The insect overwinters as pupa or adults in leaf litter or just beneath the soil surface. In the spring, female moths lay tiny, single hemispherical eggs on soybean foliage. Larvae hatch and feed for about four weeks, then pupate under litter with a new generation of adults emerging in approximately 21 days. Up to four generations may occur in parts of the southeastern USA annually (Roberts and Douce 1999b). Beneficial insects (parasitoids and predators) and diseases usually regulate the green cloverworm population below economic injury levels in most areas

where soybeans are grown. Because it attacks early in the season, plants usually compensate for foliage loss before pods are set. When defoliation exceeds the economic threshold (35 % foliage loss before flowering and 15 % foliage loss after flowering or during pod fill), chemical control is recommended.

Velvetbean Caterpillar [*Anticarsia gemmatalis* (Hübner**)]**

The velvetbean caterpillar (VBC) (Lepidoptera: Noctuidae) is native to the tropical and subtropical areas of the Western Hemisphere, where it is primarily a defoliator of soybean, but is also known to feed on tender stems and pods (Turnipseed 1973). Larvae are voracious feeders that can completely defoliate entire fields in a matter of days (Funderburk 1994b). A single larva can consume up to 110 cm^2 of foliage (Aragón et al. 1997). The insect overwinters in south Florida and in tropical areas before migrating northwards during the summer. It is considered a pest species in soybean in the southeastern USA, but economic injury levels rarely exceed threshold levels in regions north of this area. Up to four generations may be produced annually. VBC is an annual problem in the months of June through September in Florida, Georgia, and Alabama. Female moths lay tiny, oval eggs that are white in color. The egg is ribbed and white in color until just before it hatches, when it turns pink. Eggs are laid singly on the under side of leaves, although in heavy infestations eggs may be found on the upper surfaces of leaves, on the petioles, and even on the stems (Watson 1916). The egg stage usually lasts about 3 days when laid in August and September, but requires a week or more when laid later in the fall.

Newly hatched larvae feed on the egg shell initially. Larvae are typically light green in color with cream colored stripes that sometimes turn black in cool weather

(Turnipseed 1973). Larvae develop through six instars in a period that ranges from 14-25 days. During the sixth instar the VBC becomes gradually lengthened and can grow up to 48 mm. In the prepupal stage, the larvae shrink to a length of 25 mm and turn mahogany brown with few if any longitudinal lines (Watson 1916). Pupation occurs just beneath the soil surface, near the plant's base. Adult moths emerge about 10 days post pupation. They are characterized by a diagonal black line that appears on both pairs of wings (Funderburk 1994b).

Velvetbean caterpillar larvae cause damage by consuming foliage. Newly hatched larvae defoliate the leaf beginning with the lower epidermis and mesophyll and continue until the end of the second instar when the caterpillar begins to skeletonize the leaf, eating all the soft material and leaving only the veins intact (Watson 1916). After the second instar, the VBC consumes the entire leaf. Once the upper leaves and lower leaves have been consumed, foliage in the middle and lower canopy is consumed and complete defoliation may result (Roberts and Guillebeau 1999).

Several species of parasitoids affect the VBC including *Winthemia rufopicta* (Bigot) (Diptera: Tachinidae), *Euplectrus puttleri* Gordh (Hymenoptera: Eulophidae) and *Meteorus autographae* Muesebeck (Hymenoptera: Braconidae) (Daigle et al. 1990). Other natural enemies include ground beetles, tiger beetles, the striped earwig *Labidura riparia* (Pallas) (Dermaptera: Labiduridae) and the red imported fire ant *Solenopsis invicta* Buren (Hymenoptera: Formicidae). Predation has been observed to be a significant factor in management of the VBC. In a recent study, it was observed that predation was the principal mortality factor of the VBC, accounting for 52.5 to 95.2% of mortality in soybean field plots (Lee and Johnson 1990). Additionally, entomopathogens

including the fungi *Nomurea rileyi* and *Entomophthora* sp. also infest the VBC. These parasitoids, predators, and pathogens all contribute significantly to the natural control of the VBC.

Insects from the order Hemiptera:

Threecornered Alfalfa Hopper [S*pissistilus festinus* Say]

The most characteristic symptom of feeding damage caused by (threecornered alfalfa hopper) is stem girdling (Wildermuth 1915) which is caused by the repeated puncturing of the plant tissue by the insect's needle-like stylet (Moore and Mueller 1976). Third through fifth instars, as well as adults, typically inflict this type of damage to stems and petioles, with the fourth larval instars being the most injurious (Moore and Mueller 1976, Johnson and Mueller 1988).

Feeding damage by *S. festinus* was once considered not economically important (Kogan and Turnipseed 1987b) until work performed in Louisiana revealed that this was not the case (Layton 1983, Hicks et al. 1984, Mitchell and Newsom 1984a, Mitchell and Newsom 1984b). In fact, Mitchell and Newsome (1984b) reported that extensive yield losses can result from the insect feeding on petioles and racemes during the pod-setting and pod-filling stages. Additionally, Hicks et al. (1984) found that plants that had girdled stems and petioles tended to show restricted nutrient flow, reduced leaf area, decreased nodule growth and reductions in nodule number and nitrogen fixation. Significant losses reportedly occur when a minimum of 65% of plants are girdled (Mueller and Jones 1983). Stem girdling causes weakening of the main stem, causes the plant to lodge, and may even result in the eventual death of soybean plants (Wildermuth 1915). Stem feeding

damage can also provide an avenue for infection by *Sclerotium rolfsii* Saccardo (Herzog et al. 1975).

Bigeyed Bug [*Geocoris* spp.]

Bigeyed bugs belong to the insect family Lygaeidae. They are small, oval insects that can be found in many parts of the world. They are generally considered as beneficial insects because they prey upon numerous kinds of insect pests of agricultural crops including soybean. *Geocoris bullatus* (Say), the large bigeyed bug, is widely distributed in the USA and Canada, from coast to coast. These insects are characterized by having the head broader than long and prominent eyes which curve backward and overlap the front of the pronotum. Their stylus has a longitudinal groove. Additionally, in bigeyed bugs the claval commissure is very short or absent altogether. These features can be observed on both nymphs and adults and are useful in separating bigeyed bugs from similar bugs.

The most abundant bigeyed bug in the southeastern USA is *G. punctipes* (Say). McGregor and McDonough (1917) reported the life history of *G. punctipes* at Batesburg, SC, finding the average development time from egg to adult was 30 days. Nymphs consumed an average of 47 mites, and adults an average of 83 "red spider" mites on cotton per day. York (1944) reported that adult *Geocoris* required either free moisture or plant moisture as well as insect prey. Dumas et al. (1962) found more *G. punctipes* in the morning than at midday or evening, either by sweep net sampling or complete plant examination in Arkansas soybean fields. Whitcomb and Belk (1964) reported, that in Arkansas, *G. punctipes* and *G. uliginosus* were among the most abundant and important predators of *Helicoverpa zea* eggs (Boddie) on cotton from mid-June until September.

Stink Bugs

Southern green stink bug, [*Nezara viridula* (L.)]
Green stink bug, [*Acrosternum hilare* (Say)]
Brown stink bug, [*Euschistus servus* (Say)]

Soybean planted in the USA annually suffer yield losses due to escalating stink bug pest populations. Traditionally regarded as secondary pests, stink bugs have now achieved major pest status, a move that in part coincided with the reduced insecticide applications associated with the boll weevil eradication program and the widespread use of Bt-cotton. Stink bugs belong to the family Pentatomidae, a member of the insect order Hemiptera or true bugs. They are so classified because of their characteristic five segmented antennae (Pentatomid) and segmented beak that arises from the front of their head. They are typically identified by their antennae and the well developed scutellum that they possess, which may sometimes obscure their entire abdomen.

The stink bug complex in Georgia is composed of three species of true bugs (Hemiptera: Pentatomidae) *Nezara viridula* (L.) (southern green stink bug), *Acrosternum hilare* (Say) (green stink bug), and *Euschistus servus* (Say) (brown stink bug). Life histories of the stink bugs are synchronized to development and growth of crops producing seeds and fruiting structures. They develop on numerous cultivated and non cultivated hosts, and migrate into soybean fields during pod set and pod fill stages (Gore et al. 2006). The southern green stink bug, the most commonly observed species in the complex, feeds on developing soybean seeds and often causes reduced seed size (Thomas et al. 1974), reduced seed stability during storage (Miner 1961, Blickenstaff and Huggans 1962) and reduced seed quality, all of which contribute to a lower market value

(McPherson et al. 1994). The southern green stink bug is thought to be native to southeast Asia or eastern Africa and occurs throughout the tropical and subtropical continents of Europe, Asia, Africa, Australia and the Americas. In the US, its distribution is limited to the southern states, although it is now also established in California. The green stink bug is also found throughout the tropical and subtropical regions of the world (Waterhouse 1998) and it is widely distributed in North America (McPherson et al. 1994). The brown stink bug also is native to North America and in the US it can be found south of a line extending from New York to North Dakota and over to Arizona and California (McPherson et al. 1994).

All stink bugs molt through five nymphal stages before becoming adults. The reddish-black first nymphal stage of southern green stink bugs are not known to feed on plant tissue and tend to be gregarious in nature. Second instars are black and have faint white markings on the abdomen. These nymphs remain congregated near egg masses and tend to feed on young reproductive structures and shoots. Third instars disperse as a group from the egg mass, and are usually black in color with distinct white spots on the dorsal surface of the abdomen. Fourth and fifth instars disperse widely, are pale green and possess white spots on each side of the midline of the dorsal abdominal surface and on the margins. Nymphal behavior of the other two species is analogous to that of the southern green stink bug.

The members of the stink bug complex described herein overwinter as adults under leaf litter, tree bark and other protective materials. Adults may sometimes be active during warm winter spells, but no winter generations are known. In the spring, when the weather has warmed up, adults migrate to small grains, vegetables, corn, and weed hosts

where the first generation is completed. Egg clusters are laid later in the season on soybean leaves and tend to be most numerous on blooming soybean or plants that are in pod forming or pod filling stages. Egg hatch occurs in approximately six days in the summer, but may extend to two-three weeks in early spring. Development from egg hatch to adult takes about 23 days when conditions are optimal (86^{o}F or 30^{o}C) but may last from one-two months at lower temperatures. Stink bugs typically develop three-four generations per year typically and usually the third and fourth generations occur on soybean. A fifth generation may occur in south Florida, while in the northern US, only two generations typically develop per year (McPherson et al. 1994).

Both nymphs and adult stink bugs puncture soybean tissue and extract plant fluids with their specially adapted piercing-sucking mouthparts. Even though young tender growth and developing seeds are their preferred plant parts, they will also feed upon stems, foliage, and blooms. Tell-tale signs of feeding damage appear as brown-black spots at the feeding site and this has the potential to severely affect yield and seed quality (McPherson et al. 1994).

Drop cloth and sweep net sampling is typically employed to monitor stink bug populations in soybean. Early season pest populations may be randomly dispersed or may be concentrated at field borders. With soybean bloom and the onset of pod fill, female adults become attracted to the plants as they search for oviposition sites. In most states, insecticide applications only become warranted if pest populations exceed 3.3 bugs per row meter during pod development or one bug per row meter from bloom through mid pod fill (McPherson et al., 1994).

Insects from the Order Coleoptera

Mexican bean beetle [*Epilachna varivestis* (Mulsant)]

Newly emerged adult Mexican bean beetles (MBB) (Coleoptera: Coccinellidae) possess a round shape, are about 0.6 cm long and appear yellow in color. Their color changes to copper as the beetle ages. Adult beetles typically have 16 black spots that are equally divided on its elytra and is commonly used as an identifying characteristic. Additionally, six branching spines are present on each segment of the bright yellow, 0.8 cm long larvae. Pupae are also bright yellow and have only remnants of larval spines (Ratcliff et al. 2004). Adult MBB overwinter in nearby fields, along fence rows, in wood lots or in stubble and can usually be found within 400 m of host plants.

After approximately 12 days of feeding, overwintered females begin to oviposit, and clusters of 40 to 60 yellow-orange colored eggs are laid on the undersides of the leaves (Barrigossi et al. 2003). Both larvae and adults feed primarily on the undersides of the leaves, removing the epidermal layer and skeletonizing the foliage. Severely injured leaves dry up and drop from the plant (Douglass 1933, Barrigossi et al. 2001).

The beetles become active in the spring, when they fly to host plants, feed for a week or two, and then mate. Normally, 400 to 500 eggs (but occasionally three times this number) are laid in clusters on the undersides of leaves over a period of three to six weeks. Larvae hatch in 5 to 14 days and feed for two to five weeks before pupating on the undersurfaces of leaves. Adults emerge 7 to 10 days later and live from four to six weeks. In Georgia there are three-four generations annually (Funderburk et al. 1999, Roberts and Douce 1999a, Ratcliff et al. 2004).

The MBB is one of only two North American species of destructive insects in an otherwise beneficial family (ladybird beetles) of over 400 species. Damage to soybean plants occurs when adults and larvae feed on leaves. Early instars feed exclusively on the underside of leaves leaving behind a skeletonized or lacy appearance on leaves that have been fed upon (Funderburk et al. 1999). Soybean pods may also suffer scarring damage but these seldom reach economic levels. Although the MBB has mandibles that are typical of chewing insects, it is not known to swallow bits of leaf pieces; instead it masticates its food. Soybean fields under high MBB pressure take on a rusty appearance as the leaves shrivel and turn brown.

Larvae are typically easiest to control, and insecticides are commonly used as a means of control, although the cost of treatment, market price of the crop, growth stage of the crop, and percent of defoliation needs to be considered when making control decisions. In soybean, treatment may be warranted when defoliation reaches 30 % before flowering and seven or more adults and larvae can be found in 0.3 m of plants in a row. For flowering or post-flowering soybean plants, insecticide treatment may be warranted when defoliation reaches 20 % and five or more adults and larvae are present in 0.3 m of plants per row (Ratcliff et al. 2004).

Whitefringed beetles [*Naupactus* (=*Graphognathus*) spp.]

Whitefringed beetles (Coleoptera: Curculionidae) are considered serious pests of many agricultural crops including soybean (Young et al. 1950). It is a species of weevil that was first introduced into the USA through Florida in 1936. These flightless beetles originated in South America, but are now widely distributed throughout the southern USA. Females (males unknown) are typically light to dark gray or brown and possess a

light band along the outer margins of the wing covers. Two paler longitudinal lines can be found on each side of the thorax and head, one above and one just below the eye.

Adult beetles (univoltine = one generation per year) emerge from the soil from May to October and feed on foliage. Oviposition (parthenogenetic reproduction) occurs 5 to 25 days after emergence. Typically about 11 to 14 white eggs are laid on plant stems, roots and the soil surface. The eggs turn light yellow within a few days of being laid. They hatch between 11 to 100 + days after oviposition. Newly emerged larvae feed on roots, tubers, and underground stems as well as dead plant material, and complete their development in the soil. Whitefringed beetles overwinter as larvae.

Pupation occurs from late April to late July in cells constructed by the larvae; however, some larvae spend a second year feeding on plants in the soil before they pupate. Most pupal cells are 5 to 15 cm below the soil surface; however, cells have been found at a depth of 36 cm. In the summer months, the pupal stage lasts ca. 13 days; in cooler months it is longer (Young et al. 1950). Damage from root feeding by white-fringed beetle larvae can range from scattered areas of a few dead or dying plants within a field to nearly all plants being damaged. Whitefringed beetles are relatively innocuous foliage feeders which leave saw-tooth cuts on outer edges of leaves. Infested plants turn yellow and, if severely injured, wilt and die.

Host Plant Resistance in Soybean

Host plant resistance (HPR) is one tactic to manage insects that is not detrimental to the environment, and it is attractive as a pest management strategy to growers as it can reduce their cost of production (Li et al. 2004). Painter (1951) described insect resistance as the relative amount of heritable qualities possessed by the plant that influences the

ultimate degree of damage it receives in the field from insect pressure. In an earlier report, Painter (1941) proposed that host plant resistance (HPR) to insects could be categorized into three types: preference (later non-preference and now antixenosis), antibiosis, and tolerance. Plants with resistant qualities are believed to possess varying levels of these traits.

Antixenosis describes resistance in which the insect is either repelled from or not attracted to its normal host plant (Rector et al. 1999). In contrast, antibiosis refers to resistance in which the insect's normal relationship with a host plant causes physiological or developmental detriment to the insect. This includes mortality of early instars, reduced size/weight, and lower fecundity (Owens 1975, Rector et al. 1999). Tolerance describes the plant's ability to continue to grow and reproduce despite insect pressures that would adversely affect a susceptible cultivar (Painter 1951).

Several reviews cover the advancements in identifying compounds involved in soybean HPR (Kogan and Fischer 1991, Wheeler and Slansky 1991, Kessler and Baldwin 2002). Among the known chemical factors influencing antixenosis and antibiosis are isoflavonoids, phytoalexins, and proteinase inhibitors. Isoflavonoids (e.g., plaseol, afrormosin, coumesterol, and diadzein) may act individually or in combination with other factors as an antifeedant. Additionally, secondary plant compounds and nutrients may be involved in HPR, providing constitutive and induced resistance. Induced defenses are typified by a wounding response, and were first identified by the local and systemic synthesis of proteinase inhibitors which function in blocking insect digestion in response to plant injury and damage (Ferry et al. 2004).

