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ORIGINAL PAPER

Identification of QTL in soybean underlying resistance to herbivory by Japanese beetles (*Popillia japonica*, Newman)

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Abstract Soybean [*Glycine max* (L.) Merr.] was one of the most important legume crops in the world in 2010. Japanese beetles (JB; *Popillia japonica,* Newman) in the US were an introduced and potentially damaging insect pest for soybean. JBs are likely to spread across the US if global warming occurs. Resistance to JB in soybean was previously reported only in plant introductions. The aims here were to identify loci underlying resistance to JB herbivory in recombinant inbred lines (RILs) derived from the cross of Essex × Forrest cultivars (EF94) and to correlate those with loci with factors that confer insect resistance in soybean cultivars. The RIL population was used to map 413 markers, 238 satellite markers and 177 other DNA markers. Field data were from two environments over 2 years. Pest severity (PS) measured defoliation on a 0–9 scale. Pest incidence (PI) was the percentage of plants within each RIL with beetles on them. Antibiosis and antixenosis data were from feeding assays with detached leaves in petri plates. Five QTL were detected for the mean PS field trait ($16\% < R^2 < 27\%$). The loci were within the intervals Satt632–A2D8 on linkage group (LG) A2 (chromosome 8); Satt583–Satt415 on LG B1 (11); Satt009–Satt530 on LG N (3); and close to two markers OB02_140 (LG E; 20 cM from Satt572) and OZ15_150 LG (19 cM from Satt291 C2). Two QTL were detected for the mean PI field trait ($16\% < R^2 < 18\%$) close to Satt385 on LG A1 and Satt440 on LG I. The no choice feeding studies detected three QTL that were significant; two for antixenosis ($22\% < R^2 < 24\%$) between Satt632–A2D8 on LG A2 (8) and Sat_039–Satt160 on LG F (13); and a major locus effect ($R^2 = 54\%$) for antibiosis on LG D2 (17) between Satt464–Satt488. Therefore, loci underlying resistance to JB herbivory were a mixture of major and minor gene effects. Some loci were within regions underlying resistance to soybean cyst nematode (LGs A2 and I) and root knot nematode (LG F) but not other major loci underlying resistance to nematode or insect pests (LGs G, H and M).

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Introduction

Japanese beetle (JB; *Popillia japonica,* Newman) is of the Order Coleoptera and Family Scarabaeidae (Potter and Held 2002). The Japanese beetle is native to the main island of Japan where it is not a damaging pest. However, in the US and Canada, JBs represent an introduced scarab that causes losses for both crops and ornamental plants. It was first found in the US in 1916 at a nursery near Riverton, New Jersey. The beetle is currently found in coastal and adjacent midwest states from Maine to Alabama with small infestations westward beyond the Mississippi River. Three infestations of this pest have been eradicated from California and several Western state remained JB free by 2009.

Japanese beetles are voracious feeders with over 300 host plant species (Ladd 1987, 1989). Feeding typically removes all interveinal cells leaving a network. The chemicals released from plant materials by feeding are attractants to other JBs so swarms develop on particular plants. Feeding attractants include simple sugars and volatiles with a fruity or flowery smell such as terpenoids, aliphatics, and aromatics (Loughrin et al. 1996). Feeding deterrents found in non-host plants appear to be anti-nutrients that reduce longevity and fecundity, since JBs do not avoid or learn to avoid known toxins. Feeding peaks from early morning to mid-afternoon with females leaving the swarms in mid-afternoon to oviposit on the roots or feed in solitary.

The larval stages, commonly called white grubs, were especially damaging to roots (Potter and Held 2002). The larvae feed on roots for a year, overwintering there. They pupate in Spring and emerge as adults in early summer. Adult females alternately feed, mate with multiple partners and return to the soil to oviposit so that populations of JBs can increase dramatically in 1 year following infestation. The extensive intermating suggests JBs have evolved under positive selection for increased recombination and the resultant diversity (Ladd 1987).

JBs threaten sustainable soybean–corn rotation production in the US as both crops are favored hosts. However, JBs may avoid fields close to non-host species such as sorghum (Smith et al. 1988; Bohlen and Barrett 1990). Further, threat derives from their invasive nature, high rates of inter-mating and tendency of larger swarms to form each year (Yesudas 2007; Tigreros and Switzer 2009). No-till and reduced till productions favor JB grub survival. Therefore, damage may be predicted to increase as farming practices change and global warming accelerates.

