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Developing Host-Plant Resistance for Hemipteran Soybean Pests: Lessons from Soybean Aphid and Stink Bugs

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Additional information is available at the end of the chapter

1. Introduction

Soybean is world's leading agricultural crop with multiple uses including human food, animal feed, edible oil, biofuel, industrial products, cosmetics, etc. In soybean production, United States (US) is the leading producer with 33% of the world's total production of 251.5 million MT, amounting to $38.5 billion in production value [1,2]. In North-America, there has been an exponential increase of soybean acreage during the second half of last century, but there is a continuous threat of pests attacking this crop. Soybean yield is impacted by various kinds of pests such as fungi, bacteria, and insects [3]. Indeed, the strategies and input costs for pest management in soybean have changed dramatically with time [3-5]. For example, there has been a 130-fold increase in insecticide use across the North-Central US states since 2001 [4].

In regards to insects, soybean has been traditionally attacked by foliage-feeding Lepidopteran and Coleopteran pests such as soybean looper, velvet bean caterpillar, beet armyworm, bean leaf beetle, stem borer, Mexican bean beetle, and soybean leaf miner [6]. However, during the last decade, the invasion of soybean aphid [*Aphis glycines* Matsumura], brown-marmorated stink bug (BMSB) [*Halyomorpha halys* (Stål)], and (although technically not a stink bug) kudzu bug [*Megacopta cribraria* (F.)] in north-central, eastern, and southeastern US, respectively, and the emergence of red-banded stink bug [*Piezodorus guildinii* (Westwood)] as major pest in southern US have drastically changed the pest complex in soybean [4,7,8]. The threat posed by soybean aphid and stink bugs has the potential to rapidly increase as these insects continue to expand their geographical range. For example, in less than 10 years since its initial detection in Wisconsin, soybean aphid had spread across 30 US states and 3 Canadian provinces by 2009

[4], and the BMSB has already been detected in 38 US states since first being seen in Pennsylvania in 1996 [7].

Both soybean aphid and stink bug belong to order Hemiptera which also includes other economic pests such as whiteflies and leafhoppers. To minimize the damage by Hemipteran pests, host-plant resistance in soybean cultivars should constitute an integral part of an integrated pest management (IPM) program. In the current chapter, we attempt to review the recent research advances made on soybean resistance to Hemipteran pests. In the light of various challenges to manage Hemipteran pests, we have proposed strategies for successful and sustainable use of host plant resistance (HPR) in soybean against these pests.

2. Hemipteran pests of soybean

The soybean aphid, various stink bug species and kudzu bug are the major Hemipteran pests of soybean (Figure 1). Although soybean aphid and stink bugs share basic features of Hemipteran insects, there is much evolutionary divergence between them, their suborders having diverged more than 250 million years ago [9]. Aphids belong to the suborder Homoptera which have uniform, membranous forewings and hindwings. In homopteran insects, wings are held roof-like over their abdomen. Stink bugs belong to suborder Heteroptera having forewings that are leathery basally and membranous distally, in contrast to membranous hindwings. In Heteroptera, wings are folded flat over the abdomen [10]. Stink bugs can also be identified by five-segmented antennae and a conspicuous scutellum [10].

2.1. Soybean aphid

The soybean aphid is a recent invasive species in North-America [4,5,11]. This species was first detected during the summer of 2000 and is believed to have been introduced from its native Asian range [11,12]. Soybean aphid is a pest of significant economic importance as it can cause up to 58% yield losses in soybean [13]. Losses due to yield have been estimated to be $2.4 billion annually [14-15].