The discovery that soybean trichomes (pubescence) conferred resistance to the potato leafhopper [*Empoasca fabae* (Harris)] (Poos and Smith 1931, Johnson and Hollowell 1935) and the identification of soybean plant introductions with Mexican bean beetle [*Epilachna varivestis* (Mulsant)] resistance (Van Duyn et al. 1971a, Van Duyn et al. 1972) are often cited as important discoveries in the history of utilizing HPR in soybean. Additionally, the identification of three Japanese plant introductions: PI 171451, PI 227687, and PI 229358 that showed resistance to the Mexican bean beetle and later to a host of other soybean insect pests including SBL, BW, VBC, striped blister beetle (*Epilachna vittata* F.), bean leaf beetle (*Cerotoma trifurcate* Forster), whiteflies and stink bugs (Clark et al. 1972, 1976, Kilen et al. 1977, Jones and Sullivan 1979, Smith and Gilman 1981, Lambert and Klein 1984a, 1984b, Beach et al. 1985, Beach and Todd 1988b,1988a, Rowan et al. 1991) is important to the advances of HPR in soybean.

Hulburt et al. (2001) reported that near isogenic soybean lines with sharp pubescence tip morphology significantly reduced defoliation and larval weight gain in a number of lepidopterous pests, including the BW and BAW. Prior to this, several studies reported on the negative effect that dense pubescence conveyed to lepidopterous larvae (Beach and Todd 1988b, Lambert and Kilen 1989, Lambert et al. 1992a).

Breeding soybean with plant resistance to insects has been quite challenging, primarily due to the fact that the sources of resistant germplasm possess poor agronomic characteristics. Resistance is usually quantitatively inherited and this often results in the added burden of linkage drag when trying to introgress the resistance genes of interest into elite germplasm (Sisson et al. 1976, Luedders and Dickerson 1977, Kenty et al. 1996). Boethel (1999) defines linkage drag as the unintentional co-selection of

undesirable alleles that are genetically linked to alleles of interest. What typically happens is that tightly linked genomic segments containing inferior alleles affecting agronomic traits including yield, are often simultaneously transferred to the recurrent parent along with the alleles of interest. Despite the development of insect resistant cultivars with improved agronomic performance such as 'Crockett (MGVIII),' 'Lyon (MG VI)' and 'Shore (MG V)' and more than 40 breeding lines with partial insect resistance, these lines have been widely unpopular with growers due to their overall inferior yield performance.

HPR in soybean to insect pests as a pest management strategy additionally is slow to adoption by growers and producers due to the expense and time consuming nature of screening large numbers of segregating plants. Developing cultivars with insect resistance however, remains an objective of many soybean improvement programs and recent developments in the use of molecular marker technologies in soybean have provided breeders with new tools to aid in this process. Quantitative trait loci (QTLs) associated with HPR in the plant introductions PI 171451, PI 227687, and PI 229358 have been successfully mapped in the soybean genome (Rector et al. 1998, 1999, 2000). The wealth of information on QTL numbers, locations, and functions, coupled with modern techniques for QTL detection has opened up novel opportunities for the exploitation of soybean HPR using marker assisted selection (MAS) (Paterson 1996).

Molecular Breeding for Insect Resistance

The development of molecular genetic maps was initiated by the implication that restriction length polymorphisms (RFLP) could serve as an approach for the development

of numerous DNA markers (Botstein et al. 1980). Molecular markers, representing DNA variations at specific genetic loci, are widely utilized tools in both plant and animal breeding. Molecular techniques allow the detection of variation or polymorphisms that exist among individuals in a population for specific regions of the DNA. These polymorphisms can be used to establish genetic maps and to correlate differences between markers with the expression of particular traits in a population. This relationship is likely due to some degree of linkage of the QTL affecting the trait and the marker (Albert et al. 1994). Prior to their adoption in plant breeding however, quantitative traits were studied using statistical techniques that were difficult to interpret (Falconer and Mackay 1996). Molecular marker technologies including RFLP, random amplified polymorphic DNA (RADP), amplified fragment length polymorphism (AFLP), single sequence repeats (SSRs) and single nucleotide polymorphisms (SNPs) are advantageous in that they allow for the dissection of complex traits into discrete loci QTLs-- groups of tightly linked loci that are inherited together) which allows for the effects of individual alleles to be studied (Paterson et al. 1988, Paterson 1996). They are also particularly useful because they are phenotypically neutral, independent of environmental influence, and allow for rapid screening in a comparably shorter period (Smith 2004).

Different marker systems are associated with different detection costs and have varying abilities and utilities across populations. SSRs were developed in response to complexities associated with earlier marker technologies such as RFLPs, and a low level of polymorphisms across elite germplasm (Akkaya et al. 1992). RFLPs were also costly and too time consuming. SSRs (aka microsatellites) are polymerase chain reaction (PCR) based markers that were developed as single locus markers, many of which have multiple

alleles making them particularly suitable for genetic map creation and defining linkage group homology across mapping populations (Song et al. 2004).

Molecular markers have been used to explore the quantitative inheritance of HPR to insects in corn (Beavis et al. 1994), mungbean (*Vigna radiate* L.) (Young et al. 1992), potato (*Solanum tuberosum* L.) (Yencho et al. 1996), soybean (Rector et al. 1998, 1999, 2000, Terry et al. 2000, Narvel et al. 2001), and tomato (*Lycoperscion esculentum* Miller) (Maliepaard et al. 1995). The development of these molecular marker technologies associated with phenotypic HPR traits has allowed the mapping of QTLs to specific chromosomal locations, and consequently has allowed for germplasm screening at the genetic level.

Marker Assisted Selection and QTLs

DNA markers are important tools for investigating the genetics of insect resistance in soybean and for MAS of insect-resistant individuals in breeding populations (Boerma and Walker 2005). MAS requires closely linked, easily identified genetic markers that are closely linked to a QTL of interest. The process of mapping insect resistant QTLs involves crossing resistant and susceptible parents and then testing the progeny population for non-random associations between phenotype and genotype at a marker locus. If these associations reveal statistically significant associations, then this information is typically regarded as a suggestion of linkage between the marker and the gene associated with resistance (Boerma and Walker 2005).

Rector et al. (1998, 1999, 2000) identified QTLs conditioning antixenosis and antibiosis resistance to CEW in three soybean mapping populations. They identified a

major QTL for antixenosis (R^2 = 0.37) and antibiosis (R^2 = 0.22-0.28) on linkage group (LG) M using RFLP markers. In statistics, R^2 refers to the coefficient of determination and it is often cited to explain the amount of variability explained by the planned effects in a data set. Insect resistant QTLs were also identified on LG-G (antibiosis, R^2 = 0.19), LG-H (antixenosis, R^2 = 0.09-0.19), and on LG-D1b (antixenosis, R^2 = 0.10). In a follow-up study, Narvel et al. (2001) assessed the introgression of these QTLs in SIR lines by evaluating how many of the PI alleles were present at SSR marker loci closely associated with the SIRQTLs. Varying combinations of SIRQTLs near isogenic lines (NILs) have been developed in a Benning genetic background using MAS (Zhu et al. 2006). MAS may greatly increase the efficiency and effectiveness of plant breeding (Collard et al. 2005). Selecting for the appropriate allele at an appropriate marker saves time, as the number of phenotypic evaluations can be reduced in early generations or during backcrossing (Tanksley et al. 1989). By simultaneously selecting for the recurrent parent's genome and the genetic areas of interest in the donor parent, the cultivar development process can be hastened (by approximately two backcross generations) and the likelihood of linkage drag can be reduced (Hospital et al. 1992, Orf et al. 2004).

The utilization of MAS in soybean is especially useful because manual pollinations are very time consuming, labor intensive, and there is a low rate for successful takes. MAS is recognized for its efficiency and effectiveness with the introgression of single genes (Prabhu 1999) and the pyramiding of resistance genes (Hittalmani et al. 2000), and multiple QTLs (Schneider et al. 1997, Toojinda et al. 1998, Schneider et al. 2001). Therefore MAS has proven itself as an important tool in the

soybean breeder's toolbox that can be added to complement the existing strategies in the creation of elite germplasm with HPR to insects.

Value Added Trait (VAT) Soybean

A major focus of this study was to investigate what effect soybean cultivars with altered nutritional qualities would have on insect feeding preference as an indication of increased or decreased VAT cultivar susceptibility. The VAT cultivars that were used in this study and their corresponding characteristics are outlined in Table 2.1. These included two low palmitic acid varieties (Benning N87.2122.4 and Benning C1726) and one low linolenic acid variety (Benning (6) FAN). Lipoxygenase (absence) and glyphosate tolerance combined with the absence of lipoxygenase are other VATs that were included in the evaluations. A brief introduction and summary of these traits and their role in insect nutrition will help provide rationale for this study.

Fatty acids and Insect Nutrition

By definition, a fatty acid is an aliphatic monocarboxylic acid that is liberated from glycerolipids (Wilson 1987). It consists of a central hydrocarbon chain that has a methyl group and a carboxyl group at either end. They are described using the chain length, the number of double bonds they possess and the location of the first double bond (Gunstone 1996). The number of carbon atoms and connected hydrogen atoms influences the nutritional value of individual fatty acids and subsequently influences the characteristics of food products. The most common fatty acids in plants, including soybean, belong to a small group of C16-C18 fatty acids that have between zero and three

double bonds (Somerville et al. 2000). The five major fatty acids contained in soybean oil are palmitic, stearic, oleic, linoleic and linolenic (Table 2.2). About 10% of soybean oil is the polyunsaturated fatty acid (two or more carbon- carbon double bonds) linolenic acid with about 50% being linoleic acid. Another 15% is the saturated fatty acid (maximum number of hydrogen atoms) palmitic acid (11%) plus stearic acid (4%). Linolenic acid is subject to autooxidation which gives the oil an undesirable odor and flavor (Chang et al. 1983). Palmitic acid is implicated as a contributor to coronary heart disease (Willet 1994). Unsaturated fatty acids are healthier than saturated fatty acids. The monounsaturated fatty acid, oleic acid (18:1), is more stable in frying and cooking applications than are the polyunsaturated forms, linoleic (18:2) and linolenic (18:3). Chemical hydrogenation is employed to improve oxidative stability by increasing the concentrations of 18:1 fatty acid and converting the oil to a semisolid consistency, but hydrogenation also raises the concentration of trans fatty acids, which have been linked to higher health risks (Mazur et al. 1999).

Increasing the oleic acid content and simultaneously reducing linoleic and linolenic levels would increase the stability of the oil and possibly eliminate the need for chemical hydrogenation. Reducing palmitic and stearic acid would lower saturated fat content and make the oils more nutritionally acceptable for human consumption.

Polyunsaturated fatty acids (PUFA) are almost exclusively synthesized by plants, although animals are capable of converting one form to another through elongation and desaturation. It is generally understood that very few animals can synthesize PUFA *de novo* and this inability to synthesize PUFA is considered a general characteristic of insects (Downer 1978, Dadd 1983). A study by Rapport et al. (1984) did successfully

demonstrate an example of a dipteran that was reared through 10 generations in the absence of dietary polyunsaturated fatty acids, which was able to maintain a low level of linoleic acid. Other reports have cited instances where a few other insect species are able to biosynthesize linoleate (Blomquist et al. 1982, Cripps et al. 1986). As mentioned before, most insects studied lack this ability and hence, if they have a physiological need for polyunsaturates of any sort, they must exogenously acquire them from their food, directly, or as suitable precursor fatty acids. Many insects, in fact, do have a dietary requirement for small quantities of certain polyunsaturated fatty acids, and all insects known to have an essential fatty acid requirement can satisfy it by ingesting linoleic or linolenic acids, the C18 PUFA ubiquitously biosynthesized by plants. If other polyunsaturates are physiologically necessary for insects whose diet lacks them, they presumably are derived metabolically from the C18 food PUFA (Dadd et al. 1987).

These fatty acids are present in the phospholipids of many insect species and play an important role in regulating cell membrane properties in addition to serving as precursors for important hormones such as prostaglandins and other eicosanoids (Stanlay-Samuelson et al. 1988). Lepidopterans, in general, appear to require linolenic or linoleic acid in their diet. A shortage of linolenic acid in the diet of *Ephestia* spp. (Lepidoptera: Pyralidaea) results in moths emerging without scales on the wings (Stanlay-Samuelson et al. 1988). Also, mosquitoes reared with synthetic larval diets devoid of polyunsaturates correlates with the failure of such adults to fly and survive at emergence, a dysfunction which nutritional studies show can be overcome by the dietary provision of small amounts of certain individual long-chain polyunsaturated fatty acids

(Dadd et al. 1987). All herbivorous insects studied and reported in the literature require PUFA of plant origin (Blomquist et al. 1991) .

Fatty Acid Composition in Soybean Oil

The increasing demand for vegetable oil and fat that parallels the world's increasing population means that subsequently there is an increasing demand in yield and production from oilseed crops, including soybean. The majority of the world's edible fat is produced from vegetable oils (70%), with animal fats and marine oils making a substantially small contribution (Vles and Gottenbos 1989). Humans obtain their energy from three nutritional food sources: proteins, fats and oils. Fat has high energy potential and worldwide, 90% of the total non-protein energy in people's diets comes from fats and carbohydrates.

Linoleic (18:2) and Linolenic acid (18:3)

Conventional soybean contains approximately 8% linolenic acid, though cultivars today can contain anywhere between 1% and 10% linolenate depending on their source. The lower content cultivars tend to have been developed via genetic modification, with plant introductions contributing the higher linolenate content (Spear and Fehr 2007). Dutton et al. (1951) initially implicated linolenic acid as being responsible for the low oxidative stability of the oil. Subsequent investigations have shown that the three double bonds make it susceptible to oxidation, which is now known to contribute to the formation of undesirable flavors in soy food products. Apart from the undesirable flavors, there are increasing concerns about the ill effects of having a diet high in trans-fat content, and in January 2006, the U.S. Food and Drug Administration began requiring

that all foods products were to have their trans- fat content clearly indicated on the label. Low linolenic soybean oil has been pursued as a viable alternative to hydrogenated oil. At least three independent genetic loci, designated *Fan*, are associated with linolenic acid levels in soybean seed (Wilcox and Cavins 1987, Fehr et al. 1992, Rahman et al. 2001)

Palmitic acid (Palmitate) (16:0)

Palmitic acid, named after the oil palm *Elaeis guineensis* Jacq., is a type of fatty acid that is contained in soybean oil, as is stearic acid (Erickson et al. 1988). Both palmitic acid and stearic acid are classified as saturated fatty acids. Palmitic acid is the major saturated fatty acid in soybean oil and is well known to have a negative effect on nutritional quality in consumer diets. However, it is important in the manufacture of widely used cooking products such as margarine and shortening products. Palmitic acid content in conventional soybean is approximately 11%. In the USA, all commercially available food products must display a label indicating their total saturated fatty acid content. This is because saturated fatty acids raise the levels of low density lipoprotein cholesterol, which is associated with poor cardiovascular health (Kinney 1996, Wei et al. 2008). Fehr et al. (1991) produced a mutant reduced palmitic acid by treating soybean cultivar A1937 with *N*-nitroso-*N*-methyl-urea (NMU). Low palmitic acid content was found to be controlled by different alleles at two different loci: *fap1 fap 1, fapx fapx*. Palmitic acid content has been altered by mutation at the *Fap* loci (Erickson et al. 1988). A reduction in palmitic acid content improves the quality of the oil.

Stearic acid (18:0) and Oleic acid (18:1)

Stearic acid levels in soybean average 30 g kg^{-1} of the crude oil (USDA ARS 2004), whereas oleic acid is typically 180 to 240 g kg^{-1} (Wilcox et al. 1984, Diers et al.

1992). A strong negative correlation has been discovered between stearic and oleic acid in soybean (Pantalone et al. 2002). Stearic acid content has been altered by chemical mutatgenesis at the *Fas* locus (Spencer et al. 2003).

Lipoxygenase

Soybean seeds contain at least three lipoxygenase isozymes, lipoxygenases 1, 2 and 3 (Axelrod et al. 1981). These isozymes are responsible for grassy and unpleasant flavors in soybean seeds that have limited the popularity of soy products for human consumption (Rackis et al. 1979, Sessa 1979, Matoba et al. 1985). The biochemical properties of these isozymes have been well characterized. In the early 1980s, three soybean lines, each deficient in one of the lipoxygenase genes, were identified (Matoba et al. 1985). In subsequent studies, complete null mutants of soybean seed lipoxygenases were obtained. These have been shown to have reduced off flavors, particularly when L2 is absent (Wang et al. 1984).

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Table 2.1 Value added traits (VAT) and insect resistant quantitative trait loci (QTL) near-isogenic lines in the MG VII cultivar Benning background.

Lines	Characteristics
Insect QTL lines	
Benning M	Contains major SIR QTL[§] for antibiosis and antixenosis
Benning G	Contains minor SIR QTL for antibiosis
Benning H	Contains minor SIR QTL for antixenosis
Benning MGH	Contains all three SIR QTL (antixenosis and antibiosis)
VAT lines	
Benning lxy123	Elite germplasm that is lipoxygenase free
Benning RR lxy123	Lipoxygenase free germplasm with glyphosate tolerance
Benning C1726	Elite germplasm with low palmitic acid
Benning N87-2122-4	Elite germplasm with low palmitic acid
Benning (6) FAN	Elite germplasm with low linolenic acid
Checks	
Benning	Elite cultivar; recurrent parent for near isogenic lines
H7242 RR	Elite cultivar; Benning with glyphosate tolerance

[§] SIR QTL = Soybean insect resistant quantitative trait loci

Table 2.2 Fatty acid profile of soybean (Somerville et al. 2000)

Common name	Symbol*	IUPAC**	Saturation
Palmitic acid	16:0	Hexadecanoic acid	Saturated
Stearic acid	18:0	Octadecanoic acid	Saturated
Oleic acid	18:1	9-octadecenoic acid	Unsaturated
Linoleic acid	18:2	9, 12-octadecenoic acid	Unsaturated
Linolenic acid	18:3	9, 12, 15-octadecenoic acid	Unsaturated

* Initial number represents the number of carbon atoms; last number refers to the number of double bonds.
** International Union of Pure and Applied Chemistry.