By 2009, JBs were often a significant pest of soybean in the Midwest and eastern US (Delucia et al. 2008; Zavala et al. 2009). However, the damage to cost of control threshold was rarely reached because it was predicted to be 30% defoliation before bloom, factoring in the cost of insecticides (Gould 1963). However, the threshold was somewhat arbitrary and did not account for the root damage caused by the white grubs nor the effect on the rotational crop the next year. Indeed, the testing of insecticidal seed treatments has shown major yield effects that cast doubts over previous estimates of the seed yield reductions ascribed to insect pests (Bradshaw et al. 2008).

Innate genetic resistance in soybean to JBs would be a low cost control method. JB-resistant soybean germplasm releases have been developed from a plant introduction (P.I. 171451; Hammond et al. 2001). The discovery was serendipitous in that the germplasm was selected for Mexican bean beetle, but also showed resistance to Japanese beetles in field (Hammond and Cooper 1989). The genetic basis of the resistance that was introgressed from P.I. 171451 is probably located on LG M linked to Satt463, Satt220, and Satt536. The gene underlying this QTL had not been reported by 2010. However, increased insect susceptibility in CO_2 enrichment was attributed to decreased cysteine protease inhibitors (Zavala et al. 2009; Casteel et al. 2008).

Forced feeding allows the detection of antibiosis and antixenosis by soybean leaves and the chemicals within them (Rector et al. 2000). Natural products found in soybean, such as coumarin, can be shown to act as deterrents for JBs when added to artificial diets (Patton et al. 1997b). Soybean plants contain many chemicals in roots, shoots and seed that deter the feeding by many insect pests (Narvel et al. 2001). Isoflavones (phaseol, afromosin, coumestrol, daidzein, and glyceollins) and other natural products may confer resistance to soybean (Rector et al. 2000; Patton et al. 1997a). The amount of each chemical required for resistance varies among cultivars and insect pests, and is partly determined by the genotype, developmental stage and organ ingested (Chiari et al. 2004; Kassem 2004, 2006; Primomo et al. 2006; Afzal et al. 2009). Conversely, protein, oil, and sugars were known soybean components that would increase JB fecundity and longevity. However, separating the effects of attractants or nutrients from anti-nutrients or feeding deterrents can be complex.

Studies of insect resistance in soybean have concentrated on plant introduction-derived resistances to corn ear worm, aphids, cutworm and leaf hoppers sometimes combined with Bt-derived resistances (Killen and Lambert 1986; Narvel et al. 2001; Baur and Boethel 2004; Nakazawa 2005). Specific insect pest resistance has been found in P.I. 229358 ('Sodendaizu'), P.I. 1171451 ('Kosamame'), and P.I. 227687 ('Miyako White'). Both open field scores and caged or trapped feeding has been used. In traps, weight gains of insect pests and/or lethality (Komatsu et al. 2005) was an indicator resistance. In the field, feeding preference was evidenced by the extent of feeding (Rector et al. 2000). By 2008, about 30 major insect-resistant QTL had been identified, many provide a broad resistance to several insect pests (Narvel et al. 2001). Broad resistance suggests certain soybean genotypes actively synthesize and accumulate natural products to stop (antixenosis) or deter (antibiosis) insect feeding.

Here, the feeding damage caused by JB was analyzed on a group of 96 recombinant inbred lines (RILs) lacking major gene resistance. Swarming damage was measured as pest severity (PS), cultivar choice was measured as pest incidence (PI). Forced feeding allowed measurement of antibiosis and antixenosis. Genetic analysis with DNA markers allowed loci underlying partial resistance to JBs to be identified.

Materials and methods

Seed material

In 2005, the EF94 RIL seed material (at the F5:15 generation) for this study was acquired from the SIUC seed store from the 2001 increase of the released EF94 population (Lightfoot et al. 2005). Briefly, the population was 94 RILs derived at the F5 by single seed descent and bulked for the nine generations since. Near isogenic lines (NILs) derived from individual RILs were also used. The Essex cultivar parent was partially susceptible with trait means of 1.07 ± 0.12 beetle deaths (MNANTIX) and 0.025 ± 0.002 g weight gain per beetle (MNANTIB) in forced feeding; and $25.5 \pm 0.9\%$ MNPI and 3.51 units MNPS in field experiments of cultivar choice. The Forrest cultivar parent was partially resistant with trait means of 1.81 ± 0.12 beetle deaths (MNANTIX), 0.011 ± 0.002 g (MNANTIB), $16.2 \pm 0.8\%$ MNPI, 2.62 ± 0.05 units of MNPS. The seeds of the RILs, NILs and parents were grown in 2005 and 2006 at the SIUC, Agronomy Research Center (ARC) fields 19 and 26 at Carbondale, Illinois. Fields were planted to corn the previous year. Field border crops were pasture, sorghum and corn (2 sides) in 2005 and woodland, sorghum and corn (2 sides) in 2006. The 2006 seed planted were those harvested in 2005 (at the F5:16 generation).