The biology of soybean aphid in North-America has been reviewed recently [4,5]. In general, it is a typical holocyclic (asexual and sexual reproduction) heterecious (alternates between 2 hosts) aphid species. In autumn, sexual reproduction occurs on its primary host, buckthorn (*Rhamnus* spp.), and the resulting eggs undergo overwintering. The following spring, the eggs hatch, and the fundatrices (i.e. stem mothers) begin to produce female clones. After 2-3 asexual generations, winged females are formed that disperse to emerging soybean, where about 15 additional asexual generations occur, and when damage to soybean is most severe. Upon soybean maturity, sexual forms are formed and migrate back to buckthorn.
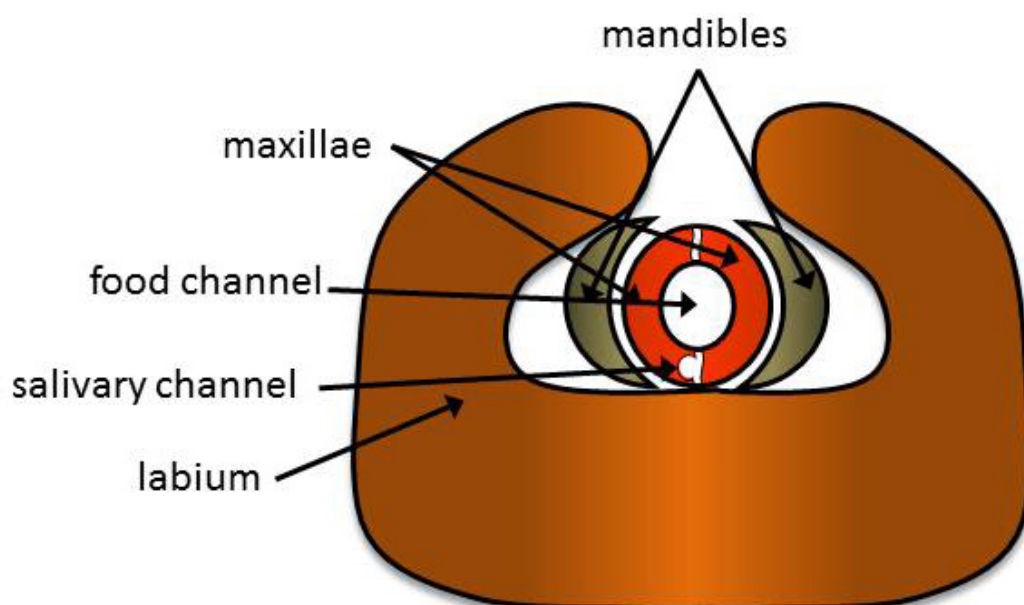
2.2. Stink bugs

In Hemiptera, stink bugs are in the family Pentatomidae. The name stink bug is attributed to the malodorous substance they emit for defense [16]. In the past, three species [southern green
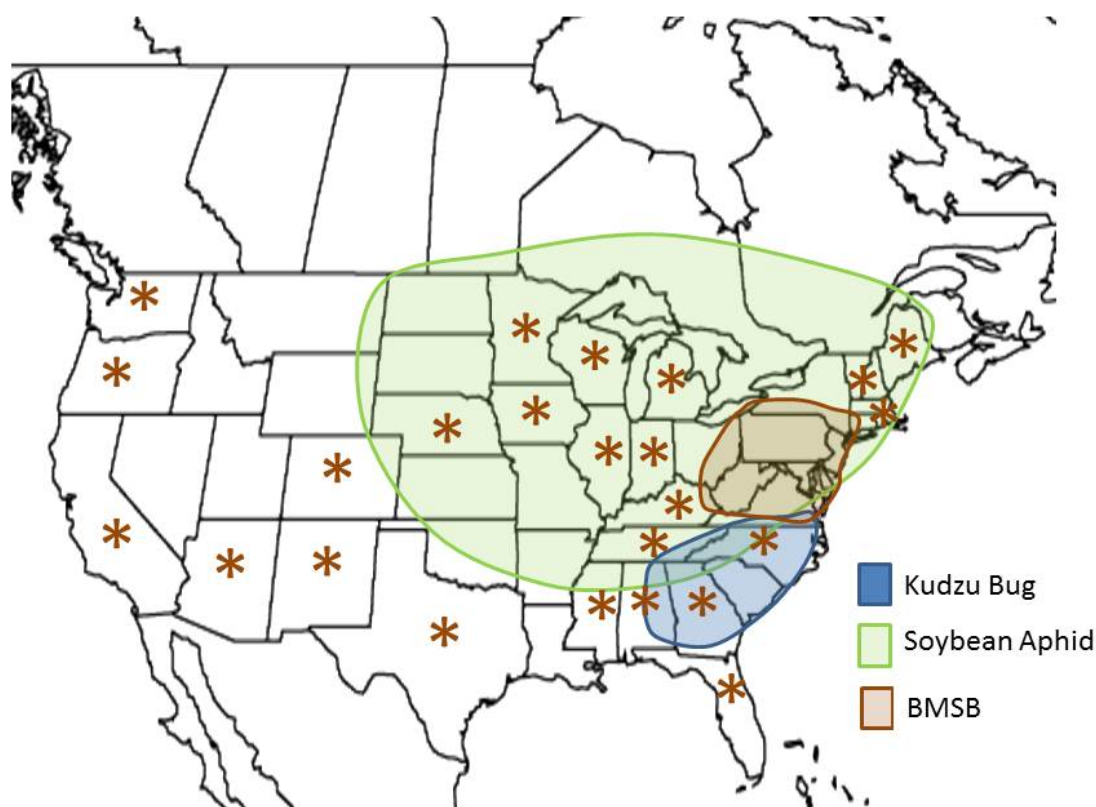
Figure 1. Approximate distribution of the main, invasive Hemipteran pests of soybean. For BMSB, shaded range is where damage is heaviest, * represent states where detection has been observed.

