SEED YIELD AND INSECT RESISTANCE OF NEAR-ISOGENIC SOYBEAN LINES WITH

INTROGRESSED RESISTANCE QTL FROM PI 229358

by

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(Under the Direction of J.N. ALL)

ABSTRACT

The development of superior soybean cultivars [*Glycine max* (L.) Merr] exhibiting

resistance to insects has been hindered greatly due to linkage drag, which refers to the

inadvertent co-introgression of undesirable alleles linked to resistance genes. Soybean insect

resistance QTLs were previously mapped in a 'Cobb' × PI 229358 population to loci on linkage

groups (LGs) M, G, H, and D1b using simple-sequence repeat (SSR) markers. Marker-assisted

selection (MAS) was utilized to create near-isogenic lines (NILs) with individual SIR QTL in a

'Benning' genetic background. The objectives of this study were to evaluate yield drag at

individual resistance QTL in Benning-derived NILs, to assess the amount of PI 229358 genome

surrounding each PI 229358 resistance locus with SSR markers, to evaluate the effects of SIR

QTL-M, -G, and –H on corn earworm (CEW, *Helicoverpa zea*) and soybean looper (SBL,

Pseudoplusia includens), and to confirm a putative SIR QTL on LG D1b.

INDEX WORDS: Corn earworm, *Glycine max,* MAS, NIL, QTL, SIR, soybean, SSR

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B.S., The University of Delaware, 2001

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment

of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2006

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August 2006

DEDICATION

I would like to thank all of my friends, family, and colleagues for their support as I worked towards my Master's Degree.

ACKNOWLEDGEMENTS

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CHAPTER 1

INTRODUCTION

Over the last century, soybean [*Glycine max* (L.) Merr.] has become the primary source of the world's vegetable oil and vegetable protein. A steady increase in North American hectarage over the last 60 years can be attributed to its conversion from a forage crop to a major seed crop. In 2004, the USA grew an estimated 30 million hectares of soybean valued at nearly $18 billion and continued to be the world leader in soybean production and exportation. Soybean was second only to corn (*Zea mays* L.) in U.S. production value (USDA, 2004).

The cultivated soybean is considered a short-season, temperate-zone plant, although it is also adapted to lower latitudes and long-season conditions in areas such as northern Brazil and the southern USA. Soybeans grown in subtropical climate zones are much more vulnerable to insect pests, and therefore require higher rates of insecticide applications than those grown in more temperate regions (Kogan and Turnipseed, 1987). Damage by defoliating insects accounts for an annual reduction in grower income in the southern USA. To control soybean pests during outbreaks, insecticide applications are necessary, often at much higher levels than in more temperate regions of the country where infestations tend to be less severe. Multiple insecticide applications on soybean are economically unappealing due to the crop's low value per hectare. In addition, the negative environmental impacts stemming from insecticide applications are well-known and documented (Turnipseed, 1972). An alternative to chemical control methods in soybean pest management is plant resistance to insects (PRI), whereby plants with native resistance are utilized in breeding programs with the aim of developing insect-resistant cultivars.

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Three Japanese soybean plant introductions (PIs), PI 171451, PI 227687, and PI 229358, were found to exhibit both antixenosis and antibiosis forms of resistance to a multitude of insect pests, including the major lepidopterous pests in the Southeast (Van Duyn et al. 1971, 1972). Quantitative trait loci (QTL) for resistance to corn earworm (CEW; *Helicoverpa zea*) were previously mapped in PI 229358 to linkage groups (LGs) M, H, G, and D1b using restriction fragment length polymorphism (RFLP) and simple sequence repeat (SSR) markers (Rector et al., 1998; 1999; 2000; Narvel et al., 2001). The development of molecular genetic linkage maps has aided efforts to introgress desirable alleles into adapted germplasm while simultaneously reducing linkage drag. The objectives of this study were (i) to evaluate linkage drag for seed yield and other agronomic traits in Benning-derived near-isogenic lines (NILs) for three insect resistance QTL; (ii) to assess the amount of PI 229358 genome surrounding each SIR QTL; (iii) to evaluate the individual effects of SIRQTL- M, -H, and -G on antibiosis and antixenosis to soybean looper (SBL; *Pseudoplusia includens*) and CEW, and (iv) to evaluate the effects of SIRQTL-M and the putative SIRQTL-D1b and their interactions in a Benning genetic background.

CHAPTER 2

REVIEW OF LITERATURE

Soybean Entomology

Soybean [*Glycine max* (L.) Merr.] grown in the southern USA is generally more likely to incur insect damage throughout the growing season than in more temperate regions of the Midwest. Major soybean pests in this region include the velvetbean caterpillar, *Anticarsia gemmatalis* Hübner, and SBL (lepidopterous defoliators), the bean leaf beetle, *Cerotoma trifurcate* (Forster) (coleopterous defoliator), the southern green stink bug, *Nezara viridula* (L.) and the green stink bug, *Acrosternum hilare* (Say) (pod-feeders), and CEW (lepidopterous defoliator and pod-feeder) (Boethel, 2004). In most years, one or more of these species attain population levels requiring some form of management (Kogan, 1989). As soybean production increased in the USA during the last 50 years, an integrated pest management (IPM) system was developed, mainly due to concerns regarding excessive insecticide applications (Kogan, 1981; Kogan and Turnipseed, 1987, Turnipseed, 1972). IPM practices entail systematic scouting for crop growth, crop damage, pest development, and natural predators, as well as the utilization of economic injury levels (EILs) to determine proper actions during pest outbreaks. The development of EILs led to the concept of economic thresholds (ETs); ETs utilized in conjunction with population monitoring have made chemical insecticide application more effective, economical, and compatible with other management tactics.

In the past, organophosphorous (OP) and carbamate compounds were routinely utilized to control pest outbreaks. Although OPs are considered the most cost-effective compounds for producers, a shift to pyrethroids and other new chemistries led to significantly less pesticide load

in the environment, since these novel insecticides are effective at much lower doses (Boethel, 2004). Resistance to pyrethroid insecticides has only been documented in SBL in areas where soybean and cotton were grown in a close proximity (Thomas et al., 1994; Boethel, 2004). According to Boethel (2004), the SBL and velvetbean caterpillar are the most damaging defoliating pests in the southern USA, since their annual migrations give rise to sporadic infestations and rapid population increases. Annually, chemical control of one or both of these pests is required to reduce economic losses. It is imperative that growers reduce the negative effects that chemical applications can have on predators and parasitoids, as the maintenance of beneficial insect populations is essential for natural control of lepidopterous pests, and is integral to successful soybean IPM. Cultural control of soybean insect pests usually involves trap cropping, or the early planting of a crop so as to localize and control insect pests before they disperse into the main crop area (Todd et al., 1994). Another management strategy, PRI refers to the ability of a plant to resist or deter insect feeding behavior (Turnipseed and Sullivan, 1976). Developing soybean cultivars with PRI, also referred to as soybean insect resistance (SIR), has been a research priority in many of the public and private soybean breeding and entomology programs in the last 40 years (Lambert and Tyler, 1999).

Plant Resistance to Insects in Soybean

The benefits associated with the deployment of soybean exhibiting insect resistance are economical and ecological in nature, and include fewer insect outbreaks and higher economic thresholds, thus resulting in reduced insecticide use and costs, as well as reduced environmental contamination (Boerma, 1999; Boethel, 1999; Kogan, 1981; Kogan and Turnipseed, 1987). Moreover, PRI is compatible with multitactic IPM programs. Soybean insect resistance was first reported by Hollowell and Johnson (1934), who detected an association between pubescence

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morphology and resistance to potato leaf hopper (*Empoasca fabae* [Harris]). Densely pubescent

genotypes were also shown to adversely affect lepidopterous larvae by reducing growth and

extending development time (Beach and Todd, 1988; Lambert et al., 1992; Lambert and Kilen,

1989). Hulburt et al. (2001) found that near-isogenic soybean lines with sharp pubescence tip

significantly reduced defoliation and larval weight gain in CEW, beet armyworm, *Spodoptera*

exiqua (Hübner), and SBL.