Field plots

In 2005, the RILs were planted as four row plots. The rows were 16 lines deep and 36 lines wide and the outer two rows were planted with RIL ExF85, a highly disease susceptible line (Iqbal et al. 2005). The plots were 3 m long and had a 2 m alley way. In 2006, the rows were 24 deep and 48 wide, 2 row plots. Individual plots were 2 m long and had a 1.3 m alley way. Herbicide treatments for the planting year 2005 had a pre- and a post-application. The pre-application was done on 06 June 2005 using Valor (flumioxazin-VALENT-EPA Reg. No. 59639-99 or 59639-98), sprayed at 140 ml/ha (2 oz. per acre) and sprayed for pre-broadleaf and grass suppression in soybean. Prowl (pendimethalin-Dow 19 AgriSci. EPA Reg. No. 68156-62719) was mixed with water at 1.68 l/ha and sprayed for pre-grass. The post-application was done on 08 July 2005. Stellar (flumiclorac + gal lactofen-VALENT-EPA Reg. No. 59639-92) was sprayed at 490 ml/ha for broadleaf and Select *AE* (Clethodim-VALENT-EPA Reg. No. 59639-3) was used as 560 ml/ha used for annual and perennial grass control. Herbicide treatment for the 2006 planting year also had a pre- and a post-application. The pre-application was done on 01 June 2006 using Valor at 140 ml/ha. Dual Magnum^{TM} (s-metolachlor + benoxacor-SYNGENTA-EPA Reg. No. 100-816) was also sprayed at 1.68 l/ha (1.5 pt. per acre) for pre-grass, annual broadleaf and grass weeds. Touchdown^{TM} (glyphosate diamonium salt-SYNGENTA-EPA Reg. No. 100-1121) was also sprayed at 1.68 l/ha for non-selective burn down before planting. Post-emergence application was done on 17 July 2006 using Stellar at 490 ml/ha and Select at 560 ml/ha.

Seed weight and yield measurements

Seeds were harvested and de-podded using a two row combine. The seeds were then sieved several times to get rid of broken seeds, seed coat debris, pods and weed seeds. Seed weight was measured for 100 seeds and the whole plot by weight.

Insect feeding in fields

The plants were scored for PS, PI, pest number (PN) and pest index (PX). PX was PS × PI. PS was calculated as the scale of damage on the 100 E × F lines (scale 0–9). The lines were damaged from 0, or very little damage, to 9 that were more than 90% defoliated. The PI was calculated as the number of individual plants within a given line that were affected by JBs and the PN was calculated based on the number of JBs that were present per plant during the feeding period. The PS field data were used to select the 30 RIL lines as high ($n = 10$), mid ($n = 10$) and low ($n = 10$) genotype groups. In addition, 20 NIL lines as worst ($n = 10$) and best ($n = 10$) were selected for the forced feeding study.

No choice feeding tests for JBs

In 2005 and 2006 two leaflets from each of 30 RILs were taken and placed in a Petri dish with a moistened filter paper disc. Two JBs were weighed and introduced into each of the Petri dishes. The Petri dishes were sealed and left on the bench under fluorescent light at 22°C for a week. The final JB weights were taken as measure of antibiosis (ANTIB) and number of alive and dead JBs noted as antixenosis (ANTIX). This data were used for analyzing feeding preference through statistical analysis systems, analytical methods (SAS 2006). The forced feeding assay data were pooled for the years 2005 and 2006 as a *t* test showed no significant difference between the 2 years (Snedecor and Cochran 1980).

Data analysis

Following Kassem et al. (2006), the programs used were Mapmaker EXP 3.0, Mapmaker QTL 1.1 (Lander et al. 1987) and QTL Cartographer 1.16 for trait distribution associations. SAS and Microsoft Excel programs were used

Table 1 Heritability of each trait, both per year and their means

Trait	Heritability (%)
PI05	90
PI06	87
PS05	89
PS06	82
ANTIB05	88
ANTIB06	92
ANTIX05	94
ANTIX06	96
MNPI	78
MNPS	85
MNANTIB	90
MNANTIX	95

for heritability estimates and correlations. Pearson's correlation coefficient analysis was performed on the mean trait data (Snedecor and Cochran 1980) in order to find correlations among traits.

