Chapter 15
Genomics of Insect-Soybean Interactions[1]

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Introduction

On-going efforts to lower costs of production, along with increased concerns over insecticide residues in the food chain and in the environment are incentives to conduct research on crop resistance to insect pests. While multiple genes for resistance to insects have been identified in many plants (Yencho et al. 2000), understanding their molecular basis remains far behind the understanding achieved for disease resistance genes. Given that plants and insects have co-evolved for millions of years, it is not surprising that a wide range of plant-insect relationships exist and that many of these have a very different genetic basis. The following review is an attempt to categorize the various plant-host relationships that exist within soybean and provide a summary of what is known about the genetics of resistance and its characterization at the genomic level.

Damage Caused by Soybean Insect Pests

Insect pests of soybean are well recognized, though their economic impact is hard to quantify, as data on insect damage are seldom collected. Georgia is perhaps the only state that historically has kept data on insect damage to the various crops grown in the state. In 2004, the last year for which data are available, there were 72,800 ha planted in soybean, for a crop valued at $43.1 million. During 2004,

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[1] **Abbreviations of commonly cited insect names**
BAW = beet armyworm, *Spodoptera exigua* Hübner; BLB = bean leaf beetle, *Cerotoma trifurcata* Forster; CEW = corn earworm, *Helicoverpa zea* Boddie; LCB = Lesser cornstalk borer, *Elasmopalpus lignosellus* Zeller; MBB = Mexican bean beetle, *Epilachna varivestis* Mulsant; PLH = potato leafhopper, *Empoasca fabae* Harris; SBA = soybean aphid, *Aphis glycines* Matsumura; SBL = soybean looper *Pseudoplusia includens* Walker; TBW = tobacco budworm, *Heliothis virescens* Fabricius; VBC = velvetbean caterpillar, *Anticarsia gemmatalis* Hübner

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Georgia soybean farmers spent $3.3 million on insect control and lost an additional $4.25 million due to insect-induced yield reduction. Thus the combined cost of insect control and yield loss was equal to 17.5 % of total crop value in 2005 (McPherson 2004).

Insect-caused injury to soybean depends on the feeding behavior and biology of the pest, the potential of the pest to vector pathogens and, in some cases, on the development stage of the plant. The majority of the principal soybean insect pests can be classified on the basis of their feeding technique into piercing-sucking, chewing, or tunneling pest species. Chewing insects can damage foliage, reproductive organs, and/or stems.

Piercing-Sucking Insects

The major pests in this category are in the order Hemiptera, which now includes the true bugs, such as stink bugs, and the aphids (suborder Sternorrhyncha) and leafhoppers (suborder Auchenorrhyncha). The green [*Acrosternum hilare* Say], southern green [*Nezara viridula* L.] and brown [*Euschistus servus* Say] stink bugs comprise the bulk of the soybean stink bug complex in North America. The primary economic damage in soybean results when nymphs and adults pierce pods and developing seeds to feed on plant juices. Damage to young pods can cause shriveled seed and pod abortion, while feeding on developing seed can result in abnormal development, wrinkling, and a stained seed coat (Turnipseed and Kogan 1987). Stink bugs are polyphagous, so they can easily move into soybean fields from alternative hosts such as cotton. Economic damage from stink bugs can outweigh damage from all other insects in the South (McPherson 2004)

The soybean aphid [*Aphis glycines* Matsumura] was first reported in the United States in 2000, and has rapidly become a major insect pest in the Midwest (Hill et al. 2004). Aphid feeding can result in yield loss, severe stunting, leaf distortion, reduced pod set, lower seed weight, and nutrient deficiencies (Mensah et al. 2005). Indirect damage is caused by transmission of viruses like soybean mosaic virus, alfalfa mosaic virus, soybean dwarf virus, and bean yellow mosaic virus (Hill et al. 2001). Growth of sooty mold on 'honeydew,' a sticky substance excreted by feeding aphids, can further reduce photosynthesis and yield (Mensah et al. 2005). Feeding by other hemipteran pests such as the potato leafhopper [*Empoasca fabae* (Harris)] and the three-cornered alfalfa hopper [*Spissistilus festinus* (Say)] can cause damage to leaves or make weakened stems more prone to lodging (Turnipseed and Kogan 1987).

Chewing Insects

These include lepidopteran and coleopteran larvae and adults that feed on soybean leaves and seed pods. Major lepidopteran pests include the green cloverworm

[*Plathypena scabra* (F.)] in the Midwest, and the corn earworm [*Helicoverpa zea* (Boddie)], soybean looper [*Pseudoplusia includens* (Walker)], and velvetbean caterpillar [*Anticarsia gemmatalis* (Hübner)] in the southern states (Turnipseed and Kogan 1987). These are primarily defoliators, with the exception of CEW, which frequently feeds on flowers, pods, and developing seed as well as leaves. Because of its feeding behavior, CEW is generally assumed to be the lepidopteran pest responsible for the greatest yield losses in the USA, even though supporting data are lacking. In addition, the ravenous appetites of VBC larvae make this species one of the most destructive defoliators in the southeastern USA, especially since they may also damage meristems after foliage becomes limited.

The Mexican bean beetle [*Epilachna varivestis* Mulsant] and the bean leaf beetle [*Cerotoma trifurcata* (Forster)] are among the most destructive coleopteran pests of soybean (Turnipseed and Kogan 1987). Both larvae and adults of the MBB and adult BLB feed on foliage, sometimes causing economic losses from defoliation, especially when populations develop during the plants' reproductive growth stage. In addition, BLB is capable of transmitting bean pod mottle virus and of injuring pods on mature plants and facilitating secondary invasion of fungi (Turnipseed and Kogan 1987). BLB larvae feed on the roots and nodules of soybean plants. Several other coleopteran species may cause infrequent yield losses.

Tunneling Insects

Lesser cornstalk borer (*Elasmopalpus lignosellus* Zeller) is the main pest that causes damage by tunneling into the stems of seedlings at the soil surface (Turnipseed and Kogan 1987). This pest tends to attack soybean growing on sandy soils in hot, dry weather, which may compound the effects of the injury caused by the larvae. Small plants are often killed outright, while surviving plants are more prone to lodging as a result of having weakened stems. The Dectes stem borer (*Dectes texanus texanus* LeConte, Cerambycidae) produces sporadic but sometimes severe damage to early planted soybean.

Seed Storage Insects

Stored soybean seed is subject to insect feeding as well. Such pests include the almond moth (*Ephestia cautella* Walker), tobacco borer beetle (*Lasioderma serricone* Fab.), red grain beetles (*Tribolium castenum* Herbst and *T. confusum* Kackuelinduval), khapra beetle (*Trogoderma granarium* Everts) and the pulse beetles (*Callosobruchus analis* Fab. and *C. chinensis* L.; Islas-Rubio and Higuera-Ciapara 2003).

Types of Resistance

Based on Effect on Insects

Painter (1941) suggested that host plant resistance to insects could be classified as non-preference, antibiosis, or tolerance. The term '*antixenosis*' was later suggested by Kogan and Ortman (1978) as a substitute for 'non-preference.' Antixenosis refers to a host plant effect on pest behavior which discourages feeding and/or oviposition. The underlying traits can be morphological (e.g., dense pubescence) or biochemical (presence of a deterrent compound or absence of an attractant). Figure 15.1 shows near isogenic soybean lines, whereby the one on the right has antixenotic insect resistance.

Antibiosis refers to a type of resistance in which a host plant has a detrimental effect on the physiology and life history of an insect pest (Painter 1951). This can be manifested as decreased growth rate, lower pupal weights, decreased fitness, or other factors that interfere with the pest's ability to survive, mature and reproduce. CEW larvae fed near isogenic lines of soybean, one of which has antibiotic resistance, are shown in Fig. 15.2.

Experimentally, a distinction between these two types of resistance is based on whether a choice (antixenosis) or no-choice (antibiosis) assay was used to quantify the resistance. In choice assays, the insect has an assortment of genotypes to feed on. In a no-choice assay, the insect is only provided one genotype as a food source. These two types of resistance are not mutually exclusive: a trait that deters a larva from feeding may slow its development and/or reduce the adult's ability to reproduce. For example, an allele at the *Pb* locus that conditions sharp pubescence tips was detected using both antixenosis and antibiosis feeding assays (Hulburt et al. 2004).