One of the most important discoveries of insect resistant soybean germplasm occurred

when Van Duyn et al. (1971, 1972) screened the majority of the USDA soybean germplasm

collection of Maturity Groups (MG) VII and VIII for resistance to Mexican bean beetle

(*Epilachnia verivestis*), and found three Japanese plant introductions (PIs) with high levels of

resistance. PI 171451 ('Kosamame'), PI 229358 ('Soden-daizu'), and PI 227687 ('Miyako

White'), were also found to exhibit high levels of resistance to a multitude of other important

soybean pests (Luedders and Dickerson, 1977; Lambert and Kilen, 1984); therefore, they have

been utilized as the donors of resistance alleles in numerous breeding programs (Boethel, 1999;

Lambert and Tyler, 1999). It has been very difficult to breed soybean for PRI due to the fact that

the resistant germplasm is of such low agronomic value. Moreover, resistance is inherited

quantitatively in all three resistance sources, which has made it nearly impossible to introgress

all of the resistance genes without linkage drag (Sisson et al., 1976; Luedders and Dickerson,

1977; Rufener et al., 1989; Kenty et al., 1996). Linkage drag refers to the inadvertent co-

selection of an undesirable allele genetically linked to a desired one (Boethel, 1999). Four

previously released cultivars, 'Crockett' (MG VIII), 'Lyon' (MG VI), 'Lamar' (MG VI), and

'Shore' (MG V), which derive resistance from at least one of the PIs, were not adopted by

producers due to uncompetitive yields and somewhat less resistance than in the donor PI (Boethel, 1999).

Mechanisms Associated with PRI

PRI is conditioned by mechanisms referred to as antixenosis and antibiosis. Although not a physiological mechanism, the term tolerance is recognized as an alternate mode of resistance (Painter, 1951; Kogan and Ortman, 1978). Antixenosis, or non-preference, refers to any morphological or biochemical trait which discourages feeding, oviposition, or colonization by insects (Kogan and Ortman, 1978). Chemical antixenosis involves secondary plant compounds which function as feeding deterrents, while physical antixenosis refers to any morphological characteristic (ie. hairs, glands, or cuticle thickness) (Smith, 2004). Antibiosis occurs when a toxin, or secondary plant metabolite, impacts insect growth, development, and/or reproduction in a detrimental fashion (Painter, 1951). Only an organism that can overcome the toxin can become a pest of the plant. Antibiosis may result from the activity of plant enzymes or proteinase inhibitors that reduce the ability of herbivores to digest the food (digestibility reducing factors) (Smith, 2004). Tolerance refers to the ability of a plant to tolerate modest insect damage without significantly impacting yield (Painter, 1951).

Although the direct physiological mechanisms conditioning plant resistance to insects have been studied frequently, there is still little known concerning mechanisms associated with resistance in soybean (Boethel, 1999). In PI 171451, PI 227687, and PI 229358, antixenosis and antibiosis resistance appear to be primarily controlled through chemical means, although morphological traits such as pubescence can also condition resistance (Hulburt, 2004). Allelochemicals found to be associated with antibiosis resistance include isoflavones (i.e., plaseol, afrormosin, coumestrol, diadzein, and glyceollin), phenolic acids, and phytoalexins

(Boethel, 1999). According to Kogan (1989), resistance can be attributed to both constitutive

and induced chemical factors. Induced defenses are exemplified by a wounding response, and

were first identified by the local and systemic synthesis of proteinase inhibitors, which block

insect digestion in response to plant damage (Ferry et al., 2004). Recent research has shown that

these induced defenses also involve the plant's ability to produce toxic or repellent secondary

metabolites as direct defenses and volatile molecules that play important role in indirect defense

(Kessler and Baldwin, 2002). Insect herbivores activate induced defenses both locally and

systemically via signaling pathways involving systemin, jasmonate, oligogalacturonic acid, and

hydrogen peroxide. In a study exploring the herbivore-induced transcriptome in tobacco

(*Nicotiana attenuata*), microarrays indicated that the jasmonic acid cascade plays an integral role

in the accumulation of transcripts in plants exposed to herbivory (Ferry et al., 2004).

Inheritance of Resistance

Kogan (1972) found evidence that soybean resistance to Mexican bean beetle was

inherited in a semidominant manner in PI 171451, PI 229358, and PI 227687. Sisson et al.

(1976) found similar results after crossing elite cultivars with these three resistant PIs and then

evaluating F_3 progeny. When PI 229358 and 'Davis' were crossed and the F_1, F_2, and F_3

progenies were examined for resistance to SBL, the data indicated that the alleles conditioning

susceptibility showed incomplete dominance in the F_2 generation (Kilen et al., 1977). Lambert

and Kilen (1984) then compared Davis with PI 171451, PI 229358, and PI 227687 and their

intercrosses. They examined the resistance of F_1 plants and donor parents to five lepidopterous

defoliators and found the presence of slight dominance for resistance. Later, Kilen and Lambert

(1986) conducted a study using velvetbean caterpillar to elucidate whether the three resistant

accessions have different genes controlling resistance. It was determined that each parent is a

unique source of resistance alleles on the basis of the recovery of highly susceptible F_3 lines from

crosses among the three resistant PIs. These findings suggested that the genes responsible for

insect resistance in soybean are inherited in a quantitative fashion, and that two or three major

genes are involved in conditioning this trait (Kenty et al., 1996; Kogan, 1972; Sisson et al.,

1976). Kenty et al. (1996) estimated the heritability of antibiosis to be 63% (based on the mean

of two replications at one location) for SBL resistance from PI 229358, and that from two to six

genes are involved. Rufener et al. (1989) estimated that the heritability (on a single-plant basis)

of PI 229358 and PI 171451 antibiosis resistance to Mexican bean beetle ranged from 33% to

48% using F_3-generation data. Recently, Komatsu et al. (2004) estimated the broad-sense

heritability of 71.3% (on a mean of F_2 progeny basis) for antibiosis in a population derived from

a cross between Fukuyutaka (susceptible) and Himeshirazu (resistant).

Development of SIR Germplasm

Cooperative efforts between soybean breeders and entomologists have resulted in the

development and release of four insect-resistant cultivars ('Crockett', 'Lyon', 'Lamar', and

'Shore') and over 40 breeding lines with partial insect resistance (Boethel, 1999). None of the

cultivars or advanced breeding lines possesses resistance levels equal to that of the donor parent

and have comparable yields to existing elite cultivars, and therefore have typically been rejected

by growers (Elden et al., 1982; Elden et al., 1992; Lambert and Tyler, 1999; Todd et al., 1984).

Since insect resistance is inherited as a quantitative trait, conventional breeding methods have

produced minimal success in transferring the full complement of resistance genes to the recurrent

parent, thereby resulting in suboptimal resistance levels. Although the unacceptable agronomic

characteristics shared by SIR cultivars may be due to metabolic costs associated with

endogenous resistance, it is believed that the major culprit associated with deficient yields has

most likely been linkage drag. In this case, tightly-linked genomic segments containing inferior alleles for several agronomic traits, including yield, are often transferred to the recurrent parent along with the resistance alleles from the PIs.

In recent years, transgenic cultivars for several crop species with resistance to insect pests have been deployed in many developed nations. *Bacillus thuringiensis* (*Bt*), a native soil bacterium, encodes crystalline (cry) proteins that are toxic to many coleopteran and lepidopteran pests (Ely, 1993). *Bt* transgenes have been used to develop genetically-modified corn, potato, cotton, and soybean lines with endogenous insect resistance. Over 10 million hectares of *Bt* crops are planted globally, mainly plants expressing toxins effective against lepidopterous defoliators (Ferry et al., 2004).

A synthetic *Bt cry1Ac* gene was incorporated into the soybean cultivar Jack (Parrott et al., 1994; Stewart, Jr., et al., 1996). The transgenic soybean plants showed resistance to SBL, velvetbean caterpillar, and CEW. Although the resistance conferred by the *cry1ac* transgene was highly effective, Stewart, Jr., et al. (1996) still found that insect survival levels were high enough that insect populations could possibly develop resistance. The creation of soybean with native gene/transgene pyramids could circumvent this potential hazard by making it increasingly difficult for lepidopteran defoliators to develop complete resistance to differing resistance mechanisms. In studies by Walker et al. (2002, 2004), soybean lines containing both the native PI 229358 allele at IRQTL-M and the *cry1Ac* transgene received significantly less insect damage than lines carrying the insecticidal transgene alone.