CHAPTER III

EVALUATION OF PEST VULNERABILITY OF 'BENNING' SOYBEAN VALUE ADDED AND INSECT RESISTANT NEAR ISOGENIC LINES[1]

[1] Samuel-Foo, M., J. N. All and H. R. Boerma. To be submitted to *The Journal of Economic Entomology*.

ABSTRACT

Selected value added traits such as improved insect resistance or improved nutritional qualities can be incorporated into elite soybean germplasm using marker assisted selection as well as traditional breeding practices. Crop enhancement with value added traits may affect vulnerability to insects, and evaluating the susceptibility levels of the various value added traits in elite germplasm would aid in developing IPM strategies. During 2007-2008, five 'Benning' lines with different value added nutritional traits and four insect resistant quantitative trait loci (QTL) lines were evaluated in an effort to determine their pest vulnerability under artificial and natural insect pest populations.

The lines showed variable susceptibility to ten different insects classified as defoliators and stem or seed feeders in replicated greenhouse and field tests. The study was carried out in three locations in Georgia: The University of Georgia Plant Sciences Farm near Athens, the Southeastern Research Station and Education Center near Midville, and the Mountain Research and Education Center of the University of Georgia near Blairsville. The green cloverworm [*Hypena scabra* (F.)] was the most common lepidopteran defoliator occurring in fields. Other caterpillar pests found included the soybean looper [*Pseudoplusia includens* (Walker)], the bollworm [*Helicoverpa zea* (Boddie)] and the velvetbean caterpillar [*Anticarsia gemmatalis* (Hübner)]. Big eyed bug (*Geocoris* spp), threecornered alfalfa hopper *Spissistilus festinus* (Say) and white fringed beetles (*Graphognathus* spp.) were also included in this study. Mexican bean beetle (*Epilachna varivestis* Mulsant) activity was evaluated at Blairsville, GA. Data indicated that there was no significantly increased pest susceptibility among the value added cultivars with improved nutritional qualities, with the insect resistant QTL lines Benning

M and Benning MGH consistently being less susceptible to lepidopterous (*Noctuidae)* leaf injury. Defoliator resistance traits in the Benning lines, however, did not influence stink bug infestation of seed.

INTRODUCTION

Technological advances in soybean crop improvement have resulted in an enhanced capability to develop cultivars with various traits that fill different market niches. Value added soybean [*Glycine max* (L.) Merr] cultivars bred with certain quality traits serve to fill these developing niches as consumer demands for a healthier soybean continue to rise. Such traits are often referred to as seed quality, output traits and value added traits (VAT) as referred to in this study. VAT can be improvements in seed size and taste, or nutrient enhancements of a crop. These improvements have been accomplished by utilizing a combination of traditional plant breeding, molecular-maker based selection, and transgenic technologies (Erickson et al. 1988, Burton et al. 1994, Boerma and Mian 1999, Boerma and Walker 2005). These VAT alter the nutritional or functional properties of the plant for use in foods, animal feeds, or industrial products (Mazur et al. 1999).

Value added lines in this study refer to improved soybean breeding lines that have been bred with the purpose of altering specific characteristics such as improved fatty acid composition (low linolenic acid, low palmitic acid). Several improved soybean cultivars are now available that meet the nutritional needs of producers and consumers alike, and research on developing VAT soybean is plentiful. While these are noteworthy accomplishments, it is important that the acquisition of information concerning potential

pest vulnerability of value added cultivars accelerate to keep pace with changing soybean production and cultural trends and consumer demands. It is imperative that along with these crop improvement developments, knowledge of insect pest preference (susceptibility or resistance) should simultaneously increase. This provided the rationale for which this study was undertaken: An examination of the occurrence and impact of common soybean insect pests on VAT near-isogenic lines in the 'Benning' genetic background.

As global consumers continue to make increasing demands on our ability to supply crops with better nutritional components, growers are looking for ways to optimize yields while reducing production inputs. Developing value added crops is one approach to meeting these needs. Genetic modification of soybean quality traits is not a recent idea, in fact, soybean breeders have been attempting to modify fatty acid composition for more than 50 years (Fehr 2007). The primary goal of this has been the creation of a superior soybean whose oil composition more readily meets the needs of consumers. Developing soybean with altered fatty acid composition is of paramount importance if soybean is to remain competitive with other oil crops (e.g., canola) whose fatty acid composition is preferred by health-conscious consumers. Soybean-based foods (soy foods) have enjoyed a surge in popularity in North American markets in recent years. It has increased in momentum since the FDA decided to allow health claims to be placed on soybean-derived products with altered nutritional qualities (Kumudini et al. 2005). The demand for soy with value added nutritional qualities has increased annually and the soyfoods industry was estimated to soon exceed $4 billion (Kaufmann 2004).

Protein meal and oil are two primary components produced from soybean seed, so increasing both protein and oil concentration in seeds are persistent goals for most soybean breeders (Burton 1997). But protein and oil are negatively correlated, so there is often the difficulty that increasing protein usually results in a decrease in oil and vice versa (Brim and Burton 1979). Index selection has been used to simultaneously increase both (Openshaw and Hadley 1984, Burton 1991). The five major fatty acids contained in soybean oil are palmitic (C16:O), stearic (C18:O), oleic (C18:1), linoleic (C18:2), and linolenic (C18:3) (Li et al. 2002). About 10% of soybean oil is the polyunsaturated fatty acid linolenic acid and another 10% is the saturated fatty acid palmitic acid. Soybean oil for food uses would be improved if both were reduced. Linolenic acid is subject to autooxidation which gives the oil an undesirable odor and flavor (Chang et al. 1983). Palmitic acid is implicated as a contributor to coronary heart disease (Willet 1994). Increasing the oleic acid content and simultaneously reducing linoleic and linolenic levels would increase the stability of the oil and possibly eliminate the need for chemical hydrogenation which contributes to undesirable trans-fat formation. Reducing palmitic and stearic acid would lower saturated fat content and make the oils more nutritionally acceptable for human consumption (Fehr 2007). Therefore, low linolenic and palmitic levels are important qualities in terms of the saturated fatty acid profile of soybean and are likely to increase in importance as consumers continue to demand a healthier soybean.

Insect pests are one of the major concerns for Georgia soybean producers (McPherson et al. 2003). Soybean that is planted in the southern regions of the USA has a greater probability of incurring insect damage throughout the growing season than in

more temperate regions such as the Midwest. Growers must be concerned with both defoliators and soil dwelling insects, since economically important species occur in both habitats (Funderburk et al. 1999). The green cloverworm (GCW) [*Hypena scabra* (F.)], the soybean looper (SBL) [*Pseudoplusia includens* (Walker)], the bollworm (BW) [*Helicoverpa zea* (Boddie)] and the velvetbean caterpillar (VBC) [*Anticarsia gemmatalis* (Hübner)] are among the lepidopteran defoliators that regularly inhabit soybean fields in this region. The BW, SBL and VBC are considered serious pests of soybean planted in the southeastern USA. They damage crops mainly by severely defoliating susceptible plants, although pod and stem feeding also occasionally occurs. Losses are estimated at $4 million annually due to a combination of crop damage and cost of control (Riley et al. 1997). GCW is generally relegated to minor pest status in Georgia soybean fields (Hudson 1995), although it is classified as a major foliage feeding pest of soybean in Midwestern states (Turnipseed and Kogan 1976, Gouge et al. 1999). Additionally, the Mexican bean Beetle (MBB) [*Epilachna varivestis* (Mulsant)], a coleopteran defoliator, may be encountered, as well as the stink bug complex which attacks developing pods and stems (McPherson and McPherson 2000, Boethel 2004).

The stink bug complex consists of *Nezara viridula* (L.) (Southern green stink bug), *Acrosternum hilare* (Say) (green stink bug), and *Euschistus servus* (Say) (brown stink bug). In the southern USA, the complex is particularly damaging in Louisiana and other Gulf States (Gilman et al. 1982). Recently, Gore at al. (2006) reported that stink bugs are perhaps the most damaging insect pests of soybean in the USA because of direct yield losses associated with pod feeding and secondary losses in seed quality caused when pathogens are introduced. In Georgia in 1996, this pest complex was responsible

for a loss to soybean producers of approximately $2.3 million. Douce and McPherson (1991) report that the losses from this group may be as much as $13 million in some years.

The MBB is considered a major insect pest on soybean grown in the southern and mid-Atlantic regions of the USA (Sisson et al. 1976). Insecticides are most often employed to effectively control MBB, though the use of host plant resistance as a control measure has been and continues to be explored (Van Duyn et al. 1972, Hart et al. 1983). In their extensive screening of the USDA Germplasm Collection for Maturity Groups VII and VIII, Van Duyn et al. (1971b) identified three plant introductions as being highly resistant: PI 229358, PI 227687, and PI171451, and several others, including PI 229321, were found to be moderately resistant to this insect pest. In a follow-up study, they demonstrated that this resistance was primarily an expression of non-preference (Van Duyn et al. 1972).

The objectives of the present study were to (i) Evaluate soybean lines differing in VAT to assess the incidence, abundance, and feeding impact of common soybean insect pests, and to evaluate whether the VAT conveyed increased or decreased pest susceptibility to the common insect pest species, and (ii) evaluate lines containing the introgressed SIR QTLS in field conditions under natural insect infestations.

MATERIALS AND METHODS

Soybean Genotypes. The VAT soybean lines with improved nutritional content used in this study were developed using a combination of conventional breeding and marker assisted backcrossing. Benning soybean, which served as the donor parent into

which the various VATs were introgressed, was developed by the Georgia Agricultural Experiment Stations, and was released in January, 1996 from a F_4 plant from the cross 'Hutcheson' x 'Coker 6738' (Buss et al. 1988, Boerma et al. 1997b). Benning is a Maturity Group VII cultivar that is noted for its productivity and disease and nematode resistance (Boerma et al. 1997a).

Benning N87-2122-4 is a low palmitic acid, backcrossed derived line that was selected from a population of Benning x N87-2122-4. N87-2122-4 serves as an important source of reduced palmitic acid genes used by breeders across the USA (Li et al. 2002). Benning C1726 is a breeding line with reduced palmitic acid levels that was developed by mutagenesis of the cultivar Century (Wilcox and Cavins, 1990). Benning (6) FAN is a low linolenic acid line that resulted from a modification at the *Fan* locus using chemical mutagenesis. Benning lxy123 and Benning RRlxy123 are near-isogenic lines that lack the three lipoxygenase enzymes which contributes to the off flavor of soybean. Benning RRlxy123 has the additional quality of being glyphosate tolerant.

The SIR QTL-containing lines Benning M, G, and H were developed via marker assisted selection at the University of Georgia and released in February of 2006 for use as sources of single and multiple SIR QTLs (Zhu et al. 2007). Benning MGH is a near-isogenic line containing three SIR QTLs originating from PI 229358. The SIR QTLs were initially identified by Rector et al. (1998, 1999, 2000) using restriction fragment length polymorphisms (RFLPs). Table 3.2 summarizes all of the soybean insect pests that were evaluated in this study.

The VAT characteristic of each near-isogenic line included in this study was verified in laboratory and greenhouse testing using chemical and molecular marker assays. Seed of the lines with altered fatty acid content (Benning N87-2122-4, Benning C1726, and Benning (6) FAN) were sent to the USDA-ARS National Center for Agricultural Utilization Research at Peoria, IL, for verification of their altered oil composition. Fatty acids were determined on a model 1255 Infratec NIR food and feed grain analyzer (Ultra Tec Manufacturing, Inc., Santa Ana, CA). The lipoxygenase-free lines were verified using seed chips in a colormetric laboratory assay modified after Suda et al. (1995). The presence of the insect resistant QTLs in the near-isogenic lines (Benning M, Benning G, Benning H, and Benning MGH) were verified using SSR molecular markers. The glyphosate resistance of near--isogenic lines (H7242 RR and Benning RRlxy123) was verified by applying Roundup® herbicide to 12 greenhouse plants.

Antixenosis Tests. Antixenosis (feeding preference) bioassays were conducted in a greenhouse at the University of Georgia (All et al. 1989). SBL eggs were supplied by the USDA-ARS Crop Protection and Management Unit in Tifton, GA and BW eggs originated from the insect supply company Benzon Research (Carlisle, PA). Two seeds representing each entry were planted in 450-mL polystyrene foam cups that had three holes punched in the bottom to allow for soil drainage and water uptake by the test plants. The cups were filled with a commercially available soil mixture (Craven Pottery, Commerce, GA) that had been amended with Osmocote® slow release fertilizer. After germination, the seedlings were thinned to allow the healthier of the two plants to be used in the assay. The cups were arranged in a stainless steel tray that measured 4.9 m long x

1.2 m wide x 8 cm deep in a randomized complete block experimental design with 20 replications. After insects were added to the test plants, the tray was filled with approximately 2 cm of water to allow for bottom watering of the plants and to avoid disturbing the insects. This also created a "moat" isolating each replication. The trays remained filled through the duration of the experiment.

About 14 d after germination, after the first trifoliolate leaf had emerged and was just expanding, individual plants were infested with four neonate larvae (<5 h old) using a size 000 soft-tipped camel's hair brush. The cups of each replication were arranged in a cluster to allow complete merging of the foliage, so that larvae could freely migrate from leaf to leaf. The insects were monitored daily, and any larvae that might have accidentally fallen from the foliage were repositioned on the closest plant. About 10 to 12 days after infestation, the percent leaf area consumed (defoliation) was visually estimated by at least three individuals and the mean of these estimates was calculated and used in the statistical analysis.

Field Procedures and Sampling Methods. Soybean seed were planted at a rate of 330 seeds per meter of row. The seed was planted using a push cone planter or a tractor pulled vacuum planter. All insect infestations were naturally occurring. Insect sampling was done using sweep net and drop cloth sampling methods. Defoliation ratings were visually scored by estimating insect defoliation from the entire plot. The specifics followed for each insect species included in the study are outlined below.

Lepidopterous (Noctuidae) Defoliator Sampling. Field plots were examined for the incidence and abundance of caterpillar pests. Field tests were planted in two locations: the University of Georgia Plant Sciences Farm near Athens, GA (Oconee

County) and at the University of Georgia Southeast Research and Education Center near Midville, GA (Burke County). In 2007, plots were two rows, 3 m in length with a 1 m spacing between rows. The plots were separated by 1-m alleys and the test was set up in a randomized complete block design with four replications and three soybean border rows on either side of the experiment. In 2008, plots were two rows, 6 m in length with 0.8 m between rows. Ranges were separated by 1.5-m alleys and were arranged in a randomized complete block design with four replications and two soybean border rows on either side of the experiment.

Plants were sampled over a 5-week interval in 2007 and 2008 when they were in the R2-R3 growth stage (full bloom to beginning pod fill) (Fehr et al. 1971). Sampling was accomplished each week by alternating between sweep net and drop cloth sampling methods. For sweep net sampling, a 25-sweep sample was taken from both rows of each plot using a 38-cm diameter sweep net as described in Kogan and Pitre (1980). Drop cloth sampling involved using a 1-m x 1-m white canvas collection sheet which was placed on the soil surface, between each two row plot. Samples were taken by extending the sheet, gently bending the plants over the drop cloth, and vigorously shaking them so that insects were dislodged and fell onto the cloth. Special care was taken to avoid disturbing the plots before sampling. Any insects found on the underside of the cloth were included in the insect counts. All plots were visually rated for percent defoliation. The data were analyzed using the GLM procedure of SAS (SAS 2003) for a randomized complete block design.

Lesser Cornstalk Borer Sampling. Infestations by LCB were evaluated by counting the number of dead seedlings in each plot within two weeks after

their emergence. Plants identified as severely wilted were included in the counts, but these were removed from the plot and individually inspected for the characteristic LCB tunneling point of entry at the plant's stem base before being included in the counts. Before attributing plant damage to LCB, the soil surrounding symptomatic plants was examined for silk feeding tubes or webbing that the larvae are known to construct (Funderburk et al.1999). Data analysis was as previously mentioned.

Stink Bug Complex Sampling. All plots were sampled weekly in 2007 and 2008 during the period starting when plants were in the R_3 growth stage (beginning pod fill) (Fehr et al. 1971) by alternating between a 38-cm diameter sweep net and a 1-m x 1-m white canvas drop cloth. In 2008, sampling at the Athens location was done over an abbreviated period (2 weeks), as herbicide drift from a nearby field caused foliage damage to the experiment, limiting the effective sampling period. Sweep net sampling was accomplished by taking a random 25-sweep sample down the center of both rows of each plot and recording the total number of stink bug adults and nymphs collected. Drop cloth sampling involved placing the canvas sheet on the soil surface near the center of a plot and vigorously shaking the soybean plant to dislodge any stink bug nymphs or adults that were present in the plot. The samples were counted on-site by at least two individuals, and the numbers of stink bug nymphs and adults collected were noted from each sample. At maturity, the lines from Athens and Midville were individually hand harvested and seed were later threshed using an ALMACO plot combine (Nevada, IA). After threshing, the seeds were weighed, counted, and inspected for stink bug damage. Seeds were taken to the laboratory and rated as undamaged or stink bug damaged (visible puncture marks observed usually accompanied by seed discoloration and seed distortion).