Fig. 15.1 Soybean (*right*) showing antixenotic resistance towards SBL (*See* also Color Insert)

Fig. 15.2 Corn earworms (*right*) showing an antibiotic effect on their growth (*See* also Color Insert)

Tolerance is technically not a type of resistance, but rather a greater ability of certain plant genotypes to maintain yields after sustaining levels of insect damage that would cause yield losses in other genotypes of the species (Haile et al. 1998). For example, narrow-leafed isolines of soybean were found that can sustain greater levels of defoliation than conventional soybeans can before yield is affected, perhaps because of the greater light-interception capacity of narrow leaves (Haile et al. 1998).

Based on Gene Expression Patterns

Insect resistance factors can also be classified as constitutive (e.g., trichome density) or induced, which involve some change in gene expression levels in response to injury caused by an insect pest. Constitutive resistance factors may or may not be uniformly effective in a plant. For example, SBL larvae can grow faster when fed newly expanded leaves from soybean accession PI 227687, than when fed older leaves from the same plant (Reynolds and Smith 1985).

Induced resistance refers to a reactive response by plants to specific or non-specific assault by predators (e.g., insects), pathogens or environmental disturbance. Induced resistance typically involves a cascade of biochemical defenses that are otherwise not present, or are only present at very low levels, within plant tissues, and innate resistance targeted towards specific pest species (Cooper et al. 2004). Innate resistance often depends on the presence of single, dominant resistance genes (R-genes) that are involved in direct or indirect recognition of the presence of a pest or pathogen. Recognition may involve detection of elicitors produced by the pest or detection of biochemical changes in host tissue or cells in response to injury caused by the pest. Salicylic acid and jasmonic acid have been implicated in the signaling that activates or suppresses resistance responses to insects and pathogens (Cooper et al. 2004).

The degree of resistance that a plant has can be determined in part by the interaction of its genotype, its environment, and the age of the plant or plant tissue. Water availability is one of the environmental factors that can alter resistance (Hammond et al. 1995; Lambert and Heatherly 1991). Resistance of soybean plants to both coleopteran and lepidopteran pests was observed to decline as the plants reach the late flowering and pod-filling stages of maturity (Hammond et al. 1995; Nault et al. 1992; Rowan et al. 1993).

Based on Traits that Confer Resistance

Biochemical: Compounds involved in resistance and susceptibility of soybean genotypes to various insect pests likely include leaf volatiles (Liu et al. 1989), variations in nutrient concentrations, feeding excitants and deterrents, and antibiotic substances (Fischer et al. 1990). Allelochemicals produced by plants can discourage colonization, feeding, and/or oviposition by one or more pest species, or interfere with metabolic processes in pests that are feeding on the host plant. Proteins such as trypsin inhibitors and secondary metabolites such as alkaloids were associated with resistance to insect pests. Mutations that alter the structure or reduce the quantity of volatile compounds that attract insect pests may also result in a higher level of perceived resistance.

Phytoalexins are antibiotic metabolites that undergo enhanced or *de novo* synthesis and accumulation in response to physiological stress (Hart et al. 1983). Flavonoids are important phenylpropanoids in *Glycine* spp., and some of them may be phytoalexins with an antiherbivory role (Burden and Norris 1992). For example, the isoflavonoid, glyceolin, was shown to function as an antifeedant against MBB and some other coleopterans, but did not have a significant effect on SBL (Hart et al. 1983). Coumestrol, another isoflavonoid, appears to contribute to antixenosis resistance in 'Davis' soybean plants (Burden and Norris 1992). Hart et al. (1983) suggested that pest deterrence in some soybean genotypes may involve the combined actions of multiple flavonoids, including glyceollin, glycinol, coumestrol, sojagol, daidzein, and genistein. There has been a notable lack of supportive research into the role of phytoalexins as deterrents to herbivory since the original research was conducted.

Physical: Physical and morphological traits that discourage or impede feeding or oviposition also contribute to host resistance. Studies of isolines that differed in the length and orientation of trichomes showed that the long, erect trichomes inherited from the resistant soybean PI 229358 contributed to antixenosis towards PLH (Turnipseed 1977). Elevated lignin content in soybean stems may reduce the damage caused by tunneling insects like the LCB.

History of Breeding Efforts

Efforts to develop soybean cultivars with insect resistance can be divided into three eras, the first two beginning with discoveries that had a major influence on subsequent breeding work, and a contemporary period that is characterized by (i) the use of biotechnological tools to address limitations of a conventional breeding approach, and (ii) the recent emergence of resistance to the soybean aphid (*Aphis glycines* Matsumura) as an important breeding objective. In the 1930s, Hollowell and Johnson found that pubescence provided resistance to the PLH, in comparison with glabrous plants (Hollowell and Johnson 1934; Johnson and Hollowell 1935). Subsequent selection for pubescence eliminated PLH as a soybean pest, and this resistance has proven durable for 70 years.

Breeding for Resistance to Defoliating Insects

Insect resistance became a major objective in several breeding programs during the 1970s after the identification of three germplasm accessions with resistance to the MBB (Van Duyn et al. 1971, 1972). PI 171451 ('Kosamame'), PI 227687 ('Mikayo White'), and PI 229358 ('Sodendaizu') also were found to be resistant to several major lepidopteran soybean pests (Clark et al. 1972; Lambert and Kilen 1984a,b). By the mid 1980s, soybean breeding programs in 10 states were using one or more of these accessions as a source of insect resistance genes (Turnipseed and Kogan 1987). Unfortunately, it proved difficult to capture the full resistance levels of these accessions in progenies derived from crosses with adapted high-yielding germplasm. For example, when five PI 229358-derived breeding lines selected for resistance to MBB and SBL were evaluated for their resistance to these two insect pests plus CEW and TBW, the results showed that, while all five lines were as resistant as PI 229358 to SBL and CEW larvae, only two of the lines equaled PI 229358 resistance to all four pests (Hatchett et al. 1979). This led the authors to suggest that the genes conditioning resistance to CEW and SBL are likely to be the same, or at least tightly linked. Smith and Brim (1979a,b) similarly found that PI 229358- and PI 227687-derived lines selected for resistance to MBB were not very effective in deterring feeding by CEW.

The presence of F_3 lines exhibiting transgressive segregation for both susceptibility and resistance suggests that these three PIs might possess at least some different genes for resistance (Kilen and Lambert 1986). Furthermore, there is some evidence that the resistance of each PI differs to different insects. PI 229358 and PI 227687 were reported to be resistant to VBC, SBL, CEW, TBW, and BAW, with the resistance of PI 227687 being greater to CEW and BAW than that of PI 229358. PI 171451 was reported resistant to CEW and TBW, but not to VBC, SBL, or BAW (Lambert and Kilen 1984b). However, similar differences were not found in a subsequent study (Gray et al. 1985). The inference is that screening segregating lines for resistance to a single insect pest may prevent selection of lines with resistance genes to other major insects. Furthermore, results may be confounded by the different soybean genotypes, and affected by the type of assay used, and perhaps even by possible geographical differences in the genetic makeup of the insect pests. The recent availability of near-isolines of soybean for different insect resistance genes may finally make it possible to start evaluating insect-specific effects of the various soybean insect-resistance genes.

It has been equally difficult to recapture the yield potential and agronomic qualities of the elite parent in crosses between these insect-resistant breeding lines and elite parents (Kilen and Lambert 1986). Although numerous breeding lines with insect resistance were released from breeding programs that used the Japanese accessions as resistance sources, only three resistant cultivars were released: ‘Lamar’, ‘Crockett’, and ‘Lyon’, and none could compete agronomically with the best contemporary cultivars (Boethel 1999; Bowers 1990; Hartwig et al. 1990, 1994; Lambert and Tyler 1999). ‘Shore’, another cultivar with both antibiosis and antixenosis towards MBB, was not derived from any of the three resistant Japanese accessions (Smith et al. 1975).