Molecular Breeding for Insect Resistance

Prior to recent technological advances in molecular biology, quantitative traits were studied using statistical techniques based on quantitative genetic theory applied to appropriate

experimental populations (Falconer and Mackay, 1996). However, the models used to study

these traits are complex, and it is difficult to interpret precisely the genetic effects of individual

loci. Molecular markers, which represent variations in DNA sequence at specific genetic loci,

have been utilized widely in plant and animal breeding. The advantage of molecular markers is

that they allow for the dissection of quantitative traits into discrete loci or QTLs, allowing the

effects of single alleles to be studied (Paterson et al., 1988; Paterson, 1996).   A QTL can also

refer to a family of similar, tightly-linked loci that are generally inherited together (Paterson,

1996). Molecular markers are generally phenotypically-neutral, unaffected by the environment,

and allow for large numbers of plants to be screened in a short time period (Smith, 2004).

Several types of markers have been developed over the past two decades which have assisted

plant breeders. These include RFLP (restriction fragment length polymorphism), RAPD

(random amplified polymorphic DNA), AFLP (amplified fragment length polymorphism), SSR

(single sequence repeat) or microsatellites, and SNP (single nucleotide polymorphism) markers.

Molecular markers have been utilized to study the quantitative inheritance of PRI in corn

(Beavis et al., 1994), mungbean (*Vigna radiata* L. Wilczek) (Young et al., 1992), potato

(*Solanum tuberosum* L.) (Yencho et al., 1996), soybean (Narvel et al., 2001; Rector et

al.,1998,1999,2000; Terry et al., 2000), and tomato (*Lycopersicon esculentum* Miller)

(Maliepaard et al., 1995). Prior to the advent of this technology, phenotypic evaluations were

necessary to determine which of the progeny from crosses made between resistant and

susceptible parents had inherited resistance genes. The identification of molecular markers

associated with phenotypic traits such as PRI has allowed researchers to map QTL to specific

chromosomal locations, and has made it feasible to select and screen germplasm at the molecular

level.

The construction of genetic linkage maps is based on the observation of the co-transmission of genes and markers closely associated on a chromosome (Morgan, 1911). The distance between the genes is calculated from the percentage of crossover events during meiosis (Sturtevant, 1913). Recombination frequency (RF) is the measure of recombination between genetic loci. RF values are measured among segregating progeny by determining marker genotypes at various loci in each progeny, and then analyzing the data with mapping software which then calculates genetic distance and constructs linkage maps.

QTLs conditioning soybean antixenosis and antibiosis resistance to CEW were first identified by Rector et al. (1998, 1999, 2000) in three mapping populations developed by crossing the susceptible cultivar Cobb to PI 171451, PI 227687, and PI 229358. To map the soybean insect resistance QTLs (SIRQTLs), progeny populations were evaluated for non-random associations between their insect resistance phenotype and genotype at a marker locus. Statistically significant associations between a marker and resistance are indicative of a nearby gene/QTL for the trait (Paterson et al., 1988). A major QTL for antixenosis ($R^2 = 0.37$) and antibiosis ($R^2 = 0.22$-0.28) was identified on LG M (SIRQTL-M) in both PI 229358 and PI 171451 using RFLP markers (Rector et al., 1998, 1999, 2000), and later SSR markers (Narvel et al., 2001). Minor SIRQTLs were identified on LG G (SIRQTL-G) of PI 229358 for antibiosis ($R^2 = 0.19$), on LG H (SIRQTL-H) in PI 227687, PI 229358, and PI 171451 for antixenosis ($R^2 = 0.09$-0.19), and on LG D1b (SIRQTL-D1b) in PI 229358 for antixenosis ($R^2 = 0.10$) (Rector et al., 1998, 1999, 2000; Narvel et al., 2001). Narvel et al. (2001) assessed the introgession of these SIRQTLs in phenotypically selected SIR cultivars, germplasm releases, and breeding lines by testing for the presence of PI alleles at SSR marker loci tightly linked to the SIRQTLs. This study also evaluated the amount of PI genome surrounding the SIRQTL-M in each line. SSR

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marker genotyping of SIR germplasm revealed that nearly all had introgressed PI parent DNA at SIRQTL-M, relatively few had introgressed SIRQTL-G and SIRQTL-H DNA, and none possessed IRQTL-D1b, indicating that marker-assisted selection (MAS) would be useful in incorporating the full complement of SIR QTLs into an elite cultivar while minimizing linkage drag (Narvel et al., 2001). Near-isogenic lines (NILs) for SIRQTLs were developed in a Benning genetic background using MAS, which created lines containing individual SIRQTLs and combinations of SIRQTLs, with the aim of elucidating main and epistatic effects of resistance loci within a Benning background (Zhu, et al., 2006). Also, Zhu et al. (2006) used recombinant substitution lines (RSLs) to fine-map SIRQTL-M to an approximately 0.52-cM interval on LG M, and have created a PI229358 BAC library to use in cloning SIRQTL-M.

Marker-assisted Selection

MAS involves the selection of plants or experimental lines within segregating populations using molecular markers. MAS is now a powerful tool in soybean breeding programs as it provides breeders with the ability to make selections based on marker data, and to pyramid desirable alleles for complex or otherwise intractable traits into adapted genetic backgrounds (Orf et al., 2004). MAS in plant breeding may be applied to parent selection, recovery of recurrent parent during backcrossing, early generation trait selection, and multiple trait selection (Dudley, 1993; Knapp, 1998). Using molecular markers, breeders can hasten the process of cultivar development through backcrossing by selecting for one or more important genetic regions of the donor parent, while simultaneously selecting for the recurrent parent's genome, thus speeding its recovery and potentially thwarting the problem of linkage drag (Orf et al., 2004). Hospital et al. (1992) determined through the use of mathematical models and computer simulations that the use of molecular markers in backcrossing could eliminate the need

for approximately two backcross generations. MAS for a gene being backcrossed is especially useful in soybean, where manual pollinations are very tedious, many pollinations are unsuccessful, and successful pollinations rarely produce more than two seeds. After an important QTL is mapped, researchers may want to backcross the gene into an elite genetic background for confirmation of its effect. Using MAS to pyramid resistance genes with similar effects may allow breeders to create cultivars with more durable resistance (van Berloo and Stam, 1999; Narvel et al., 2001). MAS has proven its effectiveness with the introgression of single genes (Prabhu et al., 1999), pyramiding (Hittalmani et al., 2000), and multiple QTL (Schneider et al., 1997; Toojinda et al., 1998). According to Orf et al. (2004), MAS has many advantages over phenotypic selection, including increased genetic gains and reduced costs, increased effectiveness of selection, and reduced time per breeding cycle. MAS has increased the feasibility of creating agronomically-acceptable SIR cultivars with the full complement of resitance alleles from the donor parent along with the ability to pyramid resistance alleles from additional SIR germplasm accessions.

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CHAPTER 3

SEED YIELD AND INSECT RESISTANCE OF NEAR-ISOGENIC SOYBEAN LINES

WITH INTROGRESSED RESISTANCE QTL FROM PI 229358[1]

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Abstract

The development of superior soybean cultivars [*Glycine max* (L.) Merr] exhibiting resistance to insects has been hindered due to linkage drag, a common phenomenon when introgressing alleles from exotic germplasm. Simple-sequence repeat (SSR) markers were utilized previously to map soybean insect resistance (SIR) quantitative trait loci (QTL) in a 'Cobb' × PI 229358 population, and subsequently used to create near-isogenic lines (NILs) with SIR QTL in a 'Benning' genetic background. SIR QTLs were mapped on linkage groups (LGs) M (SIRQTL-M), G (SIRQTL-G), H (SIRQTL-H), and D1b (SIRQTL-D1b). The objectives of this study were to: (i) evaluate linkage drag for seed yield using Benning-derived NILs selected for SIRQTL-M, SIRQTL-H, and SIRQTL-G, (ii) assess the amount of PI 229358 genome surrounding the SIR QTL in each Benning NIL, (iii) evaluate the individual effects these three QTLs on antibiosis and antixenosis to corn earworm (CEW; *Helicoverpa zea*) and soybean looper (SBL; *Pseudoplusia includens*), and (iv) evaluate the effects of SIRQTL-M and the putative SIRQTL-D1b and their interactions in a Benning genetic background. Yield data collected in five environments indicated that a significant (P=0.05) yield reduction of approximately 250 kg ha^{-1} is associated with SIRQTL-G compared to NILs without SIR QTL. Overall there was no yield reduction associated with SIRQTL-M or SIRQTL-H. A significant antixenosis and antibiosis effect was detected for SIRQTL-M in insect feeding assays, with no effect detected in antixenosis or antibiosis assays for SIRQTL-G or SIRQTL-H without the presence of PI 229358 alleles at SIRQTL-M. These results support recent findings concerning these loci. SIRQTL-D1b was found to not condition an appreciable level of antixenosis resistance to CEW.