A minimum of 1000 seeds/plot were evaluated for stink bug damage. This sample size was arrived at via a series of preliminary tests in which it was determined that 1000 seeds would provide an accurate estimate of the average damage to seeds (data not shown). Seeds were individually examined one at a time by counting out the required number of seeds and placing them in a small vial. Once this was done, the seeds were retrieved from the vial and examined under a dissecting microscope at 20 X power. Damaged seeds contained easily distinguishable stink bug damage symptoms and characteristics. Once a seed was categorized as damaged, it was placed in a separate container and later tested for seed germination.

Germination evaluations were conducted in pesticide-free greenhouses on the campus of the University of Georgia. One hundred seeds from both the stink bug damaged and undamaged samples were planted in germination trays at 82 ^{o}F, under 13 hours of supplemental light. The number of germinated seed were counted 7 days after planting and recorded for each sample. For protein and oil and fatty acid determination, a 50g seed sample of the stink bug damaged and undamaged seeds were sent to the USDA-ARS National Center for Agricultural Utilization Research at Peoria, IL. There, an 18 to 20-g sample of seed was analyzed for protein and oil composition with a model 1255 Infratec NIR food and feed grain analyzer (Ultra Tec Manufacturing, Inc., Santa Ana, CA). The protein and oil values were converted to a moisture free basis.

For statistical analyses, all three species of stink bugs collected in the fields (green, southern green and brown) were pooled, but adult and nymph stages were analyzed separately. The data collected were analyzed using the Proc GLM Procedure of SAS (SAS 2003).

Mexican Bean Beetle Sampling. The Benning value added near-isogenic lines as well as the insect QTL near-isogenic lines were planted at the University of Georgia Mountain Research and Education Center near Blairsville (Union County) GA in 2007 and 2008. This experiment was also planted in 2006, but with only nine lines; Benning C1726 and Benning N87-2122-4 were not included. The lines were planted in two row plots, arranged in a randomized complete block design with four replications. Plots were 0.96 m wide and 3.0 m long with 1.0 m alleys between replications. The entire experimental area was surrounded by other soybean lines. A deer-repellant fence and rope-net rabbit fence were constructed to encircle the growing plants to prevent varmint herbivory.

The experiment was sampled weekly over a five-week period in 2006 and 2007, beginning when the plants were in the R3 growth stage (Fehr et al. 1971). Sampling in 2008 was only extended over a three-week period due to earlier than expected cool temperatures which resulted in early leaf drop. For data collection, 20 randomly chosen trifoliolate leaves were selected and examined for the presence of MBB adults and immatures. The trifoliolate leaves were chosen from within the center area of the two row plots, and at least two trifoliates were examined per plant. The leaves were inspected on both the upper and lower sides and the number of MBB found were counted, and the life stage (adult vs larva) was recorded. Insects found along any plant stems or terminals during leaf inspections were also included in the total counts. Percent defoliation ratings were also estimated weekly on a plot basis. These ratings were performed by a single individual in 2006 and 2007, but in 2008 plots were rated by two individuals, and their ratings were averaged for increased reliability and accuracy. This visualization procedure

method is considered a reliable estimate of relative defoliation among treatments when compared with leaf area removed that is measured with an area meter (McPherson et al. 1996), or when compared to photographic representations of leaves of similar shape and size with calculated percent defoliation (All et al. 1989). The data were analyzed as indicated above for other insect species.

Miscellaneous Insects Sampling. Other insects surveyed included the threecornered alfalfa hopper (TCAH), the bigeyed bug (BEB), and whitefringed beetles (WFB). Sampling for the TCAH, BEB, and WFB was as described for the lepidopterous defoliators and stink bugs with drop cloth and sweep net samples alternating weekly. Any TCAH and BEB found on the drop cloth or in the sweep net were always counted first due to their being a flight risk. Data were analyzed as indicated above.

RESULTS AND DISCUSSION

Insect Bioassays. In this study, near-isogenic lines differing in VAT were evaluated for incidence, abundance, and increased (or decreased) susceptibility to both major and minor soybean insect pests. Results from the greenhouse antixenosis studies showed that the insect resistant QTL line Benning M was significantly less defoliated by all test insects than the checks Benning and H7242RR. (Table 3.3). Benning MGH was equally less defoliated than the checks when fed upon by the BW, SBL, VBC, and BAW. Not surprisingly, the VBC and SBL were the most voracious defoliators of the four caterpillar pests, consuming more than 50 percent of all but the insect resistant lines Benning M and Benning MGH (Table 3.3). Benning G, which contains a minor SIR-QTL for antibiosis against lepidopterous pests, was among the most defoliated lines for the

BAW, BW, and VBC. Similarly, Benning H, which contains a minor antixenosis SIR QTL, did not have a significant reduction effect on larval leaf feeding in these assays. These observations have been previously noted, and indicate that SIR QTL-G and SIR QTL-H are only effective when combined with the resistance allele(s) of SIR QTL-M (Zhu et al. 2006).

The results with the VAT lines showed that the BW equally defoliated Benning (6) FAN as well as the checks Benning and H7242RR. The remaining VAT lines all similar to the checks (Table 3.3). There were no significant differences among the VAT near-isogenic lines and checks for VBC defoliation. Additionally, the VAT lines did not have greater leaf injury than the checks when defoliated by SBL and BAW, although the low palmitic acid line Benning N87-2122-4 was among the most highly defoliated lines by SBL. In general, these insect pests did not appear to discriminate between the VAT near-isogenic lines in the antixenosis assays. Overall, these results indicated that the VAT did not have increased susceptibility to any of the major noctuid defoliators of soybean.

Field tests (Noctuidae). In field testing, the total lepidopteran insect population sampled in the test plots was slightly higher in 2007 at Midville compared to 2008 (Table 3.4). In 2007 at Athens the insect numbers were similar to 2007 at Midville. At Midville in 2007, the average defoliation for the insect QTL lines Benning M and Benning MGH was significantly less than Benning and H7242RR (Fig 3.1). Benning G and Benning H, as well as the five VAT near-isogenic lines, were equally defoliated by the caterpillar pests in the experiment. Similarly, in Midville in 2008, the defoliation of Benning M and Benning MGH was again significantly less than the checks, with Benning being the most defoliated of all the test lines (Fig 3.2). In fact, Benning showed almost twice as much

defoliation as the most susceptible VAT line, Benning C1726. There were no significant differences among the individual VAT near-isogenic lines, but as a group they were lower than both check cultivars.

At the Athens location in 2007 the insect QTL near-isogenic lines Benning MGH was the least defoliated of all the lines evaluated in this study (Fig. 3.3). Overall, there were no significant differences among the individual VAT near-isogenic lines for lepidopteran defoliation. Benning, the susceptible check, remained the most defoliated line, as was the case in the Midville locations. Initially, the VAT lines with altered nutritional characteristics were all less defoliated than the Benning susceptible check. By the time subsequent sampling was done, however, the percent defoliation had increased to almost equal that of the Benning.

Overall, the percent of defoliation was relatively low between the 11 entries in all the trials and this may have contributed to the fact that no meaningful differences were observed in abundance of insects among the individual entries at the different locations (Table 3.4). Additionally, the GCW was the most abundant foliage-feeding caterpillar recovered from sweep net and drop cloth sampling. This insect is generally regarded as a minor pest species in Georgia (Turnipseed and Kogan 1976) when compared to the VBC, SBL and BW. These insects failed to reach threshold levels during sampling and therefore low lepidopteran larval abundance of major defoliators may be a reason why there were no significant differences among the entries. This is the first time that these insect QTL near-isogenic lines have been evaluated in field conditions with natural pest populations. Walker et al. (2000) and Zhu et al. (2006) have previously reported on the

effectiveness of these insect QTLs when evaluated under artificial pest conditions using field cage studies and laboratory assays.

Lesser Cornstalk Borer. Seedlings were examined for LCB damage weekly within a 14-day period after emergence (Table 3.5). There were no significant differences observed among the near-isogenic lines and checks in either location in either year except for 2007 in Midville, where the cultivar Benning lxy123 had three times the of dead/damaged plants, than Benning C1726 (Table 3.5). LCB optimally prefers hot, dry conditions, and the wet weather encountered in 2008 in Athens and in Midville in 2007 might have reduced the damage from LCB. Gouge et al. (1999) have reported that insects that feed on seedlings are important only if stands are damaged to the extent that yields are reduced. Eighteen to 24 healthy seedlings per row meter are sufficient for optimum yields. Threshold numbers of LCB damaged plants did not occur in this study.

Stink Bugs. There was a range of 5 to 10% stink bug damaged seed at Athens and 8 to15% at Midville. There was no statistical difference in damage between any of the near-isogenic lines and Benning and H7242RR checks (Table 3.6). As with the other insects surveyed, stink bug populations were low in both years at both locations but Midville test plots generally had higher numbers of stink bugs infesting the soybean plants when compared to Athens (Tables 3.9, 3.10 and 3.11).

While there were no significant differences between the locations nor among individual near-isogenic lines and the check cultivars in terms of mean percent of stink bug damaged seeds, Benning (6) FAN and Benning C1726 produced among the least number of stink bug damaged seeds in both locations. In general, the near-isogenic lines with altered nutritional characteristics produced comparable stink bug damaged seeds as

the insect QTL lines. Benning had the most damaged seeds in Athens in 2007, but in Midville was only ~2% more damaged than the least damaged line, Benning G. H7242RR produced the most stink bug damaged of all lines in the test in Midville.

Seed viability of damaged versus undamaged seeds was tested in a 7-day germination test (Table 3.6). All of the stink bug damaged seed had substantially fewer seedlings emerge compared to the undamaged seeds. Benning G had the highest germination rate (80%) for stink bug damaged seed among all the lines, with the other lines having a seedling emergence rate ranging for stink bug damaged seed from 51-76%. The undamaged seeds averaged a 22% higher germination rate with four lines having ≥ 90% germination (Benning, Benning G, Benning lxy 123, and Benning RR lxy123). Overall, the mean germination rate was significantly different between damaged and undamaged seed, with the undamaged seed having more than a 20% higher germination rate (Table 3.6). The decreased viability of stink bug damaged seed is consistent with previous reports (Todd 1976, Russin et al. 1987). Additionally, the overall percentage of SB damaged seeds had a greater number of seed splits and broken seed coats compared to the undamaged seeds.

An examination of the protein and oil levels (Table 3.7), and the fatty acid profile (Table 3.8) for the stink bug damaged seed versus the undamaged seed, demonstrated an overall reduction in oil content accompanied by elevated protein content. On average, there was at least 1% less oil and 1% more protein in the damaged seeds compared to the undamaged seeds. This is consistent with previous reports (Daugherty et al. 1964, Miner 1966) which point out that in addition to the shriveled and discolored seeds that are often the result of stink bug feeding, seed oil and protein levels tend to increase inversely.

Fatty acid profiles were generally higher for damaged seeds compared to undamaged seeds (Table 3.8). The low palmitic acid lines Benning C1726 and N87-2122-4 both showed increases in palmitic acid levels in damaged seeds. Benning C1726 had a 1.3% increase in palmitic acid levels, while N87-2122-4 had a 0.06 % increase compared to the undamaged seeds. A similar observation was made with the low linolenic acid line Benning (6) FAN, which showed a slight increase in linolenic acid in stink bug-damaged seed vs undamaged seed. Decreasing linolenic acid levels while increasing oleic acid levels is important for improving the functionality of soybean oil (Oliva et al. 2006). Similarly, soybean genotypes with decreased palmitic acid and stearic acid levels are more desirable. In this study, linolenic acid more frequently increased (73%) in seeds that had been punctured and/or fed upon by stink bugs. Oleic acid levels decreased in 45% of the test lines, while stearic acid and palmitic acid increased in 64% and 45%, respectively, of the lines evaluated (Table 3.8). These results indicate that a high level of stink bug damaged seeds in cultivars that have been specifically altered to improve its nutritional profile could reduce the usefulness of the seeds. The 2008 Midville GA fields generally had higher numbers of stink bugs compared to both locations in 2007, but there were no significant differences among the lines in stink bug damage.

Mexican Bean Beetles. Benning M and Benning MGH were the least defoliated near-isogenic lines in all three years in this test (Table 3.12). In a comprehensive review of USDA soybean germplasm, Van Duyn et al. (1971b) identified three PIs that had high levels of resistance to MBB defoliation: PI 171451, PI 227687 and PI 229358. PI 229358 is the source of the insect resistance alleles in the insect QTL lines examined in this study. Of the three years of MBB evaluation, defoliation percentages

were generally higher in 2006 because of what appeared to be a greater number of MBB. The VAT line Benning lxy123 had the highest levels of defoliation among the test lines in every year. In 2008, Benning RR123 had equally high levels of defoliation to Benning lxy123, although they were not significantly different from the check cultivars. This seems to indicate that Benning lxy123 is susceptible to MBB insect feeding. The insect QTL lines Benning M and Benning MGH were the least defoliated of all the test lines in all years. There were no significant differences among the remaining lines in the study, an indication that no line had increased (nor decreased) levels of susceptibility to MBB feeding.

Miscellaneous Insects. Numbers of BEB, TCAH and WFB were generally low in both years at both locations. In 2008, the numbers of TCAH were much higher than the previous year in both locations. Overall no differences were noted among the cultivars.

In conclusion, these studies indicate that enhancing high yielding cultivars such as Benning with desirable nutritional or industrial food traits may not substantially increase risk for insect infestations. Greenhouse tests with noctuid defoliators had intense artificial infestations and the VAT lines had substantial damage, however, the injury was comparable to susceptible Benning. The insect QTL lines Benning M and Benning MGH tested in the antixenosis greenhouse assays were consistently significantly less defoliated than the other lines, including Benning G and Benning H. The result with Benning G and Benning H is not surprising, and our results are in agreement with previously published reports (Zhu 2006, and Warrington et al. 2008) indicating that these two minor SIR-QTLs require epistatic interactions with resistance alleles on LG M for optimization.

Field tests in northern, central and southern Georgia had normal infestations of a variety of insects that are pests of seedling, vegetative and reproductive growth stages of soybean without any VAT showing abnormal vulnerability. The overall greenhouse and field results indicate that if VAT are incorporated into elite soybean cultivars, then insect resistance should be added as well in order to maximize reduction of pest risk without using insecticides. Additionally, the insect QTL lines evaluated here performed as expected, with Benning M and Benning MGH being consistently less defoliated than the other test lines. This was the first incidence of testing these insect QTL lines under natural pest populations in normal field conditions and our results are similar to those previously reported that these introgressed SIR-QTL traits nay be are effective tools available to be used by growers as part of a comprehensive soybean pest management strategy.

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Table 3.1 Value added traits (VAT) and insect resistant quantitative trait loci (QTL) near-isogenic lines in the MG VII cultivar Benning background

Line	PedigreeValue	Characteristics
Insect QTL lines*		
Benning M	Benning (7) x PI 229358	Contains major SIR QTL[§] for antibiosis and antixenosis
Benning G	Benning (7) x PI 229358	Contains minor SIR QTL for antibiosis
Benning H	Benning (7) x PI 229358	Contains minor SIR QTL for antixenosis
Benning MGH	Benning (7) x PI 229358	Contains all three SIR QTL (antixenosis and antibiosis)
VAT lines		
Benning lxy123	'Benning' (5) x lxy123	Elite germplasm that is lipoxygenase free
Benning RR lxy123	'Benning-RR' (5) x lxy123	Lipoxygenase free germplasm with glyphosate tolerance
Benning C1726	'Benning' (5) x C1726	Elite germplasm with low palmitic acid
Benning N87-2122-4	'Benning' (6) x N87-2122-4	Elite germplasm with low palmitic acid
Benning (6) FAN	'Benning'(6) x FAN	Elite germplasm with low linolenic acid
Checks		
Benning	'Hutchenson' x 'Coker 6738'	Elite cultivar; genetic background for test lines
H7242 RR	'Benning' x 'H7242RR'	Elite cultivar with glyphosate tolerance; Benning RR

[§] SIR QTL = Soybean insect resistance quantitative trait loci
***** Zhu et al. (2007)

Table 3.2 Soybean pests surveyed in this study: a presentation of their common names, scientific names and their pest types.

Insect Order	Family	Common name	Scientific name	Pest Type
Lepidoptera				
	Noctuidae	Bollworm	*Helicoverpa zea* (Boddie)	Defoliator, pod feeder
	Noctuidae	Soybean looper	*Pseudoplusia includens* (Walker)	Defoliator
	Noctuidae	Velvetbean caterpillar	*Anticarsia gemmatalis* (Hübner)	Defoliator
	Noctuidae	Green cloverworm	*Hypena scabra* (Fabricius)	Defoliator
	Pyralidae	Lesser cornstalk borer	*Elasmopalpus lignosellus* (Zeller)	Stem feeder, soil pest
Hemiptera				
	Membracidae	Threecornered alfalfa hopper	*Spissistilus festinus* (Say)	Stem feeder
	Pentatomidae	Southern green stink bug	*Nezara viridula* (L.)	Seed, pod feeder
	Pentatomidae	Green stink bug	*Acrosternum hilare* (Say)	Seed, pod feeder
	Pentatomidae	Brown stink bug	*Euschistus servus* (Say)	Seed, pod feeder
	Coleoptera			
	Coccinellidae	Mexican Bean beetle	*Epilachna varivestis* Mulsant	Defoliator

Table 3.3 Defoliation of Benning VAT lines and insect resistant near isogenic-lines from feeding by the bollworm (*Helicoverpa zea;* BW), soybean looper (*Pseudoplusia includens;* SBL*),* velvetbean caterpillar (*Anticarsia gemmatalis;* VBC) and the beet armyworm (*Spodoptera exigua;* BAW*)* in greenhouse antixenosis tests.