stink bug, *Nezara viridula (L.)*; green stink bug, *Acrosternum hilare* (Say); brown stink bug, *Euschistus servus* (Say)] constituted the stink bug complex that attacked soybean crop in southern US [6,17]. Amongst these, *N. viridula* is the most abundant [18] which has caused the most severe damage to the field crop [17]. In more northern latitudes, the relative abundance of *A. hilare* is higher compared to that of *N. viridula* [6]. During the last decade, redbanded stink bug, *Piezodorus guildinii* (Westwood), and brown marmorated stink bug (BMSB), *Halyomorpha halys* (Stål) have established themselves as important members of stink bug complex that attack soybean in the US [7, 19,20]. From 1960 onwards, the redbanded stink bug has been detected in US soybean but without causing any economic damage. However during last decade, redbanded stink bug infestations have reached above the threshold levels. By 2009, it was the most serious stink bug species attacking soybean in southern US [21, 22]. The BMSB is native to North and South Korea, Japan, China [23] and is invasive in North America and Europe. In US, BMSB has been confirmed as pest of soybean crop with a high damaging potential [24, 25]. Although not in the Pentatomidae, the kudzu bug (Plataspididae: *Megacopta cribraria*) is another recent invasive pest, first detected in Georgia in 2009 [26] and has now spread to 8 southeastern states of US [8]. It is known to feed on both kudzu and soybean, and damage on soybean can be quite severe [27].

Figure 2. Generalized mouthpart structure of Hemiptera. Redrawn from [29.]

The biology of various stink bug species has been described in detail [10]. In general, stink bugs pass through five nymphal instars and an adult stage during their life cycle [10]. These insects overwinter as adults beneath the leaf litter of various host plants including grasses, shrubs and trees. Several species can also overwinter in homes and these infestations can be severe as seen with the BMSB [7]. In early summer (April-June), overwintered and first generation adults feed on crops like tomato, okra, crucifers, and legumes, but for BMSB, may also feed on woody trees like *Paulownia* or *Ailanthus altissima* (tree of heaven) early in the spring before moving into crops [28]. However, they will feed on soybean if early planted crop is available. In most cases, stink bugs will move into soybean to feed on the developing pods and seeds. In North America, the number of generations per year is largely dependent upon environmental factors but usually varies between 1 in the north to 5 in the south.

2.3. Hemiptera feeding and damage to soybean

Hemipteran pests inflict the damage on soybean by feeding on plant juices. These insects possess piercing and sucking mouthparts, the most characteristic feature of Hemiptera which are highly adapted for extracting the liquid contents of plants. The mouthparts' structures are held in a grove present on the anterior side of the insect's lower tip i.e. labium (also called as rostrum) (Figure 2, [29]). On the either side of maxillae, two mandibles are present. The mandibles, which are often barbed at the tip, form the main piercing structure called the stylet. Two opposing maxillae which are held together by a system of tongues and groves, form two canals: a food canal and a salivary canal. The food canal is used for uptake of plant liquids whereas salivary canal is for egestion of saliva into the plant structures. Because of the segmented structure, labium can fold itself when stylets penetrate into the plant surface.

Both the soybean aphid and stink bugs can feed on various above-ground plant parts like leaves, stems, flowers, and pods. Soybean aphid prefers to feed on the undersides of leaves [15] whereas stink bugs prefer to feed on pod and seeds [6]. During the early seedling stages of soybean, soybean aphids are mostly found on freshly growing trifoliate leaves or the stems [30]. Later in the season, soybean aphids are more likely to be found lower in the canopy, on leaves that are attached to nodes further away from the terminal bud. During feeding, soybean aphid withdraws sap from soybean leaves which results in loss of photosynthates. Heavy infestations by soybean aphid can result in yellow and wrinkled leaves, reduction in plant height, reduced pod set and lesser number of seeds within pods [15,31]. Infested leaves may turn black due to sooty mold growing on the sugary excretions or "honeydew" produced by soybean aphid [5,15]. The severity of plant losses caused by soybean aphid is largely dependent on the physiological status of the soybean plant. Soybean aphid populations that reach their peak density during the early-vegetative or mid-reproductive stages (R3-R5) are more likely to cause serious damage compared to populations that peak during late reproductive stages (R6-R7) [32].