Results

Trait distributions

All traits measured were highly heritable and ranged from 82 to 95% in the broad sense (Table 1). Mean PI and mean ANTIB each showed a nearly normal and continuous distribution. Mean PS and ANTIX were not normal and either biphasic or discontinuous indicating the effect of major loci. The mean value of PS was 3.01 and the standard deviation was 2.15 (Fig. 1). Distributions show positive skewness (0.95) and negative kurtosis (−0.54) resulting in a flattened-peaked distribution (Fig. 1a). Essex was 3.51 ± 0.05 units and Forrest was 2.62 ± 0.05 so there were both positive and negative transgressive segregants (Fig. 2). Mean PI had a mean value of 19.82 and the standard deviation was 4.89. The distribution was positively skewed (1.25) and kurtotic (2.79; Fig. 1b). Essex was 25.5 ± 0.9% and Forrest was 16.2 ± 0.8% so there were both positive and negative transgressive segregants. The mean value of ANTIB was 0.017 g and the standard deviation was 0.005 g. The distribution showed positive skewness (1.69) and kurtosis (2.16) resulting in a peaked distribution (Fig. 1c). Essex was 0.025 ± 0.002 g and Forrest was 0.011 ± 0.002 g so there were both positive and negative transgressive segregants. The mean ANTIX was 1.3 with standard deviation of 0.4. The distribution had just three major categories, 1, 1.5 or 2 dead JBs (Fig. 1d). Essex had 1.07 ± 0.12 and Forrest had 1.81 ± 0.12 beetle deaths so there were both positive and negative transgressive segregants. There were no significant correlations among the mean traits suggesting they were separate measures of the effects of soybean herbivory. Traits did not correlate significantly with seed composition or yield, including seed phytoestrogen, seed protein and seed oil content.

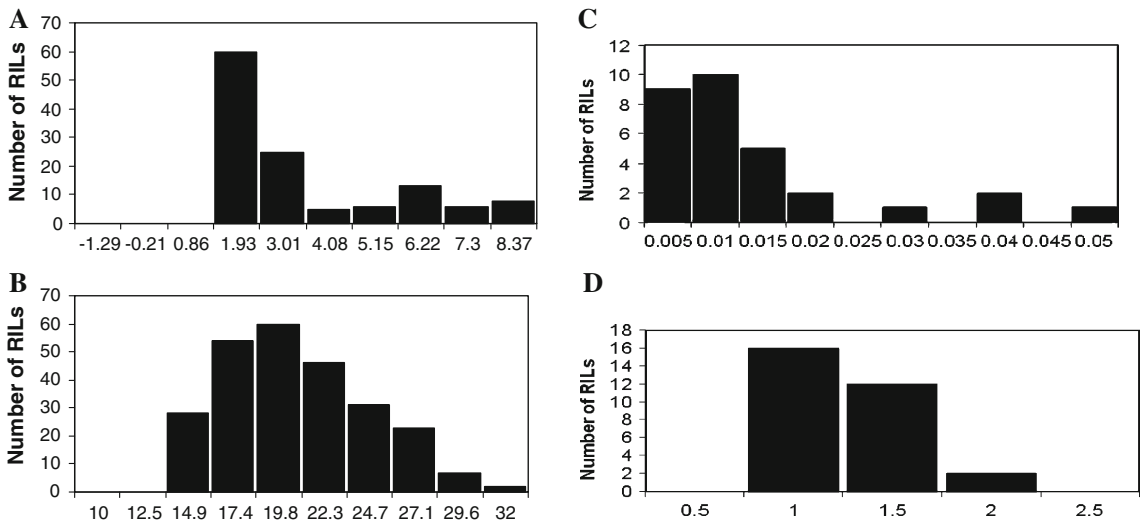

Fig. 1 Trait distribution among 100 lines for the JB related mean traits measured in the 100 RIL population of Essex × Forrest. The individual traits were: pest severity (PS); pest incidence (PI); antibiosis (ANTIB) was the weight gain of JBs during no choice feeding; and antixenosis (ANTIX) was the lethality of JBs per RIL. Means (MN) of traits are shown. Values on the *y* axis are midpoint range values. The Essex cultivar parent means were 1.07 ± 0.12 beetle deaths (MNANTIX), 0.025 ± 0.002 g weight gain per beetle (MNANTIB), 25.5 ± 0.9% MNPI and 3.51 ± 0.05 units MNPS. The Forrest cultivar parent means were 1.81 ± 0.12 beetle deaths (MNANTIX), 0.011 ± 0.002 g (MNANTIB), 16.2 ± 0.8% MNPI, 2.62 ± 0.05 units of MNPS