	Mean Percent Defoliation[§]			
Test Lines	**BW**	**SBL**	**VBC**	**BAW**
Insect QTL lines				
Benning M	20.0f	23.9e	48.3c	27.2e
Benning G	40.7bc	56.8bcd	72.2a	54.5a
Benning H	30.9de	67.0ab	52.8bc	46.5b
Benning MGH	20.7f	37.8e	53.1bc	28.2e
VAT lines				
Benning lxy123	35.9cd	67.2ab	66.3ab	44.1b
Benning RR lxy123	35.0bcde	64.7abc	61.8abc	31.8de
Benning C1726	28.2def	62.5abc	---	----
Benning N87-2122-4	26.7cef	72.7a	---	---
Benning (6) FAN	46.5ab	57.2bcd	68.8ab	40.5bc
Checks				
Benning	53.1a	59.3bcd	68.5ab	42.5b
H7242RR	40.8bc	66.1ab	72.2a	34.5cd

[§]Means within a column followed by the same letter are not significantly different (P>0.05; Duncan's multiple range test).

Table 3.4 Abundance of lepidopteran (Noctuidae) caterpillars infesting Benning insect resistant QTL and VAT lines near Midville and Athens GA, 2007- 2008.

Test Lines	Total caterpillars per row m (Mean±SE)					
	Midville GA				**Athens GA***	
	26 Sept 07	10 Oct 07	6 Sept 08	9 Oct 08	12 Sept 07	25 Sept 07
Insect QTL lines						
Benning M	0.7±0.4	0.9±1.0	0.4±0.4	0.3±0.4	0.1±2.2	0.7±0.8
Benning G	1.0±0.7	1.0±1.2	0.4±0.3	0.4±0.4	0.2±2.3	0.5±0.5
Benning H	0.7±0.6	0.9±1.0	0.4±0.3	0.5±0.7	0.2±2.7	0.9±1.0
Benning MGH	1.2±0.7	1.5±1.8	0.4±0.4	0.4±0.5	0.2±2.7	0.2±0.2
VAT lines						
Benning lxy123	0.8±0.9	0.9±1.1	0.5±0.5	0.2±0.3	0.0±2.5	0.8±0.9
Benning RR lxy123	0.5±0.4	0.9±1.1	0.4±0.4	0.3±0.5	0.0±2.1	0.5±0.6
Benning C1726	0.6±0.4	0.7±0.8	0.4±0.3	0.2±0.2	0.1±1.5	0.5±0.5
Benning N87-2127-4	0.9±0.5	0.5±0.6	0.3±0.3	0.3±0.4	0.2±2.4	0.5±0.6
Benning (6) FAN	0.9±0.6	1.1±1.2	0.3±0.3	0.5±0.7	0.0±1.8	0.6±0.7
Checks						
Benning	0.9±0.6	0.9±1.0	0.9±0.9	0.5±0.6	2.1±2.4	0.4±0.5
H7242 RR	0.7±0.7	1.2±1.5	0.5±0.4	0.3±0.4	0.3±3.1	0.1±0.7

*Athens GA 2008 data is not included as field suffered from a misdirected herbicide application from a surrounding field.

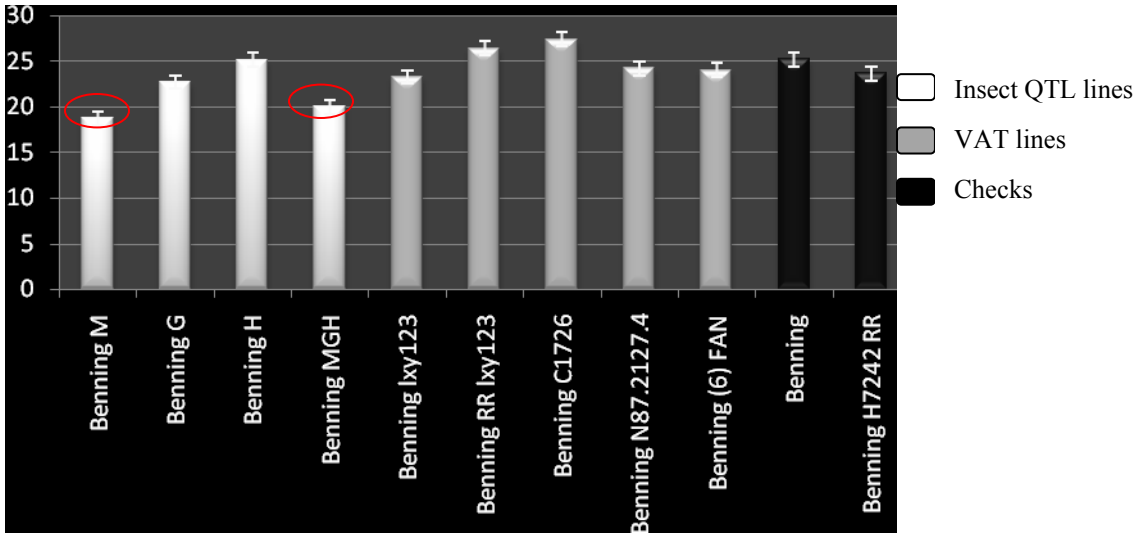

Figure 3.1 Average percent defoliation (mean ± SE) by lepidoterous (Noctuidae) pests in insect resistant QTL and VAT near-isogenic lines from 19 Sept to 10 Oct 2007, Midville GA. Plants were in the R2-R3 growth stage at the time of sampling

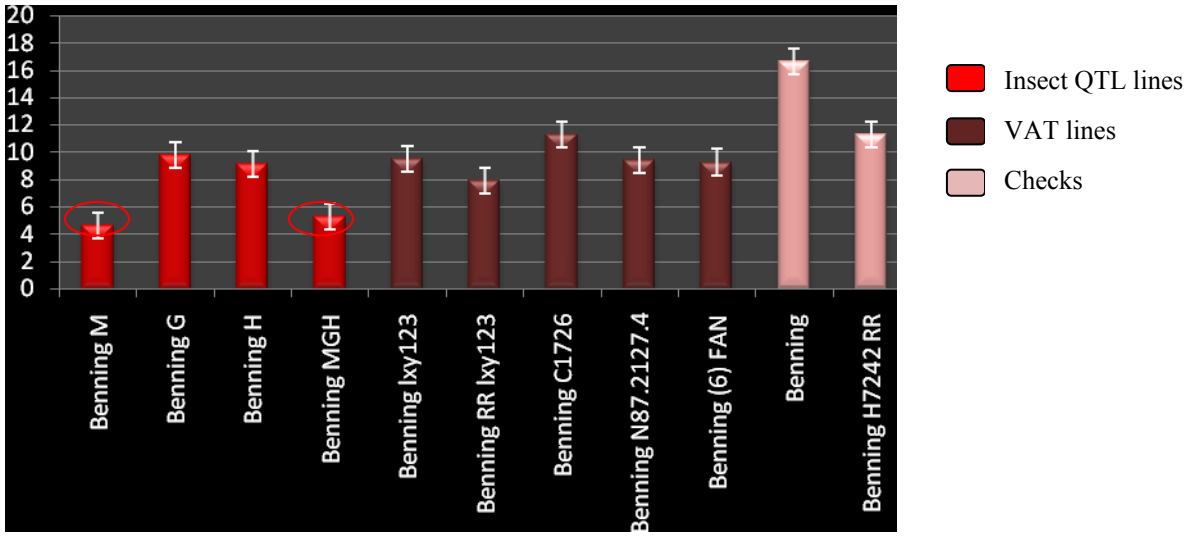

Figure 3.2 Average percent defoliation (mean ± SE) by lepidopterous pests (Noctuidae) in insect resistant QTL and VAT lines from 16 Sept to 9 Oct 2008, Midville GA. Plants were in the R2-R3 growth stage at the time of sampling

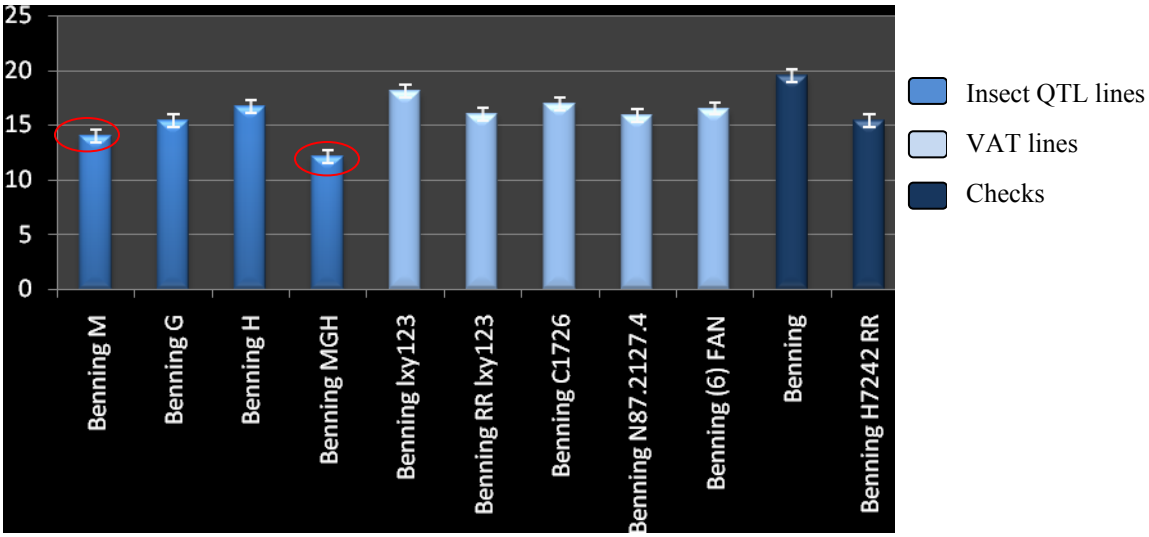

Figure 3.3 Average percent defoliation (mean ± SE) by lepidopterous pests (Noctuidae) in insect resistant QTL and VAT lines from 12 Sept to 4 Oct 2007, Athens GA. Plants were in the R2-R3 growth stage at the time of sampling

Table 3.5 Mean number of dead/damaged insect resistant QTL and VAT near isogenic lines per 100 m row due to lesser cornstalk borer (*Elasmoplapus lignosellus*) near Midville and Athens GA, 2007-2008.

	Mean no. dead/damaged plants[§]			
	Midville		Athens	
Test lines	2007	2008	2007	2008
Insect QTL lines				
Benning M	1.5ab	2.4a		32.3a
Benning G	1.8ab	7.0a		26.5a
Benning H	0.9ab	4.3a		18.9a
Benning MGH	1.5ab	4.0a		26.5a
VAT lines				
Benning lxy123	2.4a	4.6a	**	28.3a
Benning RR lxy123	0.9ab	5.5a		15.2a
Benning C1726	0.6b	4.0a		18.9a
Benning N87.2127.4	1.8ab	2.1a		38.1a
Benning (6) FAN	1.5ab	4.9a		17.1a
Checks				
Benning	1.2ab	3.4a		20.7a
H7242 RR	0.9ab	7.9a		20.7a

[§] Means within a column followed by the same letter are not significantly different (P>0.05; Duncan's multiple range test).
** Data unavailable for Athens 2007.

Table 3.6 Mean percentage of seed with stink bug damage obtained from Midville and Athens GA in 2007 plus the associated germination percentages obtained from stink bug damaged and undamaged seed in 2007. Seed samples were combined from plots and used in germination tests.

Test Lines	Stink Bug Damaged Seed (%)		Percent Germination	
	Athens	**Midville**	**Damaged**	**Undamaged**
Insect QTL lines				
Benning M	5.9	11.8	59	86
Benning G	8.4	8.1	80	97
Benning H	6.3	15.1	51	80
Benning MGH	8.1	10.2	64	83
VAT lines				
Benning lxy123	6.4	12.5	75	91
Benning RR lxy123	7.7	11.8	63	93
Benning C1726	4.9	10.5	63	88
Benning N87.2127.4	5.8	10.5	58	84
Benning (6) FAN	5.4	8.1	80	88
Checks				
Benning	10.8	10.2	65	90
H7242 RR	6.8	13.3	76	89
Overall Mean			**66.7b**	**88.1a**

[§] Means within a column followed by the same letter are not significantly different (P>0.05; Fisher's LSD test).

Table 3.7 Comparison of protein, oil and moisture content of stink bug damaged seed vs undamaged seed harvested from the same combined plots from Midville and Athens GA.

Test Lines	Damaged Seeds (%)		Undamaged Seeds (%)	
	Protein	Oil	Protein	Oil
Insect QTL lines				
Benning M	42.4	20.1	41.3	21.2
Benning G	41.5	20.4	40.4	21.6
Benning H	42.1	19.5	40.4	21.1
Benning MGH	42.7	19.4	41.0	21.3
VAT lines				
Benning lxy123	43.6	19.7	41.7	21.4
Benning RR lxy123	43.2	19.6	41.5	21.5
Benning C1726	41.8	19.5	41.1	21.0
Benning N87.2127.4	43.3	19.5	41.3	21.2
Benning (6) FAN	41.9	19.9	40.9	21.7
Checks				
Benning	42.5	19.6	40.8	20.9
H7242 RR	43.2	19.6	41.1	21.0
Means	42.6	19.7	41.0	21.3

Table 3.8 Comparison of stink bug damaged (Dam) vs undamaged (Undam) soybean seed fatty acid profiles.

Test Lines	Fatty acid profile (g kg^{-1})									
	Palmitic acid		Stearic acid		Oleic acid		Linoleic acid		Linolenic acid	
	C 16:0		C 18:0		C 18:1		C 18:2		C 18:3	
	Dam	Undam	Dam	Undam	Dam	Undam	Dam	Undam	Dam	Undam
Insect QTL lines										
Benning M	133	132	41	37	203	166	547	582	77	83
Benning G	131	139	41	39	212	220	533	528	82	74
Benning H	130	128	38	34	207	191	541	562	84	84
Benning MGH	122	131	41	40	208	194	549	562	81	73
VAT lines										
Benning lxy123	137	132	41	37	204	219	541	537	76	74
Benning RR lxy123	129	130	37	43	201	253	542	501	84	73
Benning C1726	128	115	44	37	199	211	549	562	81	75
Benning (6) FAN	127	137	39	41	201	218	581	562	45	42
Benning N87.2127.4	112	111	36	36	227	204	559	572	66	77
Checks										
Benning	129	142	37	39	175	184	574	557	85	78
H7242 RR	131	131	42	38	248	218	498	541	81	73
Means	128	130	40	38	208	207	547	551	77	73

Table 3.9 Seasonal mean of stink bugs per plot of Benning VAT and near-isogenic lines collected using sweep net (SN)* and drop cloth (DC) sampling from Midville GA in 2007.

Test Lines	SN 9/19/07[§] Nymph	SN 9/19/07[§] Adult	DC 9/26/08[§] Nymph	DC 9/26/08[§] Adult	SN 10/3/07[§] Nymph	SN 10/3/07[§] Adult	DC 10/10/07[§] Nymph	DC 10/10/07[§] Adult	Average Collected
Insect QTL lines									
Benning M	1.8bc	0.3ab	2.3a	1.0ab	3.0a	2.0a	1.0a	2.0a	1.7ab
Benning G	1.5bc	1.8ab	3.5a	0.5b	3.5a	0.5a	1.0a	1.0a	1.7ab
Benning H	2.0bc	2.0ab	2.0a	0.8ab	2.3a	0.8a	1.3a	2.3a	1.7ab
Benning MGH	0.8c	1.0ab	2.3a	1.0ab	3.0a	1.3a	1.0a	2.3a	1.6ab
VAT lines									
Benning lxy123	1.0bc	1.5ab	1.3a	0.5b	5.2a	2.0a	0.5a	0.8a	1.6ab
Benning RR lxy 123	2.5bc	0.8ab	6.5a	0.8ab	2.3a	2.3a	1.0a	1.5a	2.2a
Benning C1726	5.3a	1.5ab	1.3a	0.3b	2.3a	1.0a	0.5a	0.8a	1.6ab
Benning N87-2122-4	1.3bc	1.8ab	4.3a	2.0a	3.8a	2.3a	1.0a	0.5a	2.1ab
Benning (6) FAN	0.0c	0.0c	1.7a	2.0a	2.0a	0.5a	0.3a	1.0a	0.9b
Checks									
Benning	2.8abc	2.3a	1.3a	0.5b	5.6a	2.3a	1.3a	1.0a	2.1ab
H7242 RR	1.5bc	1.0ab	1.3a	1.0ab	2.8a	1.5a	1.3a	1.5a	1.5ab

[§]Means within a column followed by the same letter are not significantly different (P>0.05; Duncan's multiple range test).
*Per 25 sweeps

Table 3.10 Seasonal mean of stink bugs collected per plot of Benning VAT and NILs using sweep net (SN)* and drop cloth (DC) sampling from Athens GA 2007.