Key Words: corn earworm, *Glycine max,* marker-assisted selection, quantitative trait loci,

soybean looper soybean insect resistance, soybean, simple sequence repeats

Introduction

Increased soybean [*Glycine max* (L.) Merr.] production in the southern USA over the last

half-century prompted swift development of integrated pest management (IPM) programs aimed

at limiting yield losses from a host of insect pests. The subtropical climate conditions and long

growing seasons of this region make soybean especially vulnerable to economically important

insect infestations. Pesticide applications have been the primary control method employed

during insect infestations reaching threshold levels. Such chemical control measures are

economically unattractive to growers due to soybean's low value per hectare (Boethel, 1999**).**

Moreover, the detrimental environmental impacts created by repeated pesticide use in

agricultural production systems have been well-documented for some time (Boethel, 2004).

Cultivars exhibiting SIR have the potential to be a management tactic compatible with and a

supplement to threshold-triggered insecticide applications. Although U.S. soybean breeders and

entomologists have developed numerous SIR breeding lines and released four SIR cultivars,

none have been widely employed by soybean producers, primarily due to their low yield

potential. Success in producing high-yielding, agronomically acceptable SIR cultivars may

hinge upon continued advances in molecular biology, as well as in efforts by researchers to

elucidate the most efficient and cost-effective breeding techniques for SIR.

The major sources of insect resistance germplasm utilized in soybean breeding programs

to this point have been three Japanese plant introductions (PIs), PI 171451 ('Kosamame'), PI

229358 ('Soden-daizu'), and PI 227687 ('Miyako White'). Since these PIs exhibit resistance to

nearly all major soybean defoliators, they have served as the primary donor parents in breeding

for resistance to multiple insects (Clark et al., 1972; Hatchett et al., 1976; Lambert and Kilen, 1984 Van Duyn, 1971, 1972). Three mechanisms of plant resistance to insects have been described previously (Painter, 1951; Kogan and Ortman, 1978; Lambert and Kilen, 1984) and include antixenosis, antibiosis, and tolerance. Although each has been described as a distinct resistance mechanism, their effects are not mutually exclusive (Painter, 1951; Rector et al., 1999, 2000). Antixenosis, also known as non-preference, encompasses any biochemical or morphological trait which discourages or repels insect feeding, oviposition, or colonization (Painter, 1951; Kogan and Ortman, 1978). Antibiosis refers to any detrimental physiological effect on insect development, growth, and/or reproduction caused by feeding on plant tissue. Tolerance refers to a plant's ability to endure moderate tissue damage without a significant reduction in yield.

Soybean resistance to insect defoliation is a quantitative trait with multiple genes or QTL conditioning the observed phenotype. The utilization of molecular markers in QTL mapping has made it feasible to evaluate the relative phenotypic variation associated with discrete SIR loci (Tanksley et al., 1989). Rector et al. (1998; 1999; 2000) used restriction fragment length polymorphism (RFLP) markers to map QTLs associated with antixenosis and antibiosis resistance to corn earworm (*Helicoverpa zea* Boddie) in three individual crosses of the susceptible cultivar Cobb to the three SIR PIs, PI 171451, PI 227687, and PI 229358. A major SIR QTL with its resistance allele contributed from PI 229358 was detected on linkage group (LG) M (SIRQTL-M) for both antixenosis ($R^2 = 37\%$) and antibiosis ($R^2 = 22\%$), while two QTL with smaller effects were identified on LG G (SIRQTL-G) and LG H (SIRQTL-H) for antibiosis ($R^2 = 19\%$) and antixenosis ($R^2 = 16\%$), respectively. An additional SIR QTL conditioning antixenosis ($R^2 = 10\%$) was discovered on LG D1b (SIRQTL-D1b). In a follow-up

study, the SIRQTLs were mapped using SSR markers in the Cobb × PI 229358 population, which provided more precise estimates of QTL locations on each linkage group (Narvel et al., 2001). SIRQTL-M was recently fine-mapped using recombinant substitution lines (RSLs) derived from a Benning (7) × PI 229358 population to a 0.52 cM (centimorgan) interval on LG M (Zhu, et al., 2006). This increased resolution at SIRQTL-M provides improved marker precision and should facilitate ongoing efforts to clone SIRQTL-M. A bacterial artificial chromosome (BAC) library was developed from PI 229358 genomic DNA with this aim in mind (Zhu et al., 2006).

Although over 40 breeding lines and four cultivars possessing some level of SIR have been developed and released over the past 30 yr, none have successfully combined a high level of insect resistance with high seed yield. The phenomenon of linkage drag has been implicated as the cause of this limitation (Boethel, 1999; Sisson et al., 1976; Rufener et al., 1989). Linkage drag refers to the inadvertent selection and transfer of an undesirable allele genetically linked to a desired one and typically occurs while introgressing novel alleles from exotic germplasm into elite germplasm (Boethel, 1999). Since SIR is a polygenic trait, insufficient levels of resistance in many SIR releases may be attributed to the limitation of phenotypic (ie., insect screening assays) selection in transferring the full complement of QTLs present in the donor parent (Narvel et al., 2001). In addition, selection for yield and other agronomic traits may have resulted in the loss of SIR genes linked to inferior agronomic trait alleles. Consistent with the majority of exotic soybean accessions, the three Japanese donor PIs utilized in SIR breeding programs are low-yielding and of low agronomic value. Thus, the transfer of PI genome surrounding the desired SIR gene to the recurrent parent likely inhibits the yield potential of SIR cultivars.

Marker-assisted selection (MAS) has been employed in modern breeding programs as an alternative or supplement to phenotypic selection. Narvel et al. (2001) used SSR markers to determine the success of phenotypic selection for insect resistance by assessing how many of the known SIR QTLs were present in each of 15 advanced SIR genotypes and the amount of donor PI 229358 genome introgressed along with SIRQTL-M. After genotyping regions flanking SIR QTLs with SSR markers, it was concluded that phenotypic selection had been unsuccessful in transferring essential SIRQTL-G and SIRQTL-H into advanced germplasms. More importantly, phenotypic selection had maintained major segments of PI genome surrounding the major SIRQTL-M in many of the advanced breeding lines and cultivars.

After assessing the introgression of SIR QTLs into advanced breeding lines, Narvel et al. (2001) concluded that the minor QTL on LG D1b may have been a false positive in the initial mapping, since it appeared to be absent in all 15 SIR genotypes used in the study. The relatively small population size used in the original mapping study and a low logarithm of the odds (LOD) score indicated that this QTL may have been falsely identified (Beavis et al., 1998; Narvel et al., 2001). Bernardo (2004) believes that prior to the utilization of putative QTLs in MAS, they should be detected using a more stringent comparison-wise significance level. Moreover, it would be highly ineffective and costly to apply MAS for a "false positive" or non-resistant QTL.