Classical genetic studies were hampered by the inability to properly identify plants segregating for genes that provide the same phenotype. Nevertheless, research on the inheritance of insect resistance from PI 171451, PI 227687, and PI 229358 consistently pointed to quantitative inheritance involving a few major genes (Sisson et al. 1976; Kenty et al. 1996).

Kenty et al. (1996) estimated the broad-sense heritability of PI 229358-derived antibiosis towards SBL to be 21–47 %, with two to six genes being involved. A similar heritability (33–48 %) and number of resistance genes was estimated for MBB antibiosis derived from PI 171451 and 229358 (Rufener et al. 1989). Sisson et al. (1976) investigated resistance to MBB in F_3 populations derived from PI 227687, PI 229358, and PI 229321, a somewhat less resistant accession. They concluded that the resistance was quantitative, probably involving only two or three major genes expressing primarily additive gene action. Studies of other progenies derived from crosses to PI 229358 also indicated that resistance to SBL depended on a few major genes, but with partial dominance towards susceptibility (Kilen et al. 1977; Kenty et al. 1996). Investigations of VBC resistance of F_3 progenies from intercrosses among PI 171451, PI 227687, and PI 229358 also supported partial dominance of resistance (Kilen and Lambert 1986). In crosses between ‘Davis’ and PI 171451, PI 227686, and PI 229358, the average size of lepidopteran larvae from five pest species fed tissue from F_1 intercross progeny was similar to that of larvae from the resistant parent, suggesting that the genes conditioning antibiosis resistance were at least partially dominant (Lambert and Kilen 1984b). Taken collectively, these studies suggest there is a gene-dosage effect.

The limitations that hindered classical genetics studies were largely overcome by using DNA markers, which made it possible to identify the number, locations, and relative contributions of quantitative trait loci (QTLs) associated with soybean resistance.

Rector et al. (1998, 1999 and 2000) used restriction fragment length polymorphisms (RFLPs) to identify and map quantitative trail loci (QTL) associated with antibiosis and antixenosis resistance to CEW in populations derived from PI 171451, PI 227687, and PI 229358. QTL for both types of resistance were mapped in all of the PIs, and each of the PIs appeared to possess both unique and shared QTLs, as originally suggested by the classical genetic studies. This approach made it possible for the first time to quantify the contributions of the individual QTL to the resistance phenotype. The subsequent availability of abundant simple sequence repeat (SSR) markers allowed greater precision in mapping the locations of the QTL and facilitated the use of marker-assisted selection to transfer the resistance alleles into elite genetic backgrounds (Narvel et al. 2001). DNA markers made it possible to confirm the locations and effects of resistance QTL in different genetic backgrounds, and to determine which QTL contribute to resistance against a range of insect pests (Terry et al. 2000; Komatsu et al. 2005).

Of the various QTL from the three PIs, the QTL on linkage group M (QTL-M) is the major QTL conditioning resistance to defoliating insects, accounting for 37 % of the antixenotic effect and 22 % of the antibiotic effect (Rector et al. 1998, 1999 and 2000; Komatsu et al. 2005). Markers flanking the QTL are also useful to identify plants in a breeding population that have the smallest introgressed regions of donor parent DNA; therefore, mitigating the risk of linkage drag, the major historical obstacle in utilizing resistance genes from these three PIs. SSR markers were used to develop near-isogenic lines of ‘Jack’ and ‘Benning’ that carry various combinations of resistance alleles at mapped QTL, allowing the main effects and interactions of these genes on insect resistance to be studied with greater precision than previously possible (Walker et al. 2004; Zhu et al. 2006). Mean defoliation of lines with QTL-M by both SBL (16.8 vs 11.1 %) and CEW (15.4 vs 9.5 %) was about one-third lower than that of lines without QTL-M.

QTL-M appears to have a synergistic effect with the *cry1Ac* Bt gene, as determined by evaluation of Jack near-isogenic lines (Walker et al. 2004). The near-isolines with both the Bt and QTL-M resistance genes averaged 2.0 % defoliation by CEW and 5.5 % defoliation by SBL, compared with 7.0 and 10.7 % defoliation, respectively, for near-isolines with only Bt.

Another intriguing aspect of QTL-M is that a TBW strain selected for resistance to *Cry1Ac* Bt (Gould et al. 1995) is more sensitive to QTL-M than is a closely related wild-type strain of TBW (Walker et al. 2004). Wild-type TBW larvae feeding on near-isolines of Jack containing an introgressed genomic segment containing QTL-M weighed about 180 mg, while the Bt-resistant larvae raised on the same near-isoline only weighed about 80 mg, a highly significant difference. Such fitness costs associated with resistance to Bt were hypothesized to be a reason why insect resistance in transgenic crops has held up better than initially anticipated (Tabashnik et al. 2003).

Although several other QTL were identified that also confer resistance to insects (Rector et al. 1998, 1999 and 2000; Terry et al. 2000; Komatsu et al. 2005), the most relevant may be ones on linkage groups G (QTL-G) and H (QTL-H; Zhu et al. 2006). QTL-G conditions antibiosis, while QTL-H conditions antixenosis. However, the effect of these QTL is only evident in the presence of QTL-M. Near-isolines having no resistance genes or only QTL-H suffered about 30 % defoliation with CEW, those with QTL-M suffered about 24 % defoliation, but those with both QTLs only suffered 19 % defoliation, a significantly lower value.

A similar pattern is found with QTL-G. CEW larvae fed near isolines having no resistance genes or only QTL-G grew to weigh about 130 mg each. Those feeding on lines with QTL-M only grew to 80 mg each, but those feeding on lines with both QTLs only weighed 50 mg each (Zhu et al. 2006).

A retrospective genetic analysis of soybean breeding lines and cultivars phenotypically selected for insect resistance further emphasized the importance of QTL-M. (Narvel et al. 2001). Most of the 15 genotypes analyzed had PI 229358 as the donor parent, though PI 171451 was reported as the donor for three lines/cultivars. All lines were selected phenotypically for resistance to pests such as MBB, SBL, and/or VBC. Thirteen of the soybean genotypes examined had the PI 229358-derived allele at QTL-M. In contrast, PI 229358-derived alleles at QTL-G and QTL-H only were introgressed into two genotypes each. None of the derived genotypes had the PI 229358-derived alleles at all three QTLs. The inability of traditional breeding programs to consistently introgress QTL-G and QTL-H along with QTL-M helps explain why breeders failed in their attempts to recover levels of insect resistance as high as that originally found in PI 229358.

The high introgression rate of QTL-M into various conventionally bred lines and cultivars developed by several independent breeding programs (Narvel et al. 2001) indicates its action is not limited by environment or genetic background. Furthermore, it confers resistance to multiple lepidopteran pests (at least CEW, SBL, VBC, common cutworm, and LCB) and to a coleopteran pest (MBB). QTL effective against different taxonomic orders or genera appear to be rare in crop plants. In a comprehensive review on mapped insect resistance loci, Yencho et al. (2000) listed 233 QTL in six crop species. None of these were reported to confer resistance against insects from different orders or genera. Of the 29 major single-genes reviewed, 20 were reported to provide resistance to a single insect species or a closely related species within the same genus. Furthermore, QTL-M does not map to any known resistance gene cluster, suggesting that its mode of action is probably different from that of the tomato *Mi-1.2* gene, which is the first cloned gene for insect resistance and described below.