Test Lines	DC 9/25/07[§] Nymph	Adult	SN 10/04/08[§] Nymph	Adult	Average Collected
Insect QTL lines					
Benning M	0.3a	0.0a	2.8a	0.5a	0.9a
Benning G	1.3a	0.3a	3.0a	0.5a	1.3a
Benning H	0.8a	0.3a	1.3a	0.3a	0.7a
Benning MGH	1.0a	0.0a	8.5a	0.3a	2.5a
VAT lines					
Benning lxy123	0.0a	0.5a	1.3a	0.0a	0.4a
Benning RR lxy 123	0.3a	0.3a	9.8a	1.5a	2.9a
Benning C1726	0.0a	0.0a	1.5a	0.3a	0.4a
Benning N87-2122-4	0.8a	0.8a	1.3a	0.5a	0.9a
Benning (6) FAN	0.8a	0.5a	2.8a	0.3a	1.1a
Checks					
Benning	1.0a	0.8a	3.3a	0.8a	1.5a
H7242 RR	0.8a	0.3a	2.0a	0.5a	0.9a

[§]Differences were not significant (P>0.05; Duncan's multiple range test).
*Per 25 sweeps

Table 3.11 Seasonal mean of stink bugs collected per plot of Benning VAT and NILs using sweep net (SN)* and drop cloth (DC) sampling from Midville GA in 2008. There were no corresponding SB values recorded in the Athens location, due to herbicide drift onto the fields.

Test Lines	DC 9/16/08[§]		SN 9/25/08[§]		DC 10/9/09[§]		SN 10/9/08[§]		Average
	Nymph	Adult	Nymph	Adult	Nymph	Adult	Nymph	Adult	Collected
Insect QTL lines									
Benning M	6.5a	0.3a	4.0a	0.3a	4.3a	1.5a	3.3a	2.5a	2.8ab
Benning G	6.3a	0.3a	10.3a	1.8a	1.8a	0.8a	2.8a	1.5a	3.4ab
Benning H	6.0a	1.0a	5.8a	1.0a	1.0a	1.5a	6.0a	1.5a	3.0ab
Benning MGH	5.0a	0.5a	5.3a	1.5a	1.8a	2.0a	6.8a	2.0a	3.1ab
VAT lines									
Benning lxy123	2.8a	0.8a	2.0a	1.5a	1.5a	1.3a	3.0a	1.0a	1.7ab
Benning RR lxy 123	1.0a	0.0a	3.8a	0.8a	2.5a	1.5a	2.8a	3.3a	1.9ab
Benning C1726	9.5a	0.3a	5.3a	0.3a	4.3a	1.8a	7.8a	2.5a	3.9ab
Benning N87-2122-4	2.3a	0.3a	3.3a	0.5a	4.3a	2.0a	3.0a	1.5a	2.2ab
Benning (6) FAN	4.3a	0.0a	3.3a	3.3a	2.0a	2.8a	4.3a	1.5a	2.7ab
Checks									
Benning	3.0a	1.3a	6.3a	3.3a	6.5a	4.0a	8.5a	2.3a	4.4a
H7242 RR	4.0a	0.0a	6.5a	1.0a	3.8a	1.3a	4.3a	1.3a	2.8ab

[§]Differences were not significant (P>0.05; Duncan's multiple range test).
*Per 25 sweeps

Table 3.12 Percent defoliation of R4 growth stage Benning VAT and NILs by Mexican bean beetles (*Epilachna varivestis*) at the Mountain Branch Research and Education Center in Blairsville GA, 2006-2008.

Test Lines	Defoliation (%)[§] 2006	Defoliation (%)[§] 2007	Defoliation (%)[§] 2008
Insect QTL lines			
Benning M	13.3c	8.0cd	8.5c
Benning G	24.8abc	11.3abc	14.7ab
Benning H	24.8abc	9.0abc	15.1ab
Benning MGH	15.3c	5.5d	5.5c
VAT lines			
Benning lxy123	38.0a	17.25a	18.38a
Benning RR lxy123	28.5abc	13.0abc	21.7a
Benning C1726	----	14.5abc	12.9ab
Benning N87.2127.4	----	12.8abc	13.9ab
Benning (6) FAN	35.3ab	15.8ab	15.6ab
Checks			
Benning	29.0abc	11.3abc	10.1ab
H7242 RR	28.5abc	13.5abc	13.0ab

[§] Means within a column followed by the same letter are not significantly different (P>0.05; Duncan's multiple range test).
--- Lines not evaluated.

Table 3.13 Seasonal mean in Benning VAT and NILs of bigeyed bugs (*Geocoris* spp; BEB), threecornered alfalfa hoppers (*Spissistilus festinus;* TCAH*)* and whitefringed beetles (*Naupactus* spp; WFB) from Midville and Athens GA 2007-2008.

Test Lines	Midville*						Athens[§*]					
	BEB		TCAH		WFB		BEB		TCAH		WFB	
	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008
Insect QTL lines												
Benning M	0.8	1.0	2.5	10.3	0.0	0.0	2.0	1.8	1.5	5.8	1.3	3.0
Benning G	2.0	1.3	0.0	6.8	0.0	0.0	2.3	0.8	0.8	7.8	1.0	2.8
Benning H	0.8	1.0	0.8	7.0	0.0	0.0	3.5	0.3	1.0	5.3	1.5	2.0
Benning MGH	1.5	0.3	1.5	14.5	0.0	0.0	0.5	0.5	1.5	8.3	1.5	5.0
VAT lines												
Benning lxy123	0.5	1.8	1.3	6.3	0.0	0.5	3.0	1.8	1.8	6.5	1.0	4.3
Benning RR lxy123	0.5	2.3	0.8	9.3	0.0	0.0	1.3	0.5	1.3	6.3	1.5	3.3
Benning C1726	0.3	2.3	1.8	13.3	0.0	0.0	1.8	1.5	1.3	9.5	1.3	4.8
Benning N87.2127.4	1.0	1.5	1.3	9.0	0.0	0.0	1.8	1.8	1.3	6.3	3.8	5.0
Benning (6) FAN	1.3	1.8	0.0	7.8	0.0	0.0	2.0	1.0	0.3	7.5	0.8	5.0
Checks												
Benning	1.5	4.3	0.8	9.3	0.0	0.0	1.8	0.8	1.5	8.3	1.0	5.0
Benning H7242 RR	1.8	0.8	0.5	6.8	0.0	0.0	3.0	0.8	0.8	9.5	2.3	2.5

[§] In 2008, only 2 samples were taken

*Differences were not significant (P>0.05; Duncan's multiple range test).

CHAPTER IV

CONFIRMATION OF QTLs ON LG B2 AND LG E ASSOCIATED WITH INSECT RESISTANCE IN SOYBEAN [1]

[1]Samuel-Foo, M., H. R. Boerma and J. N. All. To be submitted to *Crop Science.*

ABSTRACT

Developing soybean *Glycine max* (L.) cultivars with both superior agronomic characteristics and insect resistance genes has been challenging, as these traits are quantitatively inherited and are often coupled with the inadvertent co-introgression of unfavorable alleles. In previous studies, SIR QTLs (Soybean Insect Resistant Quantitative Trait Loci) on LG B2 and LG E conditioning antibiosis and antixenosis were reported from the cross 'Cobb' x PI 227687. The QTL on LG E is particularly interesting because it maps near the classical marker for pubescence on the soybean genetic map. In this study, independent near-isogenic lines were used in an attempt to confirm these QTLs. Confirming previously reported QTLs is an important validation step before the adoption of breeding strategies and MAS programs. Using a series of insect bioassays, field testing, and simple sequence repeat (SSR) marker analysis, the objectives of this study were to confirm these previously reported SIR QTLs on LG B2 and LG E. Data from the insect feeding assays and field studies indicated that both these QTLs conditioned a significant level of antixenosis and antibiosis resistance to BW and SBL. These results are in agreement with the previously published findings concerning these loci and so validate the existence and significance of these SIR-QTLs in this NIL population.

Key Words: Bollworm, *Glycine max*, Marker assisted selection, Quantitative trait loci, Simple sequence repeat, Soybean insect resistance, Soybean looper

INTRODUCTION

Lepidopteran defoliators in the southeastern USA annually cause insect damage in soybean crops that significantly reduce profits to growers. Traditionally, pest management strategies have depended on insecticide applications to reduce these insect pests, but the high cost of chemical control combined with the desire to reduce harmful chemical inputs into the environment have increased growers' interests in alternative control measures (Kilen and Lambert 1986, Boethel 1999, 2004). Breeding cultivars that possess enhanced insect resistance has strong potential as an integrated pest management technique, but efforts to transfer resistance genes insect resistance to improved cultivars developed from insect resistant exotic plant introductions (PIs) have been largely unsuccessful. There are sundry factors that contribute to this lack of success including the fact that the resistance traits are quantitatively inherited and the PIs exhibiting insect resistance possess poor agronomic qualities that have proven difficult to overcome (Sisson et al. 1976, Boethel 1999).

To date four insect resistant cultivars have been released, but these have met with limited grower acceptance, mainly due to their low yields (Lambert and Kilen 1984). Additionally, Kilen and Lambert (1986) reported that premature pod dehiscence and plant lodging were additional traits that contributed to the overall poor performance of lines developed from crosses with the insect resistant PIs. PI 171451, PI 227687, and PI 229358 are three Japanese lines that were identified by van Duyn et al. (1971a, 1972) as exhibiting varying levels of insect resistance in large-scale screening tests. These now serve as the primary donor parents in developing insect resistant cultivars (Clark et al. 1972, Hatchett et al. 1976, Lambert and Kilen 1984). These PIs exhibit both antixenosis and antibiosis modes of host plant resistance (HPR) to soybean caterpillar pests in the

southeastern USA (Boethel 1999). *Antixenosis* or non-preference describes any biochemical or morphological trait which discourages insect feeding, oviposition, or colonization (Painter 1951, Kogan and Ortman 1978). *Antibiosis* encompasses any detrimental physiological effect on insect growth, development, and/or reproduction that occurs as a result of feeding on plant tissue (Painter 1951). *Tolerance* is the third modality that describes HPR, and this refers to the ability of a plant to recover from a moderate amount of insect damage without suffering substantial yield loss (Painter 1951, Boerma and Walker 2005).

Today the availability of DNA marker technology and associated statistical analysis has allowed for the delineation of the inheritance of quantitative traits in into unique Mendelian components (Paterson et al. 1988). Specifically, using DNA markers (e.g. RFLPs and SSRs) in QTL mapping has made it possible to evaluate phenotypic variation associated with an individual locus conditioning a quantitative trait, such as soybean insect resistant (SIR) loci (Tanksley et al. 1989). Additionally, it has allowed for the transfer of genes of interest from wild accessions or other agronomically inferior lines into superior backgrounds while reducing the linkage drag that commonly occurs with traditional breeding techniques. Rector et al., (1998, 1999, 2000) used restriction fragment length polymorphisms (RFLP) to map several antixenosis and antibiosis SIR QTLs to bollworm (*Helicoverpa zea* Boddie; BW) from the three previously mentioned PIs crossed to the susceptible cultivar Cobb. PI 229358 possessed a resistance allele on linkage group (LG) M, now widely regarded as a major SIR QTL.

A QTL for antibiosis was detected on LG B2 (R^2 = 12%) whose resistance allele was provided by PI 227687. In a follow up study, Hulburt (2001), reported antixenosis and antibiosis QTLs on LG B2 as well as on LG E. The QTL on LG B2 was identified in

the 4-cM interval of Sat_230-Satt318 while the QTL on LG E was reported to lie between Sat_112 and Satt411 in a 6-cM interval. The QTL on LG E is particularly noteworthy as this was the only QTL in the Cobb x PI 227687 population to display both antixenosis and antibiosis modes of insect resistance. The major SIR QTL-M similarly confers both modes of resistance but was only present in PI 229358 and PI 171451 and not PI 227687 (Rector et al. 1998, 1999, 2000). Of even greater interest is the fact that the SIR QTL-LG E maps within very close proximity (6 cM) to the classical *Pb* (sharp vs blunt pubescence tip) locus on the soybean consensus linkage map (Cregan et al. 1999). In a recent study, Hulburt et al., (2004) reported sharp tipped lines showed significantly less defoliation with BW, beet armyworm (*Spodoptera exigua*, Hübner), and the soybean looper (*Pseudoplusia includens*, Walker) and significant larval weight reductions for BW when compared to larvae that had fed on blunt tipped pubescent lines.

Pubescence density and orientation play a significant role in the interaction of a plant with its environment (Zhang et al. 1992). Trichomes may serve as a deterrent to insect predation and for many plant species, a negative correlation exists between trichome density and oviposition responses, larval nutrition, or insect feeding (Levin 1973). One of the earliest reports of soybean resistance to insects was the study performed by Hollowell and Johnson (1934) in which the authors reported the presence of pubescence on soybean leaves provided resistance to the potato leafhopper *Empoasca fabae* (Harris). In a later study, Wolfenbarger and Sleesman (1963) reported *E. fabae* resistance as being greater for soybean plants with normal or dense pubescence compared with glabrous plants. In general, densely pubescent soybean plants are reported to have lower levels of insect infestation (Johnson and Hollowell 1935, Singh et al. 1971). Broersma et al. (1972) reported higher *E. fabae* populations on lines that had curly

pubescence when compared with normal and dense pubescence allowing them to conclude that trichome orientation played an important role in insect resistance. Additional studies evaluating soybean trichomes have indicated that plants possessing long and erect trichomes were more resistant than plants with short and appressed trichomes, regardless of pubescent density (Turnipseed 1972, Turnipseed and Kogan 1976). More recently, Lambert et al. (1992b) reported that caterpillar pests had reduced growth and extended development time on normal or dense lines when compared to glabrous plants.

Currently there are at least 900 quantitative trait loci (QTL) reported in Soybase (Fasoula et al. 2004), a database of collective information relating to soybean genetics and genomics, but of these, only a limited number have earned confirmed QTL status. Boerma and Mian (1999) indicated that a confirmation step was not required for publication of soybean QTL studies, though the necessity of this step is generally well regarded. Confirmation of a QTL can be achieved using independent populations constructed from the same parental genotypes or closely related genotypes used in the original mapping study (Collard et al. 2005). Development and evaluation of near isogenic lines (NILs) with alternative alleles at a QTL is another method that can be used to confirm QTLs. Results from QTL confirmation studies will solidify research and add validity to breeding strategies that will be based upon them.

In agreement with the view of Fasoula et al. (2004) that the plethora of QTL data that exists will best serve plant breeders only when the reported QTL are confirmed in an independent population of meiotic events (independent from the original mapping population) provides the rationale for which this study was undertaken. The objectives of this study were to use simple sequence repeat (SSR) markers and insect bioassays to: (i)

confirm the existence of the previously reported antibiosis and antixenosis SIR-QTL on LG B2, and (ii) confirm the existence of the antibiosis and antixenosis SIR-QTL on LG E that maps near the *Pb* locus.

MATERIALS AND METHODS

LG B2 QTL STUDY

Development of Plant Materials

$BC_5F_{2:3}$ near-isogenic lines (NILs) from the cross Benning (6) × PI 227687 were developed by selecting plants based on the SSR markers at previously determined SIR QTL. The NILs were created by crossing PI 227687 which contains the LG B2 SIR QTL to the cultivar Benning, which served as the recurrent parent. Plant selections were made each generation for the LG B2 antibiosis and LG B2 antixenosis QTL using the SSR markers Satt126 (antibiosis QTL) and Satt318 (antixenosis QTL), respectively. Phenotypic screening for antixenosis and antibiosis resistance also aided in this effort. Benning is a high performing Maturity Group (MG) VII cultivar that is well suited for production in the Southeast (Boerma et al 1997; Day et al., 1999), and it served as the genetic background for the NILs. Initially, 12 NILs were evaluated for their SSR genotypes surrounding the LG-B2 antixenosis QTL (Satt318) and the LG-B2 antibiosis QTL (Satt126). From these 12 NILs, five were selected for further study (Fig. 4.1). Based on the five backcrosses to Benning, these NILs would be expected to average 98.4% Benning genome except on LG B2 near the antixenosis and antibiosis QTL.

SSR Marker Data Collection.

Recently emerged trifoliolate leaves from the five NILs, Benning, and PI 227687 were harvested from greenhouse grown plants for DNA extraction. Extraction of the leaf

DNA was done using a modified CTAB (hexadecyltrimethylammonium acid) procedure based on the protocol of Keim et. al. (1988). Briefly, leaflets were lyophilized for 2 days before being transferred to individual wells in a 96 square well-deep well plate. After being ground to a fine powder, they were suspended in 1500 µl of CTAB buffer [2% (w/v) CTAB; 1.4 M NaCl; 100 mM Tris-HCL pH8.0; 20 mM EDTA and 1% (v/v) 2-β-mercaptoethanol]. PCR (polymerase chain reaction) amplification was according to the protocol of Li et. al., (2001) with the following modifications. Each reaction mixture contained 2 µl of 30 ng template DNA, 0.5 µM each of forward and reverse primers, 2 mM of each dNTP, 2.5 mM Mg^{2+}, 1.0X PCR buffer and 0.5 units of *Taq* polymerase in a total volume of 10 µl. Primers were labeled with the fluorescent dyes 6-FAM, NED or HEX (PE-ABI, Foster City, CA), and a 384-well or a 96-well GENE AMP PCR System thermal cycler was used for DNA amplification. PCR amplicons were separated using hand poured 4.8% polyacrylamide gels that were run on an ABI PRISM 377 DNA Sequencer (PE-ABI, Foster City, CA) for 1.5 to 2 hours at 750V. Pooled PCR products (3 µl) were combined before gel electrophoresis with 2 µl formamide, 0.75 µl loading buffer and 0.30 µl Genescan ROX-500 internal DNA size standard. Each sample was denatured at 95^{o}C for 5 minutes before 1 to 2 µl of each was loaded into the appropriate gel lane, which was left after the removal of a 96-well comb from the 12-cm polyacrylamide gel. Initially, Benning and PI 227687 were screened with all the markers to identify polymorphisms. This involved the evaluation of 27 SSR markers spanning 94.4 cM of LG B2 (from Sat_177 to Satt687) (Table 4.1). Once this preliminary step was completed, the NILS and parents were screened with the polymorphic markers. Upon completion of electrophoresis, the gels were manually scored based on amplicon size data from each parent to aid in the SSR marker genotyping of each NIL.