To evaluate the potential of MAS for early generation trait selection and for facilitating the recovery of the recurrent parent during successive backcrosses, BC_6F_2 near-isogenic lines (NILs) derived from Benning (7) × PI 229358 were developed. Plant materials were selected in each generation using primarily MAS prior to each backcross with Benning (Boerma and Mian, 1999). These NILs provide the unique opportunity to study the effects of individual SIR QTL within an elite genetic background, as well as to assess the effectiveness of MAS in breeding for

superior SIR cultivars. The objectives of this study were: (i) to evaluate linkage drag associated with seed yield using Benning-derived NILs selected for SIRQTL-M, SIRQTL-H, and SIRQTL-G, (ii) to assess the amount of PI 229358 genome surrounding each SIR QTL in each Benning NIL, (iii) to evaluate the individual effects these three QTLs on antibiosis and antixenosis to soybean looper (SBL; *Pseudoplusia includens*) and CEW, and (iv) to evaluate the effects of SIRQTL-M and the putative SIR QTL on LG D1b and their interactions in a Benning genetic background.

Materials and Methods

Development of Plant Materials

For the 2004 tests, BC_6F_2-derived NILs from Benning (7) × PI 229358 were developed by selecting plants based on individual SSR genotypes at previously determined SIR QTL and through phenotypic screening for antixenosis and antibiosis resistance. Benning is a Maturity Group VII cultivar that is adapted to the Southeast (Boerma et al, 1997; Day et al., 1999). For this study, five NILs for each SIR QTL were created by selecting for homozygous PI 229358 alleles at Satt220, Satt536, and Satt175 for SIRQTL-M; Sat_334, Sat_122, and Sat_118 for SIRQTL-H; and Sct_199, Satt472, and Satt191 for SIRQTL-G during each backcross generation and in the final selection of each isoline. Each SIR QTL was represented individually by five BC_6F_2-derived NILs. Each BC_6F_2-derived was derived from a different BC_6F_2 plant. Concurrently, five lines were also selected for homozygous Benning alleles at SIRQTL-M, SIRQTL-H, and SIRQTL-G concurrently, thus creating a set of control lines.

In 2005, two BC_6F_2-derived lines representing SIRQTLs-M, SIRQTL-H, and SIRQTL-G were chosen based on their overall agronomic performances and similarity to Benning in 2004. Also included in 2005 were three Benning lines and three lines homozygous for PI 229358

alleles at all three SIR QTLs. In an effort to make clear the distinction between the individual

SIR QTL and the SIR QTL-containing NILs, the NILs will henceforth be referred to as Benning-

M, Benning-H, and Benning-G, Benning-mgh (control lines lacking the three SIR alleles), and

Benning-MGH (all three SIR alleles combined in one NIL).

Yield Assessment of SIR NILs

To evaluate seed yield of individual SIR genotypes, BC_6F_2-derived SIR near-isolines

were grown in two experiments at the Univ. of Georgia Plant Sciences Farm near Watkinsville,

GA, and in one experiment at the Univ. of Georgia Southwest Research and Education Center

near Plains, GA in 2004. The experiments were planted on 21 May and 18 June at the Plant

Sciences Farm and on 14 May at Plains. Each experiment was arranged in a randomized

complete block experimental design with three replications. A total of 25 entries (five entries

each of Benning-M, Benning-G, Benning-H, Benning-mgh, and Benning) were randomized

within each block. The experimental unit for each entry was two 6.1 m rows spaced 76.2 cm

apart. Each plot was end trimmed to 3.6 m prior to harvest. For each plot data were recorded for

maturity (date on which 95% of pods had reached their mature color), lodging (1=all plants

upright to 5=all plants prostrate), and plant height (average distance from the soil surface to tip

of three plants per plot). At maturity each plot was harvested with a self-propelled plot combine.

To determine protein and oil content, a 50-g seed sample from each plot was sent to the USDA-

ARS National Center for Agricultural Utilization Research at Peoria, IL., where an 18- to 20-g

seed sample was evaluated for protein and oil composition with a model 1255 Infratec NIR Food

and Feed Grain Analyzer (Ultra Tec Manufacturing, Inc., Santa Ana, CA). Seed quality scores

were based on visual scores (1=very good to 5=very poor). Seed weight (average weight of an

individual seed) for each plot was evaluated from a 100-seed sample. Phenotypic data were

analyzed by analyzis of variance with the Agrobase software (Agronomix Software Inc., Winnipeg, Canada). The statistical model assumed blocks and environments as random effects and NILs as fixed effects.

In 2005, seed yield of individual BC_6F_2-derived SIR NILs was evaluated in two environments. The first experiment was planted on 23 May at the Plant Sciences Farm and the second was planted on 26 May at the Southwest Research and Education Center. Each experiment was arranged in a randomized complete block experimental design with four replications. A total of 12 entries (three entries each of Benning and Benning-MGH; two entries each of Benning-M, Benning-H, and Benning-G) were randomized within each block. The experimental unit for each entry was the same as the 2004 tests. Prior to harvest, plots were end-trimmed to 3.6 m. Agronomic data for each genotype was collected and analyzed as described for the 2004 experiments.

Antixenosis Tests with Benning NILs

Antixenosis resistance in SIR NILs was assessed for CEW and SBL in a greenhouse using the same procedure described by All et al. (1989). CEW and SBL eggs were supplied by the Crop Protection and Management Unit (USDA-ARS, Tifton, GA) and Benzon Research (Carlisle, PA), respectively. Three seeds of each entry were planted in 450-mL polystyrene foam cups with three holes punched in the bottom of each to allow for drainage and water uptake. The cups were filled with Fafard 2 mix (Conrad Fafard, Agawam, MA). Each cup was thinned to one plant after 5 d. Cups with healthy seedlings were organized in a randomized complete block experimental design with 10 replications. Five lines of each SIR QTL (Benning-mgh, Benning-M, Benning-G, and Benning-H) along with one entry each of Benning and PI 229358 were included in each block. The cups were placed in a stainless steel pan measuring 4.9-m long by

1.2-m wide by 8-cm deep and filled with 2-cm of water. The cups were in contact to ensure that larvae be able to move freely about the canopy of the plants. Benning (susceptible) and PI 229358 (resistant) were also planted as a border in alternating fashion around all 10 blocks. When plants reached the V2 stage of development (Fehr and Caviness, 1977), four neonate (<5 h old) were placed on an unexpanded trifoliolate leaf of each plant with a 000-size camel's hair brush. Ten days after infestation, the percent leaf area defoliated was visually estimated by three different individuals. The defoliation scores for each cup (average of 3 individuals) were analyzed by analysis of variance with the Agrobase software (Agronomix Software Inc., Winnipeg, Canada).

Antibiosis Tests with Benning NILs

The level of antibiosis to CEW and SBL for the SIR QTL in each NIL was evaluated in a growth chamber by a procedure previously employed by Walker et al. (2002). Each chamber was maintained at 27º C with 85% ambient humidity under 14 h of fluorescent light provided by incandescent fixtures. Each chamber provided approximately 40 µmol photons $m^{-2} s^{-1}$. Newly expanded trifoliolate leaves were harvested from plants grown in the greenhouse. For the CEW assays, one leaflet was placed in a Petri dish (100×25 mm) and infested with a single neonate larva. For the SBL assays, an entire trifoliolate was placed in a Petri dish with three neonate larvae. CEW larvae were not placed in dishes together due to their cannibalistic nature. Therefore, the CEW experiment required three Petri dishes to achieve a single experimental unit. After 4 d, a new leaflet or trifoliolate was added to each dish. Once leaf tissue in any of the plates was totally consumed, feeding was ceased by placing all of the dishes in a 4ºC chamber (around 6 d after infestation). After 1 h, larvae were placed in empty Petri dishes and frozen at -20ºC overnight and then weighed. The experiment included the 25 previously described NILs

along with five entries each of Benning and PI 229358. The assays were arranged in a

randomized complete block design with six blocks. The average weight of surviving larvae from

three individual dishes (CEW) or in one dish (SBL) were recorded and analyzed by analysis of

variance with Agrobase software (Agronomix Software Inc., Winnipeg, Canada).