QTL-M from PI 229358 is also of special interest because of its contribution to both antixenosis and antibiosis resistance, its ability to enhance the effectiveness of Bt, its detrimental effect on Bt-resistant insects, its ability to ‘activate’ the effectiveness of QTL G and H, and its broad range of effectiveness against several lepidopteran and at least one coleopteran species. Furthermore, the major antibiosis/antixenosis QTL from PI 171451 maps to the same position on LG-M. Accordingly, QTL-M was the subject of intensive fine-mapping efforts, and by using soybean Willams 82 genomic sequence released by Department of Energy Joint Genomic Institute (DOE-JGi), we were able to develop a new simple sequence repeat (SSR) marker Satt729, and map it to the 0.52-cM interval. QTL-M was recently narrowed down to about 0.25-cM interval (Fig. 15.3). A more recent study

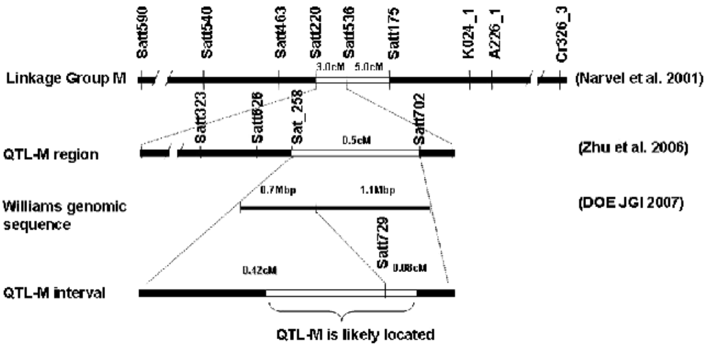

Fig. 15.3 Current map of the Linkage Group M section around QTL-M, showing the linked markers. Satt626 and Sat_258 are on the same BAC clone

by Komatsu et al. (2005) indicates that QTL-M (= *CCW-1*) is also found in the Japanese forage soybean ‘Himeshirazu.’ It is expected that cloning QTL-M will provide a key insight into the molecular mechanism involved in soybean resistance to defoliating insects, providing an effective tool for insect resistance management strategies.

As a final note, the use of molecular markers has made it possible to finally introgress QTL-M and QTL-H into elite germplasm without the loss of agronomic quality that plagued earlier attempts. QTL-G still has some linkage drag (or possible pleiotropy) associated with it, so additional breeding and selection is needed to disassociate this QTL from undesirable linked alleles (Zhu et al. 2007).

Breeding for Resistance to Aphids

The appearance of the SBA in the USA in 2000 prompted efforts to identify resistance to this new arrival. This pest rapidly spread throughout the Midwest and has now moved into other regions of North America. Hill et al. (2004) found that ‘Dowling’, ‘Jackson’, and PI 71506 have antixenosis resistance, and that Dowling and Jackson are also antibiotic, having a negative effect on aphid fecundity. This resistance was expressed at all plant stages. Palmetto and CNS, which are ancestors of Dowling and Jackson, were also resistant. Four additional germplasm accessions, ‘Sugao Zarai’, ‘Sato’, ‘T260H’, and PI 230977 were also found to exhibit antixenosis at a level equivalent to that of Dowling, Jackson, and PI 71506. Although Dowling, Jackson, and PI 71506 are ancestors of modern North American cultivars, no resistance was found among 1,425 genotypes tested (Hill et al. 2004).

Four Maturity Group III accessions from Shandong Province, China, also showed resistance to the SBA (Mensah et al. 2005). PI 567541 B and PI 567598 B have antibiosis resistance, while PI 567543 C and PI 567597 C have both antibiosis and antixenosis resistance. In another evaluation of 240 accessions, 11 were found to exhibit resistance. Of these, nine were moderately antibiotic, and two (K1639 and Pioneer 95B97) were antixenotic and strongly antibiotic (Diaz-Montano et al. 2006).

The antibiosis resistance of Dowling and Jackson to SBA was shown to be conferred by a single dominant gene in each genotype (Hill et al. 2006a,b). *Rag1*, identified in Dowling, and the resistance gene from Jackson both map to a locus that is tightly linked to Satt435 on LG-M, though it is not yet known whether these genes represent the same or different alleles (Li et al. 2007). With the help of SSR markers linked to *Rag1*, the resistance gene was successfully backcrossed into elite material adapted to the Midwest. There are concerns, however, that this single-gene resistance will not be durable (Brian Diers, personal communication).

A summary of all the known QTLs and loci for insect resistance in soybean is in Table 15.1. Both *Rag1* and QTL-M are on LG-M, approximately 10 cM apart. Besides having different flanking markers, the QTL-M-containing PI 229358 is SBA-susceptible, further reinforcing the fact that *Rag1* and QTL-M are not the same locus.

Molecular Bases for Resistance

Given the multiple types of resistance, it is reasonable to predict that several types of genetic mechanisms are involved, each with a different molecular basis. Furthermore, no insect resistance genes have been cloned from soybean, and only a limited number from other plants. To date, the *Mi-1.2* gene from tomato (*Lycopersicon esculentum*) is the only insect resistance gene cloned, though it was initially identified and cloned as a nematode-resistance gene (Kaloshian et al. 2000). Thus, this gene appears to be a general resistance gene against piercing–sucking pests.

Two conceivable functions for a gene conferring biochemically based insect resistance are: (1) involvement in the synthesis of a compound or compounds with antibiotic or antixenotic properties, or (2) a role in direct or indirect recognition of attack by an insect pest leading to an increased defense response in the plant. Examples of the first group would be genes that encode proteinase inhibitors or enzymes that enhance the biosynthesis of secondary metabolites that function as anti-feedants. The second group includes genes involved in triggering a local or systemic defense response following recognition of foreign compounds introduced into the plant tissue during insect feeding (i.e., elicitors in a classical gene-for-gene interaction), or recognition of changes in one or more of the plant's own proteins that are structurally modified as a result of injury caused by the pest (i.e., analogous to the 'guard hypothesis' of van der Biezen and Jones 1998).

Resistance to Piercing-Sucking Insects

A brief summary of what is known about *Mi-1.2* may provide some clues about the function of related genes in soybean. This gene confers resistance to diverse phloem-feeding pests such as potato aphid [*Macrosiphum euphorbiae* (Thomas)], sweetpotato whitefly (*Bemisia tabaci*), and root-knot nematodes (*Meloidogyne* spp.), from which the name of the gene is derived (Kaloshian et al. 2000). This gene deters potato aphid feeding and reduces population growth (Cooper et al. 2004), and is a member of the major class of resistance (R) genes that encode proteins characterized by nucleotide binding sites (NBS) and leucine-rich-repeats (LRRs, Milligan et al. 1998). The class of genes encoding these proteins includes many R genes involved in classic gene-for-gene interactions with pathogens. Pest or pathogen recognition is usually highly specific, and this is the case with the *Mi-1.2* gene, which provides greater resistance to European isolates of potato aphid than to North American isolates (Goggin et al. 2001).

Kaloshian et al. (2000) found that while potato aphids began probing host tissue equally rapidly and were able to locate sieve elements equally well on resistant and susceptible lines, the amount of time spent feeding once contact with a sieve element was made was 7- to 10-fold longer on the susceptible line. Insects on the resistant line made briefer and more frequent probes, indicating that the resistance mechanism involves shorter duration of contact with sieve elements, resulting in decreased salivation and ingestion of phloem fluids. Application of a salicylic acid analog, benzothiodiazole, to induce salicylic acid-dependent defenses enhanced aphid control on a tomato cultivar carrying *Mi-1.2*, as well as on a near-isogenic cultivar that lacked the gene (Cooper et al. 2004). In contrast, application of jasmonic acid enhanced resistance of the susceptible cultivar, but did not significantly increase the resistance of the cultivar with *Mi-1.2*.

A gene from *Medicago truncatula* conditions both antixenosis resistance and phloem-specific antibiosis to the blue alfalfa aphid (*Acyrthosiphon kondoi* Shinji; Klinger et al. 2005). This gene maps to a region of the genome containing resistance gene analogs that are predicted to encode resistance proteins of the coiled coil (CC)-NBS-LRR subfamily. The antibiosis resistance is inducible, since aphids on previously infested plants spent significantly less time ingesting phloem sap, and is also systemic (Klinger et al. 2005). The resistance requires an intact plant, since aphids feeding on excised shoots had enhanced survival and growth compared to those feeding on intact plants of the same resistant genotype. The resistance conditioned by this gene differs from that mediated by *Mi-1.2* in that aphid reproduction on resistant *M. truncatulata* plants was possible, albeit reduced.