Fig. 2 Japanese beetles on soybean leaflets. **a** Simultaneous mating and feeding. **b** Three leaflets with 10% PS rating caused by two JBs in 1 h. **c** The white grubs that are the larval stages of JBs (taken from Fig. 14. White grubs of (L-R) Japanese beetle, European chafer, May–June beetle. Photo courtesy of David Cappaert, http://www.ext.colostate.edu/pubs/insect/05601.htm). **d** Leaves with 80% defoliation showing the web of veins left

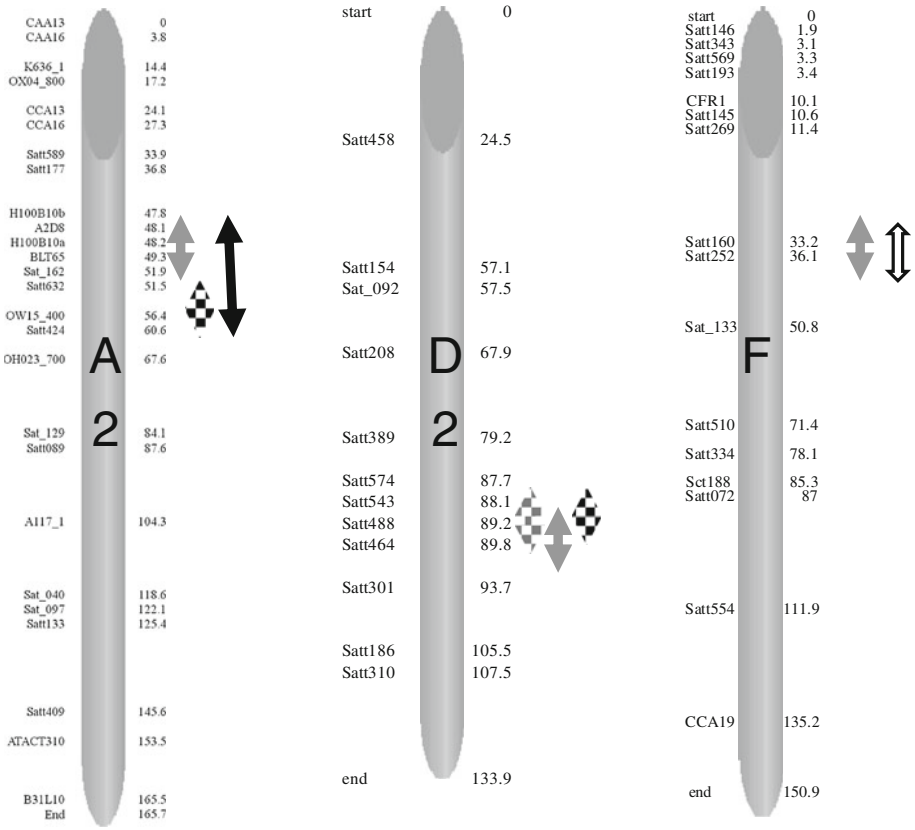

Fig. 3 Locations of the QTL found in the EF94 population on linkage groups A2, D2 and F for resistance to JB, in relation to known QTL for resistance to SCN and RKN. JB (*grey solid arrow*), Hg type 1.2.5- (*grey stippled arrows*), Hg type 0 (*black stippled arrows*), Hg type 1.3 (*black solid arrow*), RKN (*white arrow*). The size of the arrow reflects the interval significantly associated by QTL Cartographer or Mapmaker at LOD > 2.5 or ANOVA at $P < 0.0005$

QTL analysis

The 218 BARC-Satt marker data set and linkage groups developed by Kassem et al. (2006) were used to scan for QTL along with AFLP, SCAR and RAPD markers developed earlier (Chang et al. 1997).