SSR Marker Analysis

For the marker analysis, DNA was extracted from unexpanded trifoliolate leaves of the

25 NILs greenhouse-grown plants using a modified CTAB procedure (Keim et al. 1988). To

isolate DNA from leaf tissue, lyophilized leaves were ground in 1.1-mL, 96-deepwell plates and

suspended in 700μL of CTAB buffer [2% (w/v) CTAB; 1.4 M NaCl; 100mM Tris-HCl pH 8.0;

20 mM EDTA; and 1% (v/v) 2-β-mercaptoethanol]. For PCR amplification, reaction mixtures

contained 20 ng of genomic DNA, 0.5 μM of forward and reverse primers, 2 mM of each dNTP,

2.5 mM Mg^{2+}, 1X PCR buffer, and 0.5 units *Taq* polymerase in a total volume of 10 μl. The

separation of PCR amplicons was conducted using 4.8% polyacrylamide gels run on an ABI

PRISM 377 DNA Sequencer (PE ABI, Foster City, CA). Samples were prepared for

electrophoresis by combining 3 μl of PCR product, 2 μl formamide, 0.75 μl loading buffer, and

0.30 μl GENESCAN-500 ROX DNA size standard (PE Applied Biosystems, Foster City, CA).

Each was denatured for 5 min at 95ºC, and 1.5 μl of each sample was loaded in the appropriate

well of a 96 lane gel. Gels were scored visually based on marker size data from each parent to

determine the SSR marker genotype of each line.

Assessment of PI 229358 genome introgression

The previously described SSR marker methodology was used to evaluate the relative

amount of PI 229358 genome present in the genomic regions of NILs harboring SIRQTL-M,

SIRQTL-G, SIRQTL-H. Graphical genotypes were determined by SSR fingerprinting individual

NILs in a 42 to 73-cM region surrounding each introgressed SIR QTL. Distances between genetic markers were scaled to coincide with their actual estimated genetic distances, and crossover points are represented midway between the two flanking markers (Fig 3.1). It was presumed that no double crossovers in a single meiosis had occurred between markers.

Antixenosis Tests for SIRQTL-D1b Confirmation

To evaluate the resistance to CEW associated with the putative SIR QTL located on LG D1b, four lines homozygous for the different allelic combinations of SIRQTL-D1b and SIRQTL-M were identified and selected from either $BC_5F_{2:3}$ or $BC_6F_{2:3}$ lines derived from Benning (6) × PI 229358 and Benning (7) × PI 229358, respectively. SIRQTL-D1b was selected with SSR markers Satt141 and Satt290 and SIRQTL-M was selected with Satt536, Satt220, and Satt175 (Narvel et al., 2001). The selected lines were homozygous for all four combinations of either the Benning or the PI 229358 alleles at SIRQTL-D1b and SIRQTL-M.

Antixenosis resistance was evaluated with CEW in a greenhouse using the same procedure described above. Plants were organized in a randomized complete block design with 10 blocks. Four homozygous lines of each SIRQTL-D1b/SIRQTL-M allelic combination were represented within each block. Defoliation scores were analyzed by analysis of variance with the Agrobase software (Agronomix Software Inc., Winnipeg, Canada). To assess epistasis between SIRQTL-D1b and SIRQTL-M, the factorial treatment structure was analyzed by analysis of variance. The main effects of the SIRQTL-D1b and SIRQTL-M and their interaction (SIRQTL-D1b × SIRQTL-M) were determined.

Results and Discussion

Main Effects of SIR QTLs for Antixenosis and Antibiosis Resistance

Benning-derived NILs representing each of the SIR QTLs were evaluated for antixenosis and antibiosis resistance against CEW and SBL (Table 3.1). Based on the average percent leaf defoliation and larval weights, Benning was the most susceptible and PI 229358 the most resistant to CEW and SBL, as expected. For CEW, the level of defoliation and larval weight of for Benning-M lines were found to be similar to PI 229358. Although Benning-M and PI 229358 were equally defoliated by SBL, the SBL larvae that fed on Benning-M averaged 10.9 mg heavier than those that fed on PI 229358. The effect of SIRQTL-M was detected in CEW and SBL antixenosis screens as Benning-M lines were significantly less defoliated than Benning (7% less for CEW and 13% less for SBL) and the Benning-mgh control (5% less for CEW and 15% less for SBL). Similarly, larval weights were 25 mg less for CEW and 26 mg less for SBL when the insect was provided SIRQTL-M leaves rather than Benning leaves. When Benning-M lines were compared to the Benning-mgh control, Benning-M significantly (P=0.05) reduced CEW larval weights by 16 mg but not SBL larval weights. No significant (P=0.05) effects were detected for SIRQTL-G and SIRQTL-H when compared to Benning-mgh for CEW and SBL antibiosis or antixenosis. When Benning-H and Benning-G NILs were compared with the Benning recurrent parent, the weight of SBL larvae were reduced by approximately 17 mg for both SIR QTL. Overall, these results are in close agreement with those reported by Zhu et al. (2006). They used CEW and concluded that PI 229358 alleles at either SIRQTL-H or SIRQTL-G must be in combination with PI 229358 alleles at SIRQTL-M to provide significant reductions in defoliation and larval weight.

Evaluation of Yield Drag Associated with SIR QTLs

A comparison of seed yield of Benning-derived NILs (Benning-mgh, Benning-M, Benning-H, and Benning-G) and recurrent parent Benning was assessed in three environments in 2004. The Benning-G NILs averaged 250 to 335 kg ha^{-1} lower yield than Benning-H, Benning-M, Benning-mgh, or Benning (Table 3.2). The recurrent parent Benning averaged 335 kg ha^{-1} higher yield than Benning-G. Lines containing SIRQTL-G averaged the lowest mean seed weight (126 mg seed^{-1}) of all NILs, which was 9 mg seed^{-1} less than Benning. Interestingly, Benning-M NILs had significantly larger seed than all other entries.

For other agronomic traits, significant differences were detected among the SIR QTL NILs for plant height (cm), while no significant differences were detected for seed oil, seed protein, or seed quality (Table 3.2). Benning-H lines averaged 6 cm shorter than Benning and 5 to 7 cm shorter than the other SIR QTL NILs.

The SIR NILs were also evaluated in 2005 in two environments along with Benning-MGH and Benning lines. Benning-mgh, the control lines, were not included in the test. In 2005, Benning-H and Benning were significantly higher in seed yield than all other entries (Table 3.3). The lowest yielding entries were Ben-MGH and Benning-G. These NILs averaged 340 kg ha^{-1} lower yield than the recurrent parent Benning. These NILs both contained SIRQTL-G. Over the five combined environments, the Benning-G NILs averaged approximately 283 kg ha^{-1} less seed yield than Benning, while Benning-M and Benning-H were similar in yield to Benning (Table 3.4).

In 2005, significant differences for plant height, lodging, seed quality, and seed weight were found among the SIR NILs (Table 3.3). No significant differences were detected for seed oil or seed protein. Similar to 2004, the Benning-H lines were significantly shorter than all other

lines evaluated, while the Benning-G lines again showed the smallest seed weight. In two previous QTL mapping studies, Mian et al. (1996) and Lee et al. (2001) each detected a QTL at the RFLP locus A235 on LG G associated with seed weight in the two mapping populations PI97100 × 'Coker 237' and 'Pureunkong' × 'Jinpumkong', respectively. Interestingly, this RFLP locus maps within 2 cM of the SSR markers Satt191 and Satt472 that flank SIRQTL-G.

The amount of PI 229358 DNA flanking each SIR QTL in the NILs was estimated using SSR markers. Graphical genotypes were constructed for SIRQTL-M, SIRQTL-G, and SIRQTL-H NILs as a means of assessing the relative amounts of PI 229358 genome remaining in each NIL near the introgressed SIR QTL (Fig 3.1). SSR marker loci residing within the genomic regions not included in the graphical genotypes were monomorphic between Benning and PI 229358. It was assumed that a marker locus located between two markers shown in Figure 1 with the same parental alleles were also of the same genotype. Each of the five lines carrying SIRQTL-G and SIRQTL-H had the same SSR genotypes in regions flanking the presumed location of each SIR QTL.