Resistance to Defoliating/Tunneling Insects

Much less is known about genes which condition resistance to defoliating insects. Mechanical wounding such as that induced by caterpillars is known to activate jasmonic-acid-regulated gene expression, and some caterpillars regurgitate compounds that are thought to induce a novel defense pathway in plants (Reymond et al. 2000; Baldwin and Preston 1999; Walling 2000). Insect herbivory in lima bean can induce the upregulation of isoprenoid production (Bartram et al. 2006). However, it

Table 15.1 QTLs and genes reported to confer insect resistance in soybean

QTL or gene	Source	Insect target	Linkage group	Flanking markers	Segment size (cM)	% Variation explained	Confirmed?	References
CCW-2	Hime-shirazu	Defoliators	M	Sat_567 Satt463	17	16	ND[a]	Komatsu et al. 2005
QTL A1	Cobb	Defoliators	A1	A0831 Satt382	11	16	ND	Hulburt 2001
QTL B2#1	PI 227687	Defoliators	B2	A343b Satt126	25	12	ND	Rector et al. 2002; Hulburt 2001
QTL B2#2	PI 227687	Defoliators	B2	Sat_230 Satt126	4	17	ND	Hulburt 2001
QTL C1[b]	PI 227687	Defoliators	C1	A132T-1 A670T	–	12	ND	Rector et al. 1999; Hulburt 2001
QTL D1b	PI 229358	Defoliators	D1b	Satt141 Satt290	4	10	No	Rector et al. 1998, 1999; Narvel et al. 2001
QTL E, *Pb*, QTL U2	PI 227687 Minsoy	Defoliators	E	Satt124 Satt411	3	20–26	Yes	Terry et al. 2000; Hulburt 2001; Hulburt et al. 2004
QTL F#1	Cobb	Defoliators	F	B212V-1 A757V-2	–	20	ND	Rector et al. 1999
QTL F#2[c]	PI 227687	Defoliators	F	Sat_090 Sat_074	24	12	ND	Rector et al. 2000; Hulburt 2001
QTL G	PI 229358	Defoliators	G	Satt472 Satt191	4	14	Yes	Rector et al. 2000; Narvel et al. 2001; Zhu et al. 2006
QTL H	PI 171451 PI 227687 PI 229358	Defoliators	H	Sat_122 Satt541	0.5[d]	15	Yes	Rector et al. 1998, 1999; Narvel et al. 2001; Zhu et al. 2006
QTL J	Cobb	Defoliators	J	A064V K411T-1	41	19	ND	Rector et al. 2000

Table 15.1 Continued

QTL or gene	Source	Insect target	Linkage group	Flanking markers	Segment size (cM)	% Variation explained	Confirmed?	References
QTL M *CCW-1*	PI 229358	Defoliators Borers	M	Sat_258 Satt702	0.5	21–37	Yes	Rector et al. 1998, 1999, 2000; Narvel et al. 2001; Walker et al. 2004; Komatsu et al. 2005; Zhu et al. 2006
QTL O	Cobb	Defoliators	O	Satt358	–	19	ND	Hulburt 2001
QTL U10^{e}	Minsoy	Defoliators	H	Satt192 Satt302	42	6–9	ND	Terry et al. 2000
Rag1	Dowling	Suckers	M	Satt435 Satt463	12	–	Yes	Hill et al. 2006a; Li et al. 2007
Rag-Jackson	Jackson	Suckers	M	Satt435 Satt463	10	–	Yes	Hill et al. 2006b; Li et al. 2007

[a] ND- Confirmation study either not done or not reported

[b] Originally identified as QTL C2 in LG C2 by Rector et al. 1999

[c] Positive allele was incorrectly identified by Rector et al. 2000

[d] Distance between markers = 8 cM in Song et al. 2004

[e] Probably the same as QTL-H. The flanking markers for QTL-H are nested within those for QTL-U10.

is important to note that these studies were conducted on plant genotypes without any special resistance to defoliating insects, so these responses probably represent a general response to herbivory, rather than a response that limits the resulting herbivory to a greater extent than takes place in susceptible genotypes.

Smith and Fischer (1983) investigated the nature of the chemical basis of SBL resistance in PI 227687 using artificial diets containing leaf powder or extracts. They reported the most pronounced allelochemic effects on larval weight and mortality resulted when larvae were fed diets containing compounds extracted using methanol, though chloroform extracts also affected larvae. Later Caballero et al. (1986) found two fractions from methanol leaf extracts that were active against SBL, and isolated coumestrol, phaseol, and afrormosin from these fractions. Subsequent experiments showed that antibiotic effects of PI 227687 on SBL larvae were enhanced by mechanical wounding of foliage 24 hr prior to allowing larvae to consume the tissue (Smith 1985). On the basis of these experiments, Smith (1985) speculated that antibiosis was due to combined effects of a feeding deterrent and a growth inhibitor. Rutin, a glycosyl flavone, was isolated from leaves of PI 227687. Incorporation of rutin into the diet of VBC larvae inhibited their growth (Hoffman-Campo et al. 2006).

In this regard, resistance to defoliating insects in soybean may be similar to CEW resistance in maize. CEW is inhibited by several C-glycosyl flavones, predominantly maysin, found in maize silks. Maysin and related compounds are synthesized via a complex metabolic pathway that results in a variety of end products, only a few of which have an antibiotic effect towards CEW. In a loose sense, any gene along the pathway that eventually leads to maysin production could be considered an insect resistance gene. For example, the *p1* locus is a Myb-homologous transcription activator that up-regulates the entire pathway. However, the genes near the ends of the metabolic branches that actually result in the production of maysin and related compounds have not been identified (Cortés-Cruz et al. 2003; Zhang et al. 2003).

Resistance to Seed Storage Insects

Many of the seed storage proteins found in soybean and other legumes double as protease or amylase inhibitors. Protease inhibitors are particularly relevant, as midgut proteases are essential for insect larval survival, and their inactivation will have detrimental effects on insect growth. For example, the Kunitz trypsin inhibitor from soybean was shown to provide effective resistance to the Angoumois moth (*Sitotroga cerealella* Oliver; Shukle and Wu 2003). It also gives resistance to brown planthopper (*Nilaparvata lugens* Stal) when engineered and expressed in rice (Lee et al. 1999). Soybean vicilins were shown to inhibit development of cowpea weevil (*Callosobruchus maculatus* Fabricius; Yunes et al. 1998). The mode of action appears to be the ability of the vicilins to bind to chitin in larval midgut microvilli (Sales et al. 2001).

Physical Resistance

Sharp pubescence tip (sharp as opposed to blunt, Fig. 15.4) is associated with antixenosis to CEW, BAW, and SBL, and with antibiosis to CEW and BAW, as determined in bioassays with sharp-tipped and blunt-tipped near-isolines of ‘Clark’ and ‘Harosoy’ (Hulburt et al. 2004). The effect of this type of resistance is as large as that of QTL-M. According to the latest consensus soybean linkage map of LG-E (Song et al. 2004), *Pb* is 0.7 cM away from Satt411 on one side, and 2.3 cM from Sat_124 on the other. The *Pb* locus determines whether pubescence tips are sharp (*Pb*) or blunt (*pb*).