PS of JBs

Five QTL were detected for the mean PS field trait ($16\% < R^2 < 27\%$; Table 2; Fig. 3). The loci were within the intervals Satt632–A2D8 on linkage group (LG) A2 (chromosome 8); Satt583–Satt415 on LG B1 (11);

Table 2 Intervals detected by CIM associated with mean traits underlying resistance to Japanese beetles

Trait	L.G. (Chr)	Marker/interval	Peak cM position	LOD	R^2	Essex allele (mean ± SEM)	Forrest allele (mean ± SEM)
MNANTIX[a]	A2 (8)	Satt632–A2D8	45.2	10.3	0.28	1.38 ± 0.13	1.625 ± 0.13
	F (13)	Sat_039–Satt160	30.1	3.2	0.20	1.30 ± 0.12	1.53 ± 0.13
MNANTIB[b]	D2 (17)	Satt464–Satt488	89.6	2.7	0.54	0.021 ± 0.003	0.010 ± 0.001
MNPI[c]	A1(5)	Satt385–Satt526	46.7	2.5	0.18	22.1 ± 1.4	19.0 ± 0.7
	I (20)	Satt440–Satt292	112.7	2.2	0.16	21.4 ± 0.8	18.4 ± 0.7
MNPS[d]	A2 (8)	Satt632–A2D8	45.2	9.0	0.27	3.51 ± 0.06	2.61 ± 0.04
	B1 (11)	Satt583–Satt415	83.5	4.0	0.19	3.00 ± 0.06	3.50 ± 0.07
	N (3)	Satt009–Satt530	30.5	3.1	0.18	3.15 ± 0.04	2.72 ± 0.05

The allelic means units for each trait were: [a]number of beetle deaths (MNANTIX), [b]grams of weight gain per beetle (MNANTIB), [c]percent MNPI, and [d]units of MNPS on a 0–9 scale

Table 3 Single markers from sparse areas of the EF94 map associated with traits in mean of years or single years and not the mean

Trait	Marker	LG (Chr.)	cM	*P*	R^2	Essex allele (mean ± SEM)	Forrest allele (mean ± SEM)
PS05	SIUC_B20C11	M (7)	4.8	0.002	0.11	3.39 ± 0.06	2.00 ± 0.03
MNPS	$OB02_{140}$	E (20 cM to Satt573)	15.1	0.006	0.12	3.26 ± 0.04	2.52 ± 0.04
MNPS	$OZ15_{150}$	C2 (19 cM to Satt291)	25.1	0.0008	0.30	1.90 ± 0.02	3.54 ± 0.05
PI05	SIUC_B08D14	B1 (11)	88.8	0.002	0.19	23.2 ± 0.6	18.3 ± 0.3
PI06	SIUC_B08D14	B1 (11)	88.8	0.006	0.11	19.2 ± 0.2	23.5 ± 0.4

Beneficial alleles were opposite in different years for some traits

Satt009–Satt530 on LG N (3); and close to two markers not closely linked to others OB02_140 (LG E) and OZ15_150 (LG C2; Table 3). Beneficial alleles were from Forrest on A2, N and E but from Essex on B1 and C2. Allelic effects ranged from 1.1 to 1.7 units difference in PS.

PI of JBs

Two QTL were detected for the mean PI field trait ($16\% < R^2 < 18\%$) close to Satt385 on LG A1 and Satt440 on LG I. Beneficial alleles were from Forrest. Allelic effects ranged from 3.0 to 3.1% reduction in PI. One locus was detected where the beneficial allele was different in the two locations (Table 3). Marker SIUC_B08D14 on LG B1 (11) had Forrest as a beneficial allele in 2005 and Essex in 2006. Therefore, this locus was not significantly associated with the mean trait.

Antixenosis to JBs

The no choice feeding studies detected two QTL that were significant for antixenosis ($22\% < R^2 < 24\%$) between Satt632–A2D8 on LG A2 (8) and Sat_039- Satt160 on LG F (13; Table 2; Fig. 3). Beneficial alleles causing more JB deaths were from Forrest. Allelic effects ranged from 0.22 to 0.25 increase in JB mortality.

Antibiosis to JBs

A major locus effect ($R^2 = 54\%$) was detected for antibiosis on LG D2 (17) between Satt464–Satt488 (Table 2; Fig. 3). The beneficial allele causing lower JB weight gains were from Forrest. The allelic effect was a 0.011 g difference in JB weight over the forced feeding period.