Graphical genotypes surrounding SIRQTL-G cover a 42-cM region based on six polymorphic SSR markers, with approximately 26-cM of PI genome surrounding the QTL for the SIRQTL-G NIL. Seventy-three centiMorgans and five polymorphic SSR markers were evaluated for the Benning-H graphical genotypes, with 23-cM of PI 229358 genome remaining in all five of the SIRQTL-H NILs. Benning-M graphical genotypes represented a 58-cM region based on seven polymorphic SSR markers surrounding SIRQTL-M. Three of the five Benning-M NILs (L1, L2, and L3) contain approximately 41-cM of PI 229358 genome, spanning from Satt220 to Satt306. NIL L4 and L5 have PI 229358 alleles between Satt220 and Satt175, roughly an 11-cM distance, indicative of an additional cross-over event in this region upstream

of SIRQTL-M (Fig. 3.1). No significant differences in agronomic performance or resistance to CEW or SBL could be detected among these lines, again reaffirming that SIRQTL-M is likely located in the region between Satt220 and Satt536 on LG M (Zhu et al., 2006). As expected, the Benning-mgh lines possess Benning alleles at all marker loci used to genotype Benning-M, Benning-G, and Benning-H NILs in the region containing the appropriate SIR QTL (data not shown).

Our results indicate there was not a yield reduction associated with SIRQTL-M or SIRQTL-H NILs. Thus, these SIR QTL could be introgressed from these NILs into other cultivar backgrounds without an expected yield drag. On the other hand, our results indicate that a significant seed yield reduction was associated with SIRQTL-G. It is also apparent that seed weight is reduced in these NILs. The smaller seed weight could be conditioned by a seed weight QTL residing in the region flanking SIRQTL-G. The approximately 26-cM segment of PI 229358 genome surrounding this QTL occurs at the distal portion of LG-G, where informative SSR markers are limited. In order to make substantial progress in our efforts to eliminate the yield drag associated with SIRQTL-G, it will be imperative to create and identify lines containing cross-overs within this specific region of LG G. This task would be made easier by the development of additional PCR-based markers in this region of LG G. The Georgia Agricultural Experiment Stations has recently approved the release of SIR germplasm (Benning NILs with introgressed SIRQTL-M, SIRQTL-G, SIRQTL-H, and SIRQTL-MGH from PI 229358) with the aim of providing this material to soybean breeders and entomologists for further study and for use in development of SIR cultivars.

Evaluation of SIRQTL-D1b

Sixteen NILs representing four combinations of the putative SIRQTL-D1b and the major SIRQTL-M were evaluated for antixenosis resistance with CEW (Table 3.5). Analysis of variance for antixenosis showed no significant main effect for the SIRQTL-D1b (P>0.05), while a significant main effect for SIRQTL-M (P < 0.0001) was detected. There was no significant (P > 0.05) epistatic interaction between the two SIR QTLs.

Based on defoliation ratings, NILs with SIRQTL-M alleles from PI 229358 averaged 13.9% less defoliation by CEW than those in which the QTL was homozygous for the Benning allele. The observed main effect of SIRQTL-M was expected based on its detection in two mapping populations (Rector et al., 1998, 1999, 2000) and its confirmation in Jack and Benning backgrounds as a major QTL conditioning insect resistance (Walker et al., 2002; Zhu et al., 2006). The data indicate that the SIRQTL-D1b previously identified in a Cobb × PI 229358 population does not condition a detectable level of resistance to CEW defoliation in a Benning background, even in the presence of the PI 229358 allele at SIRQTL-M.

According to Romagosa et al. (1999), the magnitude of a putative QTL should be assessed by evaluating the outcome of its selection in a novel genetic background; thereby the true breeding value of a QTL may be assessed. In the original mapping study, Rector et al. (1999) found differences in CEW defoliation levels among lines with alternative alleles at the SIRQTL-D1b locus, indicating that SIRQTL-D1b conditioned a significant level of CEW resistance. Narvel et al. (2001) later mapped SIRQTL-D1b to the same location on LG D1b with SSR markers, so it is unlikely that the general position of this QTL was misidentified (Rector et al., 1999; Narvel et al. 2001). One explanation for our inability to confirm SIRQTL-D1b is that it may have been a false positive in the original mapping population (Type I error), given that

only a moderately stringent probability level (P=0.01) was used in testing for significant associations (Fasoula et al., 2004). Narvel et al. (2001) speculated that this QTL may have been falsely identified after discovering that it had not been introgressed into any of 15 conventionally-derived SIR cultivars and breeding lines. This result is not totally unexpected due to the fact that the QTL was originally mapped in a small population (100), had a relatively low LOD score (2.3), and explained approximately 10% of the variation in CEW defoliation (Beavis, 1998; Fasoula et al., 2004). Although the effects of SIRQTL-D1b could not be detected in Benning, it is feasible, based on data reported by Day et al. (1996, 1998) that the Benning allele at SIRQTL-D1b conditions a higher level of antixenotic resistance to CEW than the Cobb allele, thereby reducing the magnitude of the difference between the Benning and PI 229358 allele at SIRQTL-D1b. Both Cobb and Benning have frequently been used as the susceptible checks in our insect resistance screening experiments. In these experiments, Benning is commonly less defoliated than Cobb by both CEW and SBL. Therefore, there is a possibility that there is a real difference between the PI 229358 allele at SIRQTL-D1b and the Cobb allele at that locus. From a practical breeding standpoint, however, these results indicate that there is no value associated with the introgression of the PI 229358 allele at SIRQTL-D1b into a Benning background.

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Table 3.1. Mean percent defoliation of SIR QTL NILs by corn earworm and soybean looper and larval weights from feeding assays.

Near-isogenic line	Corn earworm		Soybean looper	
	Defoliation (%)	Larval weight (mg)	Defoliation (%)	Larval weight (mg)
PI229358	24.6a†	22.1a	32.0a	18.0a
Benning-M	28.2ab	20.8a	33.7a	28.9b
Benning-H	32.7c	42.7b	47.0b	38.2b
Benning-G	31.5bc	40.5b	45.7b	38.1b
Benning-mgh	33.4c	37.2b	48.3b	34.9bc
Benning	35.3c	45.5b	46.8b	55.0d

† Means with the same letter are not significantly different based on F LSD (0.05).

Table 3.2. Mean agronomic performance of five SIR QTL NILs (five lines/NIL) at three 2004 environments.

| Near-isogenic line | Seed yield (kg ha^{-1}) | Plant height (cm) | Lodging (score) | Seed | | | |
				Oil (g kg^{-1})	Protein (g kg^{-1})	Quality (score)	Weight (mg seed^{-1})
Benning	2840a†	99a	4.0a	196a	382a	1.72a	135b
Ben-mgh	2756a	98a	4.0a	194a	385a	1.68a	127c
Ben-M	2779a	100a	4.4a	193a	388a	1.69a	146a
Ben-H	2755a	93b	4.2a	192a	387a	1.69a	139b
Ben-G	2505b	99a	3.9a	195a	386a	1.68a	126c

†Means with the same letter are not significantly different based on F LSD (0.05)

Table 3.3. Mean agronomic performance of five SIR QTL NILs (3 entries for Benning and two lines each of Benning-MGH, Benning-M, Benning-H, and Benning-G) at two 2005 locations

Near-isogenic line	Seed yield (kg ha^{-1})	Plant height (cm)	Lodging (score)	Seed			
				Oil (g kg^{-1})	Protein (g kg^{-1})	Quality (score)	Weight (mg seed^{-1})
Benning	3426a†	93b	1.5bc	20.5a	41.0a	2.5ab	143bc
Ben-M	3241b	99ab	2.1a	20.1a	41.4a	2.4bc	151a
Ben-H	3499a	91b	1.6bc	20.2a	40.9a	2.2c	147ab
Ben-G	3095bc	103a	1.4c	20.2a	41.4a	2.5ab	128d
Ben-MGH	3073c	95ab	1.7b	20.4a	40.9a	2.7a	138c

† Means with the same letter are not significantly different based on F LSD (0.05).

Table 3.4. Mean yield (kg ha^{-1}) of five SIR NILs and Benning in 2004 and 2005.

Near-isogenic line	2004 (3 locations)	2005 (2 locations)	2004/2005 (5 locations)
Benning	2840a†	3426a	3133a
Benning-mgh	2756a	---	
Ben-M	2779a	3244b	3067a
Ben-H	2755a	3500a	2949a
Ben-G	2505b	3096bc	2741b
Ben-MGH	---	3073c	

† Means with the same letter are not significantly different based on an F LSD (0.05).