A QTL mapped to linkage group E (QTL-E) is associated with both antixenosis ($R^2 = 20\,\%$) and antibiosis ($R^2 = 26\,\%$) resistance to CEW in a Cobb × PI 227687

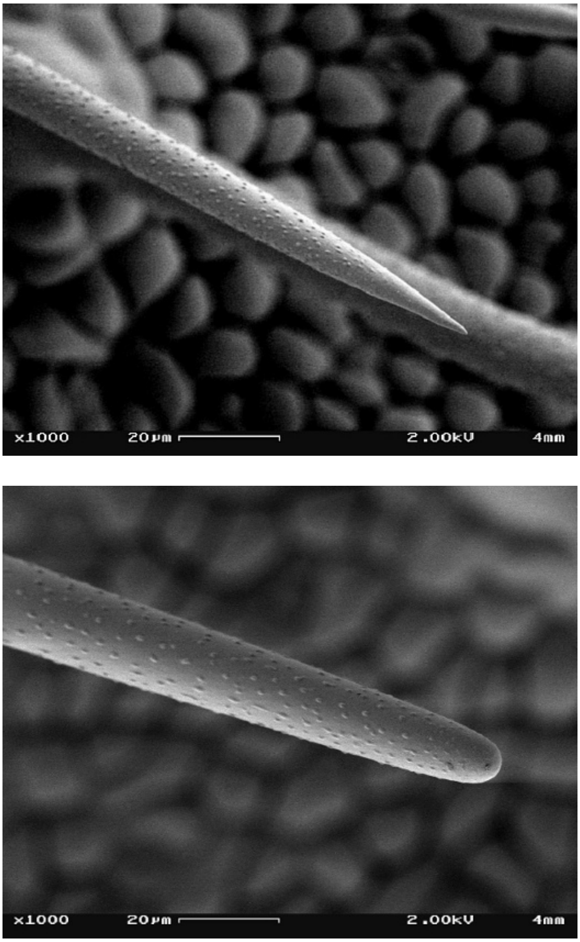

Fig. 15.4 Sharp (*top*) and blunt (*bottom*) pubescence

mapping population (Hulburt et al. 2004; Boerma and Walker 2005). This QTL maps to an interval spanning the *Pb* locus, which determines whether pubescence tips are sharp (*Pb*) or blunt (*pb*). The pubescence of PI 227687 has sharp tips, suggesting that *Pb* and QTL-E are the same locus. Other lines of evidence also suggest QTL-E and *Pb* are the same. Insect resistance and *Pb* remain associated in Harosoy and Clark near-isolines for *Pb*. A major QTL for insect resistance was found on LG-E (previously U2) in a cross between Minsoy and Noir 1 (Terry et al. 2000), which has since been found to be also segregating for pubescence tip.

Sharp-tipped pubescence should be very useful for improving insect resistance in soybean, since it contributes to both antixenosis and antibiosis, and provides some level of resistance to several lepidopteran pests. This mode of resistance is not related to that conditioned by QTL-M. Sharp pubescence is rare among soybean accessions, but is found in most wild soybean (*G. soja*) accessions (Broich and Palmer 1981). Because PI 227687 has very poor agronomic qualities, there is a high probability of linkage drag. Therefore, it has been used less frequently than PI 229358 as a donor parent in breeding programs aimed at achieving resistance to insects.

Conclusions and Future Prospects

It is clear that a variety of insect resistance mechanisms are operational in soybean. Initial genomic efforts were geared towards mapping major genes for resistance, and to use molecular markers to dissect the interactions among the various QTL for insect resistance. Future research will determine the extent to which genetic resistance will relate to increased soybean productivity. Syntenic relationships among plant species and their resistance genes allow information and knowledge about resistance genes from one plant to be applied to others. A better understanding of insect resistance in soybean should facilitate development of insect-resistant cultivars of important food legumes like the common bean (*Phaseolus lunatus* L.), for which funding of genomics research has historically been more limited than for soybean.

For insect-resistant soybean to be adopted by growers, cultivars must have superior yield and other agronomic characteristics. Genomic approaches are enabling the development of elite genotypes with specific insect resistance traits. The availability of such tagged genes and the understanding of how the QTLs interact with each other should facilitate breeding for insect resistance, and achieve the decades-old goal of obtaining modern, high-yielding cultivars that are as resistant as the original Plant Introductions from Japan.

Furthermore, pyramiding these QTLs with different modes of resistance, such as sharp pubescence, promises to achieve economically viable levels of resistance to a broad spectrum of defoliating insects. Enough progress has been made towards cloning QTL-M, that this QTL may be the first locus for resistance to a defoliating insect ever cloned, thus providing an understanding of the biological basis to resistance to defoliating insects.

References

Baldwin, I.T. and Preston, C.A. (1999) The eco-physiological complexity of plant responses to insect herbivores. Planta 208, 137–145.

Bartram, S., Jux, A, Gleixner, G. and Boland, W. (2006) Dynamic pathway allocation in early terpenoid biosynthesis of stress-induced lima bean leaves. Phytochem. 67:1661–1672.

Boerma, H.R. and Walker, D.R. (2005) Discovery and utilization of QTLs for insect resistance in soybean. Genetica 123, 181–189.

Boethel, D.J. (1999) Assessment of soybean germplasm for multiple insect resistance. In S.L. Clement and S.S. Quisenbury (Eds.) Global Plant Genetic Resources for Insect-Resistant Crops. CRC Press, Boca Raton, FL, pp. 101–129.

Broich, S.L. and Palmer, R.G. (1981) Evolutionary studies of the soybean - the frequency and distribution of alleles among collections of *Glycine max* and *Glycine soja* of various origin. Euphytica 30: 55–64.

Burden, B.J. and Norris, D.M. (1992) Role of the isoflavenoid coumestrol in the constitutive antixenosic properties of "Davis" soybeans against and oligophagous insect, the Mexican bean beetle. J. Chem. Ecol. 18, 1069–1081.

Bowers, G.R. (1990) Registration of 'Crockett' soybean. Crop Sci. 30, 427.

Caballero, P., Smith, C.M., Fronczek, F.R. and Fischer, N.H. (1986) Isoflavones from an insect-resistant variety of soybean and the molecular structure of afrormosin. J. Nat. Prod. 49, 1126–1129.

Clark, W.J., Harris, F.A., Maxwell, F.G. and Hartwig, E.E. (1972) Resistance of certain soybean cultivars to bean leaf beetle, striped blister beetle and bollworm. J. Econ. Entomol. 65, 1669–1671.

Cooper, W.C., Jia, L. and Goggin, F.L. (2004) Acquired and R-gene mediated resistance against the potato aphid in tomato. J. Chem. Ecol. 30, 2527–2541.

Cortés-Cruz, M., Snook, M. and McMullen, M.D. (2003) The genetic basis of C-glycosyl flavone B-ring modification in maize (*Zea mays* L.) silks. Genome 46, 182–194.

Desclaux, D., Roumet, P. (1996) Impact of drought stress on the phenology of two soybean cultivars. Field Crops Res. 46, 61–70.

Diaz-Montano, J., Reese, J.C., Schapaugh, W.T. and Campbell, L.R. (2006). Characterization of antibiosis and antixenosis to the soybean aphid, (Hemiptera: Aphidae) in several soybean genotypes. J. Econ. Entomol. 99. 1884–1889.

Fischer, D.C., Kogan, M. and Paxton, J. (1990) Effect of glyceollin, a soybean phytoalexin, on feeding by three phytophagous beetles (Coleoptera: Coccinellidae and Chrysomelidae): dose versus response. Environ. Entomol. 9, 1278–1282.

Goggin, F.L., Williamson, V.M. and Ullman, D.E. (2001) Variability in the response of *Macrosiphum euphorbiae* and *Myzus persicae* (Hemiptera : Aphididae) to the tomato resistance gene *Mi*. Environ. Entomol. 30, 101–106.

Goldberg, R.B., Bake, S.J., Perez-Grace, L. (1989) Regulation of gene expression during plant embryogenesis. Cell 56, 149–160.

Gould, F., Anderson, A. Reynolds, A., Bumbarner, L. and W. Moar, W. (1995) Selection and genetic analysis of a *Heliothis virescens* (Lepidoptera: Noctuidae) strain with high levels of resistance to *Bacillus thuringiensis* toxins. J. Econ. Entomol. 88, 1545–1559.

Gray, D.J., Lambert, L. and Ouzts, J.D. (1985). Evaluation of soybean plant introductions for resistance to foliar feeding insects. J. Miss. Acad. Sci. 30, 67–82.

Haile, F.J., Higley, L.G. and Specht, J.E. (1998) Soybean cultivars and insect defoliation: Yield loss and economic injury levels. Agron. J. 90, 344–352.

Haile, F.J., Higley, L.G., Specht, J.E. and Spomer, S.M. (1998) Soybean leaf morphology and defoliation tolerance. Agron. J. 90, 353–362.

Hammond, R.B., Bledsoe, L.W. and Anwar, M.N. (1995) Maturity and environmental effects on soybean resistant to Mexican bean beetle (Coleoptera: Coccinellidae). J. Econ. Entomol. 88, 175–181.