Discussion

Analysis in the Rosaceae of QTL for JB resistance suggested that there was a significant environmental dependence but the genetic component was underlain by specific biochemical pathways that produce antixenotic products (Patton et al. 1997b). However, in EF94 soybean JB resistance was a highly heritable genetic trait by all measures (Table 1) suggesting a multi-factored genetic interaction.

PS was a measure of the tendency to swarm on particular genotypes. Several QTL of small effect were detected. Such QTL might underlie the amount or quality of odorants produced during herbivory that serve to attract other JBs to a feeding swarm (Loughrin et al. 1996). Known attractants include simple sugars and volatiles with a fruity or flowery smell such as terpenoids, aliphatics, and aromatics. The

five QTL each encompass an interval with genes encoding enzymes in these broad synthetic pathways. Biochemical mechanisms triggered by herbivory and signals initiated during the wounding responses (Patton et al. 1997a, b; de Bruxelles and Roberts 2001; Underwood and Rausher 2002) might underlie the QTL detected in soybean if taste rather than toxicity or nutritive values are altered.

PI was a measure of a tendency toward cultivar choice within the EF94 population. Only two QTL were detected and they were of large effect. One QTL that was detected in single years was not found in the mean of those years because the beneficial allele was opposite across the 2 years, suggesting a G × E interaction at that locus. JBs are not known to choose among host plants on the basis of genotype, being unable to detect or avoid toxins (Ladd 1987, 1989; Loughrin et al. 1996). However, it is possible that since soybeans and JBs have co-evolved in Japan, JBs are able to detect certain chemicals in soybean that serve as attractants or repellents.

Antibiosis showed the JBs grew larger when a single locus on LG D2 was inherited from the Essex parent. Consistent with this the Essex parent was more nutritive for JBs than Forrest. This locus appeared to be a major gene effect and might be underlain by a locus altering leaf composition, of nutrients, anti-nutrients or toxins. QTL in this region include those for resistance to SCN (Schuster et al. 2001; Kazi et al. 2009), SDS (Kazi et al. 2008) Sclerotinia (Arahana et al. 2001) and corn ear worm (Chase et al. 2001). Therefore, genes in this region involved in broad pest resistance might underlie the antinutritive effects recorded for JBs. Alternately, a single locus might underlie resistance to the three insect-like pests JBs, CEW and SCN Hg types 1.2.5- and 1.3-. This locus might be ascribed to a gene symbol *Rpj1* with permission from the Soybean Genetics Committee.

Antixenosis showed the JBs had greater mortality rates when two loci on LG 2 and F were inherited from the Forrest parent. Consistent with this the Essex parent caused less death for JBs than Forrest. These loci appeared to be major gene effects and might be underlain by a loci altering leaf toxin content. Loci in the A2 region included *Rhg4* for resistance to SCN (Webb et al. 1995; Meksem et al. 2001; Kazi et al. 2009) and a QTL for leaf area (Ashley et al. 1998). The F region encompassed QTL for resistance to root knot nematodes (Boerma et al. 1997). Therefore, genes in these regions involved in nematode resistance might underlie the lethality effects recorded for JBs. These locus might be ascribed to gene symbols *Rpj2 and Rpj3* with permission from the Soybean Genetics Committee.

The QTL reported previously for antibiosis and antixenosis to Mexican bean beetle located on LGs M from P.I. 171451 (Narvel et al. 2001) were not detected in JB resistance assays. There was a QTL for PS detected in 2005 on LG M by marker SIUC_B20C11 which might reflect the activity of the same locus. There were very few polymorphic markers on LG M in the EF94 population (Kassem et al. 2006) so another possibility is that the M locus might be fixed. The loci for soybean resistance to herbivory by other insects on LG D1b, G and H were not detected although many markers were polymorphic on those LGs.