Table 3.5. Main and epistatic effects of SIRQTL-D1b and SIRQTL-M on defoliation by corn earworm in Benning near-isogenic lines.

SIRQTL-M/ SIRQTL-D1b	SIRQTL-M (Benning)	SIRQTL-M (PI 229358)	Mean
SIRQTL-D1b (Benning)	28.0a†	14.2b	**25.6^{ns}**
SIRQTL-D1b (PI229358)	31.2a	15.7b	**26.6**
Mean	**31.6*****	**20.5**	

† Numbers followed by the same letters are not significantly different at the 0.05 probability level.

*** significantly different from its counterpart at the 0.001 probability level based on analysis of variance; ^{ns} not significantly different at the 0.05 probability level.
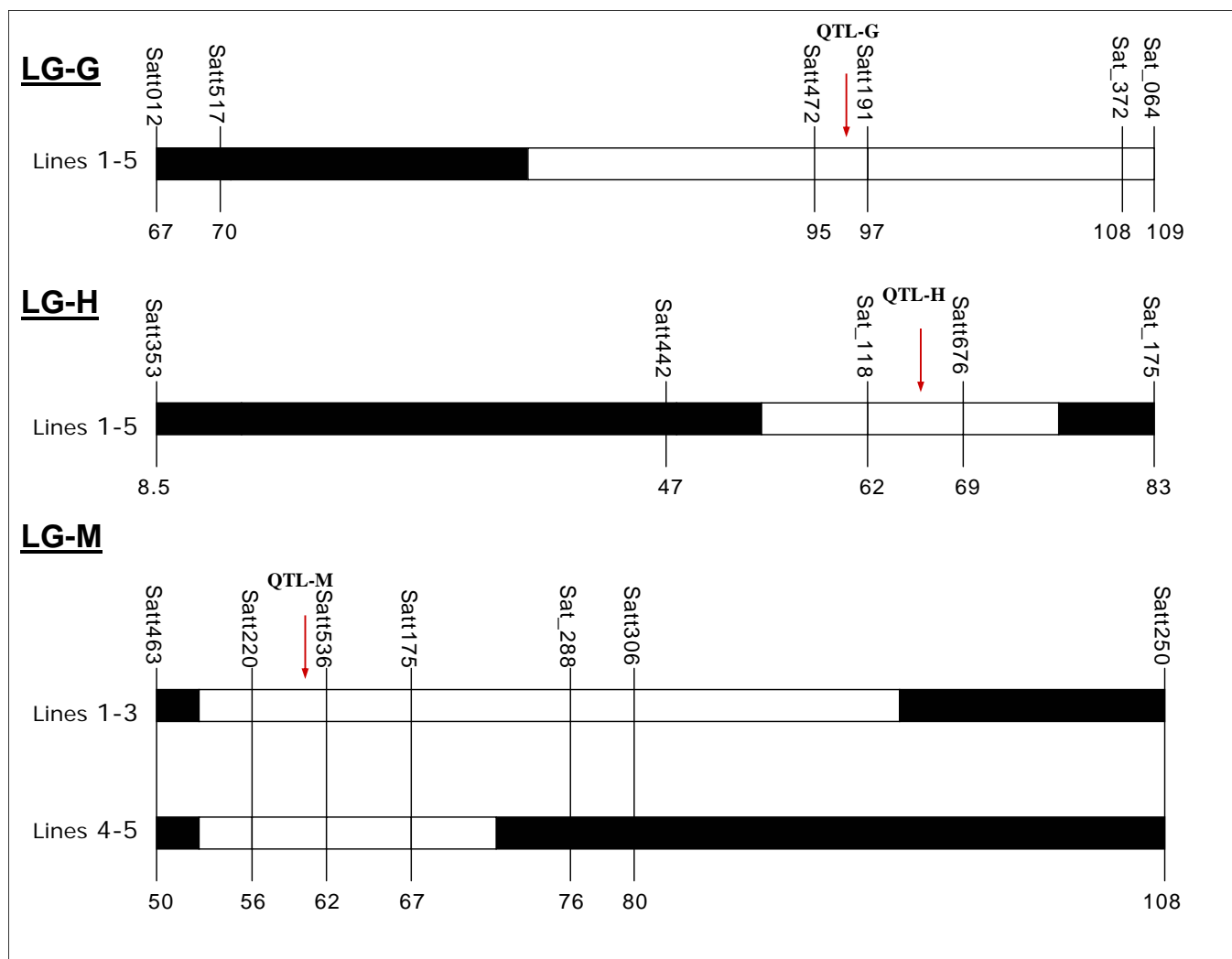
Figure 3.1. Graphical genotypes of NILs representing amount of PI 229358 genome flanking SIRQTL-G, SIRQTL-H, and SIRQTL-M. Numbers shown under each SSR locus is the approximate centimorgan (cM) location of the marker on the USDA-ARS concensus linkage group (Song et al. 2004). The most likely position of the SIR QTL is indicated by an arrow. White segments indicate PI 229358 origin, black segments indicate Benning origin. It was assumed that genomic regions defined by a single marker extended halfway to its flanking marker(s).

CHAPTER 5

SUMMARY

Efforts to develop high yielding insect resistant soybean cultivars have been largely unsuccessful, potentially due to linkage drag from the agronomically-poor resistance donors. Simple sequence repeat (SSR) markers identified QTL associated with CEW resistance in PI 229358 on linkage groups (LGs) M, H, G, and D1b. Marker-assisted selection was used to select for SIRQTL-M, SIRQTL-H, SIRQTL-G, and SIRQTL-D1b during the development of BC_5F_2 or BC_6F_2 near-isogenic lines (NILs) derived from Benning (7) × PI 229358. The NILs were evaluated for antixenotic and antibiotic resistance to CEW and SBL in greenhouse and no-choice Petri dish bioassays. Agronomic traits were assessed to determine whether any SIR QTL NILs were associated with poor agronomic performance due to linkage drag. The NILs were genotyped using SSR markers and graphical genotypes of the regions flanking each SIR QTL were created. Also, an experiment was conducted to confirm a putative QTL on LG D1b for CEW antixenosis resistance.

The average percent defoliation and the larval weights of CEW and SBL for Benning-M lines were similar to those of the resistant parent, PI 229358. The Benning-M NILs were significantly less defoliated than both Benning and Benning-mgh lines in antixenosis assays with CEW and SBL. In the antibiosis assays, CEW and SBL larvae fed Benning-M leaves had significantly reduced larval weights compared to those which fed on Benning leaves. In addition, CEW larvae fed Benning-M leaves had significantly reduced weights when compared to those fed leaves from the control Benning-mgh, while SBL larval weights were not significantly different. Our data support the recent findings by Zhu et al. (2006), which

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concluded that SIRQTL-H and SIRQTL-G are effective not solely in providing significant reductions in defoliation and larval weight unless in combination with SIRQTL-M.

Seed yield data from three environments in 2004 indicated that a reduction in seed yield of approximately 330 kg ha^{-1} was associated with SIRQTL-G when compared to Benning. The NILs containing SIRQTL-G also averaged 9 mg seed^{-1} less for seed weight than Benning. In 2005, the SIR NILs were evaluated for agronomic traits and again Benning-G lines had reduced seed yield and seed weight. Benning-MGH was statistically similar to Benning-G in mean seed yield, likely due to the presence of SIRQTL-G. By genotyping each set of lines using SSR markers, the amount of PI 229358 remaining in each NIL surrounding each resistance locus was found not to be associated with performance for SIRQTL-M and SIRQTL-H. It was also determined that the SIRQTL-D1b does not condition any appreciable level of antixenotic resistance to CEW, even in the presence of the SIRQTL-M.

The development of Benning-derived NILs containing PI 229358 alleles at previously identified SIR QTL allowed for yield drag and insect resistance to be evaluated for individual and multiple SIR QTL. Linkage drag for seed yield was not present in the NILs containing SIRQTL-M or SIRQTL-H. The study also reiterated the value of MAS as a breeding tool. Altogether, our findings should aid the in development of soybean cultivars with acceptable yields and high levels of resistance the few major lepidopteran defoliators of soybean.