Hart, S.V., Kogan, M. and Paxton, J.D. (1983) Effect of soybean phytoalexins on the herbivorous insects Mexican bean beetle and soybean looper. J. Chem. Ecol. 9, 657–673.

Hartwig, E.E., Kilen, T.C. and Young, L.D (1994) Registration of 'Lyon' soybean. Crop Sci. 34, 1412.

Hartwig, E.E., Lambert, L. and Kilen, T.C. (1990) Registration of 'Lamar' soybean. Crop Sci. 30, 231.

Hatchett, J.H., Beland, G.L. and Kilen, T.C. (1979) Identification of multiple insect resistant soybean lines. Crop Sci. 19, 557–559.

Hill, C.B., Li, Y. and Hartman, G.L. (2004) Resistance to the soybean aphid in soybean germplasm. Crop Sci. 44, 98–106.

Hill, C.B., Li, Y. and Hartman, G.L. (2006a) A single dominant gene for resistance to the soybean aphid in the soybean cultivar Dowling. Crop Sci. 46, 1601–1605.

Hill, C.B., Li, Y. and Hartman, G.L. (2006b) Soybean aphid resistance in soybean Jackson is controlled by a single dominant gene. Crop Sci. 46, 1606–1608.

Hill, J.H., Alleman, R., Hogg, D.B. and Grau, C.R. (2001) First report of transmission of Soybean mosaic virus and Alfalfa mosaic virus by *Aphis glycines* in the New World. Plant Dis. 85, 561.

Hoffman-Campo, C.L., Ramos Neto, J.A., Neves de Oliveira, M.C. and Jacob Oliveira, L. (2006). Detrimental effect of rutin on *Anticarsia gemmatalis.* Pesq. agropec. bras. 41, 1453–1459.

Hollowell, E.A. and Johnson, T.W. (1934) Correlation between rough-hairy pubescence in soybean and freedom from injury by *Empoasca fabae*. Phytopath. 24, 12.

Hubick, K.T., Farquhar, G.D., Shorter, R. (1986) Correlation between water-use efficiency and carbon isotope discrimination in diverse pea nut (Arachis) germplasms. Aust. J. Plant Physiol. 13, 803–816.

Hulburt, D.J. (2001) Identifying additional insect resistance quantitative trait loci in soybean using simple sequence repeats. M.S. Thesis, University of Georgia, Athens, GA.

Hulburt, D.J., Boerma, H.R. and All, J.N. (2004) Effect of pubescence tip on soybean resistance to lepidopteran insects. J. Econ. Entomol. 97, 621–627.

Islas-Rubio, A.R. and Higuera-Ciapara, I. (2003) XXI: Soybean: Post-harvest operations. In: Mejía, D. And Lewis, B. (Eds). Compendium on Post-harvest Operations. Information Network on Post-harvest Operations (INPhO). FAO, www.fao.org/inpho.

Johnson, T.W. and Hollowell, E.A. (1935) Pubescent and glabrous character of soybeans as related to injury by the potato leafhopper, J. Agric. Res. 51, 371–381.

Kaloshian, I., Kinsey, M.G., Williamson, V.M. and Ullman, D.E. (2000) Mi-mediated resistance against the potato aphid *Macrosiphum euphorbiae* (Hemiptera: Aphidae) limits sieve element ingestion. Environ. Entomol. 29, 690–695.

Kenty, M.M., Hinson, K., Quesenberry, K.H. and Wofford, D. (1996) Inheritance of resistance to the soybean looper in soybean. Crop Sci. 36, 1532–1537.

Kilen, T.C., Hatchett, J.H. and Hartwig, E.E. (1977) Evaluation of early generation soybean for resistance to soybean looper. Crop Sci. 17, 397–398.

Kilen, T.C. and Lambert, L. (1986) Evidence for different genes controlling insect resistance in three soybean genotypes. Crop Sci. 26, 869–871.

Klinger, J., Creasy, R., Gao, L., Nair, R.M., Calix, A.S., Jacob, H.S., Edwards, O.R. and Singh, K.B. (2005) Aphid resistance in *Medicago truncatulata* involves antixenosis and phloem-specific, inducible antibiosis and maps to a single locus flanked by NBS-LRR resistance gene analogs. Plant Physiol. 137, 1445–1455.

Kogan, M. and Ortman, E.E. (1978) Antixenosis - a new term proposed to define Painter's "nonpreference" modality of resistance. Bull. Entomol. Soc. Am. 24, 175–176.

Komatsu, K., Okuda, S., Takahashi, M., Matsunaga, R. and Nakazawa, Y. (2005) QTL mapping of antibiosis to common cutworm (*Spodoptera litura* Fabricius) in soybean. Crop Sci. 45, 2044–2048.

Lambert, L. and Heatherly, L.G. (1991) Soil water potential: effects on soybean looper feeding on soybean leaves. Crop Sci. 31, 125–1628.

Lambert, L. and Kilen, T.C. (1984a) Multiple insect resistance in several soybean genotypes. Crop Sci. 24, 887–890.

Lambert, L. and Kilen, T.C. (1984b) Influences of three soybean genotypes and their F1 intercrosses on the development of five insect species. J. Econ. Entomol. 77, 622–625.

Lambert, L. and Tyler, J. (1999) An appraisal of insect resistant soybeans. In: J.A. Webster and B.R. Wiseman (Eds.), Economic, Environmental, and Social Benefits of Insect Resistance in Field Crops. Thomas Say, Lanham, MD. pp. 131–148.

Lee, S.I., Le,e S.H., Koo, J.C., Chun, H.J., Lim, C.O., Mun, J.H., Song. Y.H. and Cho, M.J. (1999) Soybean Kunitz trypsin inhibitor (SKTI) confers resistance to the brown planthopper (*Nilaparvata lugens* Stal) in transgenic rice. Mol. Breed. 5, 1–9.

Li, Y., Hill, C.B., Carlson, S.B., Diers, D.W., and Hartman, G.L. (2007) Soybean aphid resistance genes in the soybean cultivars Dowling and Jackson map to linkage group M. Mol. Breed. 19, 25–34.

Liu, S.H., Norris, D.M. and Lyne, P. (1989) Volatiles from the foliage of soybean, *Glycine max*, and lima bean, *Phaseolus lunatus*: their behavioral effects on the insects *Trichoplusia ni* and *Epilachna varivestis*. J. Agric. Food Chem. 37, 496–501.

Liu, F., VanToai, T.T., Moy, L., Bock, G., Linford, L. D., Quackenbush, J. (2005) Global transcription profiling reveals novel insights into hypoxic response in Arabidopsis. Plant Physiol. 137, 1115–1129.

Mensah, C., DiFonzo, C., Nelson, R.L. and Wang, D. (2005) Resistance to soybean aphid in early maturing soybean germplasm. Crop Sci. 45, 2228–2233.

McPherson, R.M. (2004) XVIII. Soybean insects. In: Gillebeau, P., Hinkle, N., and Roberts, P. (Eds). Summary of Losses from Insect Damage and Cost of Control in Georgia 2004. Georgia Cooperative Extension Service. Pp 37–38.

Milligan, S.B., Bodeau, J., Yaghoobi, J., Kaloshian, I., Zabel, P. and Williamson, V.M. (1998) The root-knot nematode resistance gene *Mi* from tomato is a member of leucine zipper, nucleotide binding, leucine-rich repeat family of plant genes. Plant Cell 10, 1307–1319.

Miranda, M., Borisjuk, L., Tewes, A., Dietrich, D., Rentsch, D., Weber, H., Wobus, U. (2003) Peptide and amino acid transporters are differentially regulated during seed development and germination in faba bean. Plant Physiol. 132, 1950–1960.

Momen, N.N., Carlson, R.E., Shaw, R.H., Arjmand, O. (1979) Moisture stress effects on the yield components of two soybean cultivars. Agron. J. 71, 86–90.

Narvel, J.M., Walker, D.R., Rector, B.G., All, J.N., Parrott, W.A. and Boerma, H.R. (2001) A retrospective DNA marker assessment to the development of insect resistant soybean. Crop Sci. 41, 1931–1939.