Pleiotropy or linkage among QTL may have been detected between *Rhg4 Rhg3, Mj* and the JB resistance QTL found on LG A2, D2 and F, respectively. Loci underlying resistance to soybean cyst nematode (*Rhg* genes) are either linked or pleiotropic to genes underlying resistance to sudden death syndrome; at *rhg1*/*Rfs2* (Triwitayakorn et al. 2005); and at *rhg3/rfs10* (Kazi et al. 2008, 2009). Proven examples of pleiotropy are rare since they require the isolation in transgenic plants of at least one gene and measurement of two traits. However, there are examples from insect resistances in tomato. The tomato *Mi*-1 gene confers resistance against root knot nematodes (*Meloidogyne* spp.), whitefly (*Bemisia tabaci*; Nombela et al. 2003) and a biotype of the potato aphid (*Macrosiphum euphorbiae*; Martinez de Ilarduya et al. 2003). The phenyl propanoid pathway leading to maysin has been associated with resistance to multiple insect pests in maize (Abel et al. 2000) that are controlled by alleles at the *P1*gene (a transcription factor) that was epistatic over the *a1* (dihydroflavanol reductase; E.C. 1.1.1.219) and *whp* (white pollen 1; unknown protein) allelic variations (Szalma et al. 2005). Arabidopsis induces common pathways to insect pests (Mewis et al. 2005). However, linkage is more likely across the majority of clustered QTL. Also, possible is that the extensive epistasis that has been detected in soybean (Chase et al. 2001) due to the homeologous genomes (Shultz et al. 2007) may cause the loci to appear multi-functional.

QTL maps suggested the region for resistance to SCN and Japanese beetle on LG A2 overlapped. Therefore, genes underlying the two QTL might be linked or pleiotropic. Recombination is suppressed in this region due to the *I* gene associated deletions and the subsequent introgression of *Rhg4* into the region encompassed by SIUC–A2D8 and BLT65. The SCN susceptible cultivars 'Williams 82' and 'A3244' encoded a cluster of nine genes within this region (Lightfoot and Meksem 2001; Kazi et al. 2009; Campbell et al. 2009). The genes linked to the RLK, primary candidate for SCN resistance *Rhg4*, may be involved in resistance to JBs. The linked set of genes at the *Rhg4* locus included an EST (gi 5677126) expressed in leaves, a brassinosteroid-regulated protein (gi 347458) abundant in etiolated hypocotyls of very young seeds, a paralog of EST (gi 12493776) from *G. soja* abundant in etiolated seedling, a predicted gene of no known expression, a duplicate pair of paralogs of an EST (gi 15662259)

that was similar to a UDP-glucose:anthocyanin 5-*O*-glucosyltransferase, a paralog of an EST (gi15662259), the presumed *Rhg4* receptor like kinase abundant in roots, an EST (gi 21256330) similar to a serine protease abundant in roots, and the gene (gi 2970554) encoding aspartokinase-homoserine dehydrogenase (Gebhardt et al. 1998). Each gene may be a candidate to underlie the resistance to JBs. The dual activity of many insect resistance genes would mean that the *Rhg4* nematode resistance gene was a strong candidate. However, since nematodes parasitize plant cells by cytoplasm to cytoplasm, or membrane to membrane, contact through a stylet whereas insect pests ingest crushed cells, a common mechanism would infer the involvement of the induction of a common metabolite or small molecule.

The RLK protein at *Rhg4* may be implicated in sensing the nematode and setting up a response that includes the induction of the phenylpropanoid and glucosinolate pathways (Mahalingham and Skorupska 1996; Mewis et al. 2005). This response could confer broad insect resistance like the *Mi* gene (Nombela et al. 2003; Martinez de Ilarduya et al. 2003).The duplicate pair of paralogs of an EST (gi 15662259) that were similar to a UDP-glucose:anthocyanin 5-*O*-glucosyltransferase could be involved in the production of chemicals toxic to insect pests such as maysin in maize (Abel et al. 2000; Szalma et al. 2005). Aspartokinase-homoserine dehydrogenase is a bifunctional enzyme found in plant chloroplasts that catalyses the first and third steps of methionine and threonine biosynthesis. Both amino acids can be used to produce defense compounds through special nitrogen metabolism (Gebhardt et al. 1998). Equally intriguing, the EST (gi 5677126) expressed in leaves and the defense-related brassinosteroid-regulated protein (gi 347458) abundant in etiolated hypocotyls of very young seeds are expressed in the organ that the JBs feed upon. Further tests of function will use fine mapping in NILs followed by candidate gene TILLING and transformation (Lightfoot 2008).

The development of host plant resistance in a wide range of plant species (Patton et al. 1997a) may cause JBs to increase their feeding on soybean leaves. Global warming and increased CO_2 concentrations would increase the nutrition value of soybean leaves to JB populations (Casteel et al. 2008). Better understanding of the pleiotropic effects of genes, QTL interactions (Walker et al. 2004) and their effects on related traits like seed composition to JB herbivory will help develop new varieties with resistance to JB without introgression from PIs (Hammond and Cooper 1989). Pyramiding or stacking of the QTL discovered here can lead to the development of better varieties of soybean cultivars.

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