Assessment of PI 227687 genome introgression. To evaluate the relative amount of PI 227687 genome present in the genomic regions of the NILs containing the putative SIR QTL-B2 the regions flanking the QTL of interest were genotyped with the polymorphic SSR markers spanning LG B2. A graphical genotype was developed by SSR fingerprinting individual NILs in a 94-cM region including both putative SIR QTL. Genetic marker distances were appropriately scaled to distances based on the USDA Soybean Consensus Genetic Map (www.soybase.org) and cross over points were delegated to the midpoint between the two adjacent markers (Fig. 4.1). We assumed that no double crossovers occurred between markers in a single meiosis event.

Antixenosis Tests. Antixenosis (feeding preference) bioassays were conducted in a greenhouse on the campus of the University of Georgia in Athens GA, using the procedure that was originally described by All et al. (1989). SBL eggs were supplied by the Crop Protection and Management Unit (USDA-ARS, Tifton GA) and BW eggs originated from the insect supply company Benzon Research (Carlisle, PA). Two seeds of an entry (NIL or parent) were planted in 450-mL polystyrene foam cups that had three holes punched in the bottom to allow for soil drainage and water uptake by the test plants. The cups were filled with a commercially available soil mixture (Craven Pottery, Commerce, GA) that had been amended with Osmocote slow release fertilizer. After germination, one seedling was removed from the cup to allow the healthier of the two to be used in the assay. The cups were arranged in a randomized complete block design with 20 replications in a stainless steel tray that measured 4.9 m long x 1.2 m wide x 8 cm deep. After insects were added to the test plants, the pan was filled with approximately 2 cm of water to allow for water uptake into the cups and to avoid disturbing the insects. The trays remained filled through the duration of the experiment. Approximately 14 days

after germination, after the first trifoliolate leaf had emerged and was just expanding, individual plants were infested with four neonate larvae (<5 h old) using a size-000 soft tipped camel's hair brush. The cups were arranged together in the tray to allow complete merger of the foliage so that larvae could freely migrate from leaf to leaf. Approximately 10 to 12 days after infestation, the percent leaf area consumed by the insects (defoliation) was visually estimated for each plant by at least three individuals. The means of these estimates for each plant were analyzed using PROC ANOVA in SAS (SAS institute 2003).

Antibiosis Tests. Petri-plate antibiosis feeding assays to BW and SBL for each NIL and the parents were evaluated in a growth chamber, using a procedure that was modified from Walker et al. (2002). The growth chamber was maintained at 27^{o}C with 85% ambient humidity. A combination of fluorescent lights and incandescent fixtures provided a 14-h photoperiod for the assays. Newly expanded trifoliolate leaves were harvested immediately prior to assay set up, from greenhouse plants that were specifically grown for this purpose. Petri plates, either 100 x 25 mm (for SBL) or 1000 x 25 mm (for BW), were prepared with a layer of plaster of Paris. At least 24 hours after the dishes were prepared, three white filter paper discs were inserted in each dish and moistened with distilled water to provide humidity for the leaves and insects during the experiments. For the SBL assays, one entire leaflet was placed in a Petri dish (100 x 25 mm) to which two freshly hatched neonatal larvae were added. The edges of the closed plate were sealed in a layer of Parafilm to prevent moisture and larval escape.

About 5 days after the initiation of the experiment, the initial leaves were removed together with the outermost filter paper to reduce the amount of insect excreta in the plate. Clean up and transfer of leaves was initiated in the group of plates in which the

leaves had been most consumed or had yellowed. A new trifoliolate leaflet was added to each plate and the larvae were gently transferred to the leaves in the re-moistened Petri plate. Once the leaf tissue of the susceptible parent Benning had been completely consumed, approximately 4 days after leaf replacement, the experiment was terminated by transferring all the Petri dishes to a -20^{o}C freezer where they remained overnight (about 9 days after initial infestation). The following day, the larvae were weighed using a Mettler A30 balance. For the BW assays, 1000 x 25 mm Petri plates were used, due to the cannibalistic behavior common in these larvae, and the assays began with two trifoliolate leaflets and two neonatal larvae. The bioassays were arranged in a randomized complete block design with 20 replications and the entire experiment was duplicated 2 weeks later. The average weight of the surviving larvae were recorded and analyzed by PROC ANOVA in SAS (SAS Institute 2003).

SIR QTL-LG E STUDY

Field evaluation of plant material.

BC_5F_2-derived NILs for SIR QLT LG E from Benning (6) × PI 227687 were developed and used in this experiment. In this case, each BCF_1 plant was selected based on the phenotype of the pubescence tip which was segregating. As before, the MG VII cultivar Benning, served as the recurrent parent. For this study, three sharp tipped (G05-6493, G05-6495 and G05-6496) and three blunt tipped (G05-6483, G05-6486 and G05-6488) NILs were created by selecting for homozygous sharp or blunt pubescent lines from among the BC_5F_2-derived lines. Benning, which has blunt pubescence tips, served as the susceptible check in this study. A total of eight entries including three sharp and three blunt NILs as well as two entries of blunt-tipped Benning were planted for evaluation. In 2006, the experiment was planted at three locations; the University of Georgia Plant

Sciences Farm near Athens GA in a late May and late June planting and at the University of Georgia Southwest Research and Education Center near Plains GA in a late May planting. In 2007 and 2008 the experiment was also planted at two locations; the University of Georgia Plant Sciences Farm and at the University of Georgia Southeastern Research and Education Center near Midville GA.

Field plots were examined for the incidence and abundance of caterpillar (Noctuidae) pests in 2007 and 2008 when they were in the R2-R3 stage of development (full bloom to beginning pod fill) (Fehr et al. 1971). Insect sampling was accomplished each week by alternating between sweep net and drop cloth sampling methods. For sweep net sampling, a 25-sweep sample was taken from both rows of each plot using a 38 cm diameter sweep net as described in Kogan and Pitre (1980). Drop cloth sampling involved using a 1-m x 1-m white canvas collection sheet which was placed on the soil surface between each two row plot. Samples were taken by extending the sheet once it was in place and then gently bending the plants over the drop cloth and vigorously shaking them so that insects are dislodged and fall onto the cloth. Special care was taken to avoid disturbing the plots before sampling. Any insects found on the underside of the cloth were included in the field counts recorded. All plots were visually rated for percent defoliation. The data were analyzed using the GLM procedure of SAS (SAS 2003) for a randomized complete block design.

Within 2 weeks of seedling emergence, the experiments were also evaluated for the feeding effects of lesser cornstalk borer [(LCB) *Elasmopalpus lignosellus* (Zeller)] by noting the number of dead plants as well as the number of live plants, in each stand. Plants identified as severely wilted were included in the counts, but these were removed from the plot and individually inspected for the characteristic LCB tunneling point of

entry at the plant's stem base before being included in the counts. The data were analyzed using PROC ANOVA in SAS (SAS Institute, 2003).

Phenotypic evaluation and classification of pubescence genotypes.

Soybean NILs and checks were evaluated in greenhouse experiments to gauge the level of resistance against BW and SBL. Each experiment consisted of 12 entries which included 3 entries each of susceptible Benning (blunt pubescent tip; *pbpb*) and PI 227687 (sharp pubescent tip; *PbPb*) lines as well as one entry each of the sharp tipped lines (*PbPb,)* G05-6493, G05-6495, G05-6496, and the blunt tipped lines (*pbpb*), G05-6483, G05-6486, G05-6488, NILs. The entries were evaluated in a randomized complete block experimental design in a greenhouse at the University of Georgia in Athens, GA. Parent and NIL trichomes were characterized for tip morphology by sampling a trifoliolate leaf from 12 randomly selected plants of each line at 15 days after planting [plants in V3 stage; (Fehr and Caviness, 1971)]. Each leaf was evaluated for pubescence tip morphology by use of the 20x magnification on a light microscope. Pubescence was characterized as sharp if the trichome tip ended in a pointed apex or as blunt if the trichome tip was rounded in appearance.

Antixenosis and Antibiosis tests. Antixenosis resistance was evaluated with BW and SBL using the same procedure as described above. Experimental lines were arranged in a randomized complete block experimental design with 20 replicates. Antibiosis effects were also evaluated as mentioned previously. The experimental lines were evaluated in a randomized complete block experimental design with 20 replications. Each Petri plate bioassay included a single insect to avoid cannibalism effects, as is known to occur with BW larvae. Defoliation scores and larval weights were analyzed using PROC

ANOVA in SAS (SAS Institute, 2003) and Tukey's Standardized range test was used for means separation.

RESULTS AND DISCUSSION

LG B2 Study

A total of 32 SSR markers that map on the USDA Soybean Consensus Genetic Map for LG B2 were tested for polymorphism between Benning and PI 227687. Of these, 27 were polymorphic and were scored on the five NILs (Table 4.1). The remaining markers were identified to be either monomorphic or they performed inconsistently (missing bands or stutter bands) in this preliminary evaluation, and were omitted from further consideration. Twelve near isogenic lines (NILs) representing putatively different allelic combinations of the putative antixenosis and antibiosis SIR QTL on LG B2 were genotyped to identify unique allelic combinations for the two QTL. From this evaluation five NILs with different allelic combinations for the two QTLs (MG 200, MG 206, MG 208, MG 246, and MG 271) were selected. MG 200 is heterogenous for PI 227687and Benning alleles at both SIR QTL (located at Satt126 and Satt318). MG 208 is homozygous for PI 227687and Benning alleles at both SIR QTL (Satt126 and Satt318). MG 246 NIL is heterogenous at the antibiosis QTL (Satt126) and homozygous for PI 227687 alleles at the antixenosis QTL (Satt318). Both MG 206 and MG 271 are homozygous at the antibiosis QTL (Satt126) and heterogenous for both alleles at the antixenosis QTL (Satt318).

The five NILs were evaluated in feeding bioassays with BW and SBL larvae. The results from the BW antixenosis and antibiosis evaluations identified Benning as the most susceptible and PI 227687 as the most resistant lines based on both BW defoliation and

BW larval growth in the study (Table 4.2). There were no significant differences among the NILs for BW defoliation, but as a group they all were significantly less defoliated than the susceptible recurrent parent Benning. The NILs averaged 34% less defoliation than Benning. A similar pattern was seen for BW larval weight gain (antibiosis assays). After feeding for 9 days on all the test lines, the five NILs averaged approximately 4 mg less weight than the susceptible Benning. BW larvae fed on PI 227687 leaf tissue were 10 mg lighter in weight than the larvae that fed on Benning.

In general the results from the SBL insect assays on the NILs were similar to that of BW. The NILs were significantly less defoliated (14 - 21%) than Benning (73%). The resistant donor parent PI 227687 was the least defoliated entry (9% defoliation). The data recorded from the SBL larval bioassays indicated that larvae that fed upon Benning were significantly heavier than both the NILs and PI 227687. Larvae that fed on Benning leaf tissue averaged from 4.8 to 8.0 mg heavier than larvae fed leaf tissue from the NILs and 15.1 mg heavier than those fed on PI 227687.

Overall these results indicate an antixenosis and antibiosis effect of the SIR QTLs on LG B2. Across the two insect species, the NILs were significantly less defoliated than Benning (34% less for BW and 55% less for SBL). Similarly, larvae gained approximately 4 mg (BW) and 6 mg (SBL) less weight when offered leaves from the NILs containing the SIR QTL on LG B2 when compared to the susceptible Benning. These results confirm the presence of an antixenosis and antibiosis QTL as reported by Hulburt (2001). Therefore, these results, obtained from a different genetic background than the original mapping population, indicate that the putative QTL for antibiosis and antixenosis reported by Rector et. al, (1998, 1999, 2000) and Hulburt (2001) exist, and that their effects on BW and SBL feeding and development are inhibitory. Based on the

data generated in this study, it is not possible to verify the exact location of these QTLs on LG B2. The availability of lines homozygous for the Benning alleles at one QTL and homozygous for the PI 227687 alleles at the other QTL is required to more precisely determine the QTL locations. These QTLs hold potential for SIR QTL pyramiding into superior cultivars potentially producing higher and broader levels of insect resistance for use by growers in soybean pest management.

LG E study

Six NILs differing in pubescence tip morphology were evaluated in greenhouse experiments for antixenosis and antibiosis resistance with BW and the SBL. These same lines were also tested in the field for LCB damage over a 3-year period. Insect defoliation levels with natural insect populations in experiments conducted in Athens and Midville GA were recorded in 2008. Benning, a blunt tip cultivar, known to be susceptible to lepidoterous insects, served as a susceptible check in all tests.

The pubescence morphology of greenhouse grown plants used in this study was evaluated and was in accordance with previously collected data (Table 4.3). The shape of the trichome apex significantly affected BW larval development in terms of weight gain, despite not having an effect on BW defoliation. Although the sharp and blunt NILs were equally defoliated by BW, the larvae that fed on the blunt-tipped NILs averaged twice the weight gain compared to the larvae that fed on the sharp-tipped NILs

In SBL insect bioassays, both the sharp and blunt NILs showed greater resistance to defoliation and larvae weight gain than the susceptible Benning. Although there were no statistically significant differences in the SBL insect bioassays, the sharp NILs averaged numerically less defoliation and provided for less SBL larval weight gain than the blunt NILs. For SBL both the sharp and blunt tipped NILs averaged less defoliation

and less larval weight gain than the susceptible check Benning. Both sets of NILs showing greater resistance than Benning was also observed based on the BW larval weight gain data. A possible explanation of these findings is a potential cross over been the gene for pubescence tip (*Pb*) and an insect resistance QTL on LG E that is conditioning the observed insect resistance.

In 2006 there was a severe LCB infestation in our early planted experiment at the University of Georgia's Plant Sciences Farm (Table 4.4). Although the sharp and blunt pubescent tipped lines had not been previously evaluated for LCB resistance, both the sharp and blunt NILs averaged approximately 40% dead/damaged plants from LCB while Benning averaged 76% dead/damaged plants. This 36% reduction in LCB damage for both the sharp and blunt NILs and was totally unexpected. Unfortunately, subsequent field tests in 2007 and 2008 never achieved adequate infestations of LCB larvae to confirm this finding. Data collected in 2006 showed that both the blunt and sharp pubescent lines yielded less on average (3486 kg ha^{-1} and 3274 kg ha^{-1}, respectively) than the Benning lines (3732 kg ha^{-1}) (Table 4.4). At maturity, the sharp pubescent lines averaged 4 cm taller than both the blunt pubescent lines and Benning, while an analysis of their protein and oil content revealed all three lines to be similar. The sharp NILs averaged 20 mg/seed lighter than Benning and 14 mg/seed lighter than the blunt-tipped NILs.

In 2008, the lines were rated for weekly defoliation levels to naturally occurring caterpillar populations in the field over a 4-week period from 9/15/08-10/3/08 at both locations. Common defoliators found occurring in the fields via drop cloth and sweep net sampling included SBL, BW, velvetbean caterpillar (*Anticarsia gemmatalis)* and green cloverworm (*Plathypena scabra).* Initially there was no difference for either group of

NIL, but subsequently there was a significant difference noted between the defoliation levels of the entries. The sharp tipped NILs were significantly less defoliated than the blunt tipped NILs (4 vs. 9% at Midville and 3 vs. 8% at Athens) across locations (Table 4.5). Benning, the susceptible check was defoliated at a similar level as the blunt tipped NILs. These results confirm the presence of an insect resistance QTL in the same region of LG E as the *Pb* locus and the likelihood that sharp pubescence is the cause of this resistance

Trichomes are generally regarded as being one of the more important morphological parameters in terms of plant resistance to insects (Khan et al. 1986). The present study was based on the findings of Hulburt et. al., (2004) which reported the existence of a SIR QTL on LG E in the interval Sat_112 – Satt411 that is associated with both antibiosis and antixenosis insect resistance. This putative QTL was identified in a population of 'Cobb' x PI 227687 and was mapped to a location near the *Pb* locus which conditions sharp vs. blunt pubescence (Cregan et al. 1999). Based on insect bioassays and field tests with the NILs derived from a Benning x PI 227687 population, our results support the presence of an insect resistance QTL near the *Pb* locus on LG E.

The possibility exists that the SIR QTL on LG E is not the *Pb* locus. This hypothesis is based on the resistance shown by both sharp and blunt tipped NILs in some insect bioassays. Although the sharp tipped NILs clearly had significantly less insect damage in some insect bioassays, the blunt pubescent lines performed as well as the sharp pubescent lines on more than one occasion. This could result from a crossover between the *Pb* locus and the insect QTL resulting in blunt tipped pubescent lines with a SIR QTL from PI 227687 in this region of LG E. These lines would be expected to possess the same level of insect resistance as the sharp tipped NILs. Additional

investigations are needed to elucidate if this crossover event could be present in the NILs evaluated in this study. From a pest management perspective, the confirmed QTL on LG E, whether the *Pb* locus or a closely linked SIR QTL, provides an additional resistance QTL to breeders for use in developing insect resistant cultivars.