Nault, B.A., All, J.N. and Boerma, H.R. (1992) Resistance in vegetative and reproductive stages of a soybean breeding line to three defoliating pests (Lepidoptera: Noctuidae). J. Econ. Entomol. 85, 1507–1515.

Nielsen, N.C. (1996) Soybean seed composition. In: *Soybean: Genetics, Molecular Biology, and Biotechnology.* Biotechnology in Agriculture, D.P.S. Verma, R.C Shoemaker (Eds.) No. 14, CAB International, Wallingford, UK, pp. 127–163.

Painter, R.H. (1941) The economic value and biological significance of insect resistance in plants. J. Econ. Entomol. 34, 358–367.

Painter, R.H. (1951) Insect Resistance in Crop Plants. Macmillan & Sons, New York.

Rector, B.G., All, J.N., Parrott, W.A. and Boerma, H.R. (1998) Identification of molecular markers associated with quantitative trait loci for soybean resistance to corn earworm. Theor. Appl. Genet. 96, 786–790.

Rector, B.G., All, J.N., Parrott, W.A. and Boerma, H.R. (1999) Quantitative trait loci for antixenosis resistance to corn earworm. Crop Sci. 39, 531–538.

Rector, B.G., All, J.N., Parrott, W.A. and Boerma, H.R. (2000) Quantitative trait loci for antibiosis resistance to corn earworm in soybean. Crop Sci. 40, 233–238.

Rennie, B.D., Tanner, J.W. (1989) Fatty acid composition of oil from soybean seeds grown at extreme temperatures. J. Am. Oil Chem. Soc. 66, 1622–1624.

Reymond, P., Weber, H., Damond, M. and Farmer, E.E. (2000) Differential gene expression in response to mechanical wounding and insect feeding in *Arabidopsis*. Plant Cell 12, 707–719.

Reynolds, G.W. and Smith, C.M. (1985) Effects of leaf position, leaf wounding, and plant age of two soybean genotypes on soybean looper (Lepidoptera: Noctuidae) growth. Environ. Entomol. 14, 475–478.

Rowan, G.B., Boerma, H.R., All, J.N. and Todd, J. (1993) Soybean maturity effect on expression of resistance to Lepidopteran insects. Crop Sci. 33, 618–622.

Rufener, G.K., II, St. Martin, S.K., Cooper, R.C. and Hammond, R.B. (1989) Genetics of antibiosis resistance to Mexican bean beetle in soybean. Crop Sci. 29, 618–622.

Sales, M.P., Pimenta, P.P., Paes, N.S., Grossi-de-Sa, M.F. and Xavier, J. (2001) Vicilins (7S storage globulins) of cowpea (*Vigna unguiculata*) seeds bind to chitinous structures of the midgut of *Callosobruchus maculatus* (Coleoptera : Bruchidae) larvae. Braz. J Med. Biol. Res. 34. 27–34.

Shukle, R.H. and Wu, L. (2003) The role of protease inhibitors and parasitoids on the population dynamics of *Sitotroga cerealella* (Lepidoptera: Gelechiidae). Environ. Entomol. 32, 488–498.

Sisson, V.A., Miller, P.A., Campbell, W.V. and Van Duyn, J.W. (1976) Evidence of inheritance of resistance to the Mexican bean beetle in soybeans. Crop Sci. 16:835–837.

Smith, C.M. (1985) Expression, mechanisms and chemistry of resistance in soybean, *Glycine max* L. (Merr.) to the soybean looper, *Pseudoplusia includens* (Walker). Insect Sci. Applic. 6, 243–248.

Smith, C.M. and Brim,C.A. (1979a) Field and laboratory evaluations of soybean lines for resistance to corn earworm feeding. J. Econ. Entomol. 72, 78–80.

Smith, C.M. and Brim,C.A. (1979b) Resistance to the Mexican bean beetle and corn earworm in soybean genotypes derived from PI 227687. Crop Sci. 19, 313–314.

Smith, C.M. and Fischer, N.H. (1983) Chemical factors of an insect resistant soybean genotype affecting growth and survival of the soybean looper. Entomol. Exper. Applic. 33, 343–345.

Smith, T.J., Camper Jr., H.M. and Schillinger, J.A. (1975) Registration of Shore soybean. Crop Sci. 15, 100.

Song, Q.J., Marek, L.F., Shoemaker, R.C, Lark, K.G., Concibido, V.C., Delannay, X., Specht, J.E. and Cregan, P.B. (2004) A new integrated genetic linkage map of the soybean. Theor. Appl. Genet. 109, 122–128.

Tabashnik, B.E., Carriere, Y., Dennehy, T.J., Morin, S., Sisterson, M.S., Roush, R.T., Shelton, A.M. and Zhao, J.Z. (2003) Insect resistance to transgenic Bt crops: Lessons from the laboratory and field. J. Econ. Entomol. 96:1031–1038.

Terry, L.I., Chase, K., Jarvik, T., Orf, J., Mansur, L. and Lark, K.G. (2000) Soybean quantitative trait loci for resistance to insects. Crop Sci. 40, 375–382.

Turner, N.C. (2000) Drought resistance: A comparsion of two frame works. In: *Management of Agricultural Drought: Agronomic and Genetic Options.* N.P. Saxena, C. Johansen, Y.S. Chauhan, R.C.N. Rao (Eds.), Oxford and IBH, New Delhi.

Turnipseed, S.G. (1977) Influence of trichome density on populations of small phytophagous insects on soybean. Environ. Entomol. 6, 815–817.

Turnipseed, S.G. and Kogan, M. (1987) Integrated control of insects. In: J.R. Wilcox (Ed.), Soybeans: Improvement, Production, and Uses. ASA, CSSA and SSSA, Madison, pp. 779–817.

van der Biezen, E.A. and Jones, J.D.G. (1998) Plant disease resistance proteins and the gene-for-gene concept. Trends Biochem. Sci. 23, 454–456.

Van Duyn, J.W., Turnipseed, S.G. and Maxwell, J.D. (1971) Resistance in soybeans to the Mexican bean beetle. I. Sources of resistance. Crop Sci. 11, 572–573.

Van Duyn, J.W., Turnipseed, S.G. and Maxwell, J.D. (1972) Resistance in soybeans to the Mexican bean beetle. II. Reactions of the beetle to resistant plants. Crop Sci. 12, 561–562.

Walker, D.R., Narvel, J.M., Boerma, H.R., All, J.N. and Parrott. W.A. (2004) A QTL that enhances and broadens Bt insect resistance in soybean. Theor. Appl. Genet. 109, 1051–1057.

Walling, L.L. (2000) The myriad plant responses to herbivores. J. Plant Growth Reg. 19, 195–216.

Yencho, G.C., Cohen, M.B. and Byrne, P.F. (2000) Applications of tagging and mapping insect resistance loci in plants. Ann. Rev. Entomol. 45, 393–422.

Yunes, A.N.A., de Andrade, M.T., Sales, M.P., Morais, R.A., Fernandes, K.V.S., Gomes, V.M. and Xavier-Filho, J. (1998) Legume seed vicilins (7S storage proteins) interfere with the development of the cowpea weevil (*Callosobruchus maculatus* (F)). J. Sci. Food Agric. 76, 111–116.

Zhang, P., Wang, Y., Zhang, J., Maddock, S., Snook, M. and Peterson, T. (2003) A maize QTL for silk maysin levels contains duplicated Myb-homologous genes which jointly regulate flavone biosynthesis. Plant Mol. Biol. 52, 1–15.

Zhu, S., Walker, D.R., Boerma, H.R., All, J.N. and Parrott W.A.(2006) Fine mapping of a major insect resistance QTL in soybean and its interaction with minor resistance QTLs. Crop Sci. 46, 1094–1099.

Zhu, S., Walker, D.R, Warrington, C.V., Parrott, W.A., All, J.N., Wood, E.D. and Boerma, H.R. (2007) Registration of four soybean germplasm lines containing defoliating insect resistance QTLs from PI 229358 introgressed into 'Benning'. J. of Plant Registrations 1, 162–163.