A review of the literature on soybean QTLs will return many studies reporting the existence of QTLs. Despite the fact that many QTL have been mapped in soybean, only few have been confirmed. Glover et al (2004) reported that the confirmation of QTL after initial mapping is a critical step before the selection of the QTL with markers in breeding programs. NILs are particularly useful for QTL confirmation because they are developed to segregate for QTL in an otherwise homogeneous background. In this study, NILs were used to validate the existence of antixenosis and antibiosis SIR QTLs on LG B2 and LG E of the soybean genome to BW and SBL. Additional studies will to help explain the unusual phenomenon occurring on LG E and to help pinpoint the exact location of the QTLs on LG B2.

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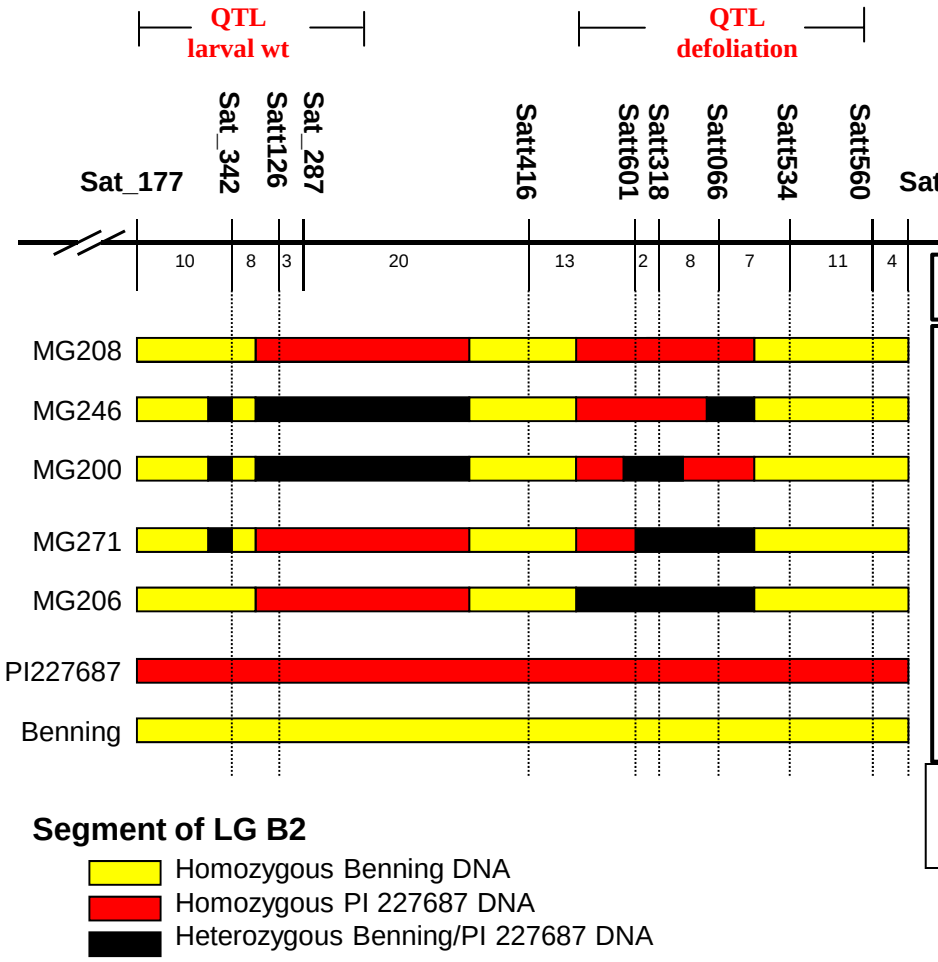
Table 4.1 Simple sequence repeat markers on LG B2 evaluated in this study.

LG	Position (cM)[§]	Marker
B2	5.9	Sat_177
B2	11.0	Sat_264
B2	15.5	Sat_342
B2	19.2	Satt467
B2	23.3	Satt126
B2	26.6	Sat_287
B2	42.0	Satt083
B2	43.2	Sct_034
B2	45.7	Satt416
B2	58.4	Satt601
B2	60.8	Satt318
B2	62.9	Sat_083
B2	63.3	Satt556
B2	63.3	Satt070
B2	63.4	Satt474
B2	63.4	Satt272
B2	63.4	Satt122
B2	63.5	Sat_189
B2	63.5	Sat_230
B2	63.7	Sct_094
B2	68.4	Satt066
B2	75.7	Satt534
B2	77.8	Sct_064
B2	80.8	Satt063
B2	86.8	Satt560
B2	90.6	Sat_424
B2	100.3	Satt687

[§] Based on the 2008 USDA consensus soybean linkage map.

Figure 4.1 Graphical genotypes of the NILs used in the study depicting probable regions of the SIR QTL and the amount of flanking PI 227687 and Benning genome. The most likely location of the SIR QTLs on LG B2 is indicated above the diagram. Numbers shown beneath each SSR locus represents the approximate cM location of the marker based on the 2008 USDA-ARS consensus linkage group.



Segment of LG B2

Bollworm		Soybean Looper	
Def(%)	Lar. wt(mg)	Def(%)	Lar. wt(mg)
23.8b	17.1bc	7.9cd	14.7c
25.2b	19.7bc	10.4b	17.3bc
24.6b	14.4cd	7.1d	15.7bc
23.6b	21.6b	9.2bc	17.9b
23.3b	17.3bc	9.5bc	16.6bc
8.7c	9.4d	2.6e	7.6d
58.9a	73.3a	12.6a	22.7a

Means with the same letter are not significantly different based on Tukey's Standardized Range, P = 0.05

Segment of LG B2

- Homozygous Benning DNA
- Homozygous PI 227687 DNA
- Heterozygous Benning/PI 227687 DNA

Table 4.2 Mean percent defoliation by bollworm (BW) and soybean looper (SBL) of NILs containing the putative SIR QTL on LG E and differing in pubescence tip morphology. Insect larval weights from antibiosis tests are also included.

NIL	Phenotype	Bollworm		Soybean Looper	
		Defoliation (%)[§]	Larval weight (mg)[§]	Defoliation (%)[§]	Larval weight (mg)[§]
G05-6493 G05-6495 G05-6496	Sharp	30.5a	4.3c	44.9b	11.2b
G05-6483 G05-6486 G05-6488	Blunt	30.2a	8.6b	49.7b	13.4b
Benning	Blunt	37.2a	13.5a	69.8a	17.7a

[§]Means with the same letter are not significantly different based on Tukey's Standardized range P=0.05

Table 4.3 Percent of dead/damaged plants due to lesser cornstalk borer in a May planting at the University of Georgia Plant Sciences farm in 2006. Yield and agronomic data are also presented for the NILs and Benning from a June planting at the Plant Sciences Farm and a May planting from the Southwest Research and Education Center. Data were combined across locations with locations and replications considered as random effects and genotypes as fixed effects in the model.

	Phenotype	Yield (kg ha^{-1})	Maturity (days)	Height (cm)	Seed wt Protein (mg)	(mg/seed)	Oil (mg/seed)
G05-6493	Sharp						
G05-6495	Sharp	3274b	23.7a	108.3a	140.7b	405a	208a
G05-6496	Sharp						
G05-6483	Blunt						
G05-6486	Blunt	3486ab	25a	103.7b	154.3a	406.7a	206.3a
G05-6488	Blunt						
Benning	Blunt	3732a	23.5a	104ab	160.5a	407.5a	208a

[§]Means with the same letter are not significantly different using Tukey's Standardized test range at P = 0.05.

Table 4.4 Mean percent defoliation by caterpillar pests of NILs and Benning differing in pubescence tip in field tests in two locations in 2008.

NIL	Phenotype	Defoliation (%)		LCB
		Midville	Athens	Damaged plants(%)
G05-6493				
G05-6495	Sharp	4.0b	3.1b	39b
G05-6496				
G05-6483				
G05-6486	Blunt	9.4a	7.1ab	40b
G05-6488				
Benning-1	Blunt	10.5a	9.5a	76a

[§]Means with the same letter are not significantly different.

CHAPTER V

SUMMARY

Efforts to improve the nutritional and insect resistant characteristics of soybean have resulted in the release of value added trait cultivars. Although increasing yield remains the primary objective of most soybean improvement programs, creating lines with improved nutritional quality is becoming a breeding priority as interest in soybean oil for human consumption is becoming popular among consumers. Having diets with positive health benefits available is becoming a niche market for specialty soy growers. Similarly, having cultivars with introgressed insect resistant traits would improve the financial burden to growers as they seek to employ pest management strategies. This also has the added advantage of allowing them to be good stewards of the environment.

This research examined the feeding preference of common soybean insect pests on eleven value added soybean cultivars to assess what effect their improved nutritional qualities had on their pest vulnerability. The cultivars in this study included: '*Benning*' a germplasm known for its strong agronomic properties and disease and nematode resistance. This line served as the genetic background in which all the cultivars were developed. *Benning C1726* and *Benning N87-2122-4*, are two cultivars with reduced palmitic acid levels. A reduction in palmitic acid reduces the amount of saturated fatty acid in soybean making it more attractive to health conscious consumers. *Benning (6) FAN* is a low linolenic germplasm, having high linolenic acid levels contributing to the low oxidative stability of the oil. *Benning H7242 RR* is a glyphosate tolerant

cultivar which is attractive to growers due to the ease of controlling yield robbing weeds in plots. *Benning lxy123* is a cultivar that lacks lipoxygenase. Lipoxygenase is the name for a family of isozymes that is responsible for the off-flavor of soybean seeds, making it unpopular with consumers. In addition to the absence of lipoxygenase, *Benning RRlxy123*, is glyphosate tolerant. Benning M, G and H are cultivars that contain antibiosis and antixenosis SIR QTLs. *Benning M* contains a major SIR QTL while *Benning G* and *Benning H* both contain minor QTLs. *Benning MGH* is a pyramided cultivar containing all three SIR QTLs.

In this study, these Benning VAT and near isogenic lines were field tested with naturally occurring insect populations over two years in different locations to assess their vulnerability to a host of common soybean insect pests including the soybean looper, bollworm, green cloverworm, velvetbean caterpillars, stink bugs, Mexican bean beetles, three cornered alfalfa hoppers, and whitefringed weevils. The VAT lines were also tested in a series of greenhouse insect feeding bioassays. Healthy seedlings in the V3-V4 stage were subjected to intense artificial infestations using neonatal BW, SBL, VBC and BAW larvae. Defoliation ratings were taken typically nine days after infestation and were statistically analyzed for differences in resistance to feeding injury. The VAT lines in these assays had comparable injury to 'Benning' but greater feeding injury than 'Benning' lines with insect resistant QTLs. Overall, the results from these field studies and greenhouse assays indicated that enhancing high yielding cultivars such as 'Benning' with desirable nutritional or industrial food traits does not substantially increase risk for insect infestations. The fact that these VAT lines did not suffer increased pest vulnerability adds validity to their continued development and use by growers. Greenhouse tests with noctuid defoliators had intense artificial infestations and the VAT lines had substantial damage, however the injury was comparable to susceptible 'Benning' but greater

than ‘Benning’ lines with insect resistant QTLs. Field tests in North, Central and South Georgia had normal infestations of a variety of insects that are pests of seedling and vegetative and reproductive growth stages of soybean without any VAT showing abnormal vulnerability. The overall greenhouse and field results indicate that if VAT are incorporated into elite soybean cultivars then insect resistant should be added as well in order to maximize reduction of pest risk without using insecticides.

In a separate project, we set out to confirm the existence of previously reported SIR QTLs on LG B2 and LG E of the soybean genome. These antibiosis and antixenosis QTLs condition lepidopterous resistance and had previously been mapped using restriction fragment length polymorphisms (RFLPs) and later simple sequence repeat (SSR) DNA markers. Developing soybean, *Glycine max* (L.) cultivars with both superior agronomic characteristics and insect resistance genes has been challenging as these traits are quantitatively inherited and often the inadvertent co-introgression of unfavorable alleles occur simultaneously. In work originally performed in our lab, marker assisted selection (MAS) was utilized to create soybean near isogenic lines with SIR QTLs in a Cobb x PI 227687 population. In these NILs, minor QTLs for antixenosis and antibiosis insect resistance were identified on LG B2 and LG E that seemed to explain previously unreported genetic and phenotypic variation. In this study, these QTLs were evaluated for resistance to lepidopteran defoliators in a series of greenhouse and detached leaf insect bioassays, as well as in field testing using NILs that were developed in a ‘Benning’ genetic background. Utilizing NILs is well regarded as a means of verifying the existences of QTLs. Data were collected for larval damage to plants in an antixenosis (preference) feeding assay and larval weight after the insects had fed upon detached leaves were also recorded. Simple sequence repeat DNA marker analysis was also employed in the LG B2 confirmation

study. This involved genotyping 27 polymorphic DNA markers against our test population and then manually scoring the resultant acrylamide gels.

For the LG B2 test, the NILs were on average significantly less defoliated than Benning, the susceptible check by both BW and SBL. In the antibiosis assays, insects that fed on the NILs also weighed less than Benning. This indicated that the effect of the SIR QTL on LG B2 was detected in both antixenosis and antibiosis insect assays with these two common soybean insect pests. These results are consistent with previously published reports.

In the LG E study, six near isogenic lines differing in pubescence tip morphology were evaluated in greenhouse experiments for antixenosis and antibiosis resistance against bollworm and the soybean looper. These same lines were also field tested for lesser cornstalk borer susceptibility. Although the sharp and blunt NILs were equally defoliated by BW, the larvae that fed on the sharp pubescent NILs as a group gained significantly less weight compared to those on the susceptible Benning. Insects offered sharp tipped trichomes weighed approximately 9 mg less than those offered blunt tipped Benning leaves. Interestingly, insects offered blunt tipped Pb NILs also showed a decrease in weight gain (approximately 5mg less) as compared to the susceptible Benning.

In SBL insect bioassays, both sets of pubescent NILs (Sharp and Blunt) performed better than the susceptible Benning in antibiosis and antixenosis testing. Trichomes are generally regarded as being one of the more important morphological parameters in terms of plant resistance to insects (Khan et al. 1986). Hulburt et. al. (2004) reported the existence of a SIR QTL on LG E in the interval Sat_112 – Satt411 that is associated with both antibiosis and antixenosis insect resistance. This putative QTL was from the cross Cobb x PI 227687 and was mapped in a location near the *Pb* locus which conditions sharp vs. blunt pubescence (Cregan et

al. 1999). The present study was initiated to confirm the existence of the putative SIR QTL on LG E. Based on the results of insect bioassays and field tests with the NILs derived from a Benning x PI 227687 population, the results support the recent findings of an insect resistance QTL near the *Pb* locus on LG E. Field test results were also supported by these findings. Both the sharp and blunt lines averaged approximately 40% less susceptibility to LCB tunneling in seedling plants. The sharp tipped NILs were significantly less defoliated than blunt tipped counterparts including Benning while the blunt tipped lines were not significantly different from Benning. Based on the results of insect bioassays and field tests with the genotypes in this Benning x PI 227687 cross, these results support the existence of a SIR QTL on LG E and also suggest that a crossover event must have occurred beneath the sharp *Pb* QTL and is contributing to the reduced defoliation levels observed with both sets of NILs.

The possibility exists that the SIR QTL on LG E is not the *Pb* locus. This hypothesis is based on the resistance shown by both sharp and blunt tipped NILs in some insect bioassays. Although the sharp tipped NILs had consistently less insect damage in insect bioassays, the blunt pubescent lines performed as well as the sharp pubescent lines in certain tests. This could result from a crossover between the *Pb* locus and the insect QTL resulting in blunt tipped pubescent lines with a SIR QTL from PI 227687 in this region of LG E. These lines would be expected to possess the same level of insect resistance as the sharp tipped NILs. Additional investigations are needed to elucidate if this crossover event could be present in the NILs evaluated in this study. From a pest management perspective, the confirmed QTL on LG E, whether the *Pb* locus or a closely linked SIR QTL, provides an additional resistance QTL to breeders for use in developing insect resistant cultivars. In this study, NILs were used to validate the existence of antixenosis and antibiosis SIR QTLs on LG B2 and LG E of the soybean genome to BW and SBL.

Additional studies could to help explain the unusual phenomenon occurring on LG E and to help pinpoint the exact location of the QTLs on LG B2.

Overall, data from the insect feeding assays and field studies indicated that both these QTLs conditioned a significant level of antixenosis and antibiosis resistance to BW and SBL. These results are in agreement with the previously published findings concerning these loci and so validate the existence and significance of these SIR-QTLs.

APPENDIX

LIST OF ABBREVIATIONS

AFLP	Amplified fragment length polymorphism
BAW	Beet armyworm (*Spodoptera exigua*)
BW	Bollworm [aka Corn earworm (*Helicoverpa zea*)]
GRIN	Genetics resources information network
HPR	Host plant resistance
IPM	Integrated pest management
LCB	Lesser cornstalk borer (*Elasmopalpus lignosellus*)
LG	Linkage group
MAS	Marker assisted selection
MBB	Mexican bean beetle (*Epilachna varivestis*)
MG	Maturity group (soybean)
NIL	Near isogenic lines
PI	Plant Introductions
PUFA	Polyunsaturated fatty acid
QTL	Quantitative trait loci
RFLP	Restriction fragment length polymorphism
SBL	Soybean looper (*Pseudoplusia includens*)
SIR	Soybean insect resistance
SSR	Simple sequence repeat

VBC Velvetbean caterpillar (*Anticarsia gemmatalis*)