Except the first instar which is a non-feeding stage, all other developmental stages of stink bugs feed upon plants. In soybean, the most damage is caused by adults and/or fifth instars stages [10]. Due to their preference for pods and seeds, reproductive stages of soybean are the most susceptible to damage by stink bugs [17]. Further, stink bugs prefer to feed upon pods present on upper half of the plant. However in case of severe infestation, these insects may also feed on lower pods. Stink bugs cause injury to soybean seeds as they insert the stylets through the pod wall into the seed for feeding on plant juices. In immature seeds, discolored necrotic areas may occur around these punctures [10]. Mature seeds show puncturing marks, discoloration, and internal irregular white spots which may have a chalky appearance [33]. Heavy feeding on mature seeds may result in smaller size, irregular shape, including wrinkled areas around punctures (Figure 3) [33, 34]. Stink bug damage in soybean results in decreased pod number, fewer seed per pod, lower seed weight, changes in fatty acids composition, and lower seed quality [10]. The germination of soybean seeds may be prevented due to single puncture in radicle-hypocotyl axis of seeds [35]. On the other hand, several punctures in cotyledons may not prevent germination but affect the vigor. Heavy infestation of stink bugs can result in foliar retention, delayed maturation and abnormal growth of the soybean crop [10].

Hemipteran pests also cause indirect damage to crops by vectoring the transmission of microbial pathogens. The virus transmission by soybean aphid to various crops has been reviewed [4]. In soybean, soybean aphid has the potential to transmit *Soybean mosaic virus* [4] but so far, there is no report of significant damage. Stink bugs generally transmit fungal pathogens [36] but recently, they were also found to transmit bacterial pathogens [37]. Besides vectoring of fungal pathogens, feeding by stink bugs provides the entrance points for microbial pathogens [10].

3. Host Plant Resistance (HPR)

In agriculture, HPR represents the ability of a certain plant variety to produce a larger yield of better quality compared to other varieties of the same crop at the same level of insect
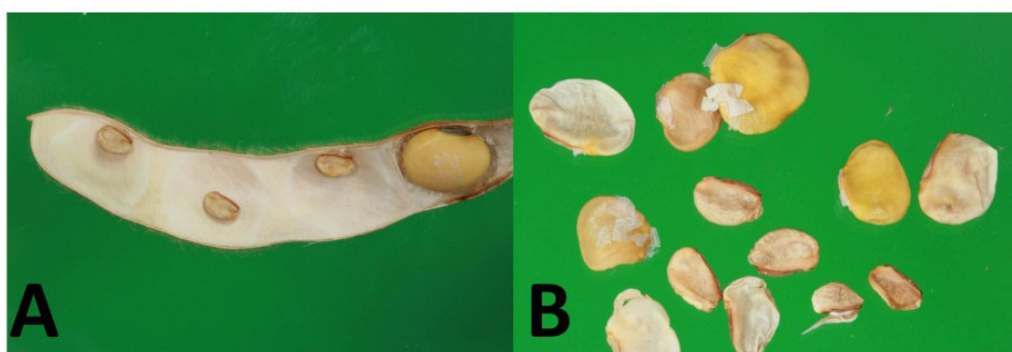
Figure 3. Picture of BMSB damage to soybean in Columbus, Ohio. A) Damaged seeds found in the pods compared to undamaged seed (far right); B) Sample of shriveled seeds collected from BMSB infested soybean. (Picture courtesy of R. B. Hammond)

infestation [38]. R. H. Painter, a pioneer researcher on HPR described it as the relative amount of heritable qualities possessed by the plant that influence the ultimate degree of damage done by the insect [39]. Painter's definition was extended to emphasize the relative nature of HPR [40]. HPR refers to the "…sum of the constitutive, genetically inherited qualities that result in a plant of one cultivar or species being less damaged than a susceptible plant lacking these qualities." [40]. Thus, plant resistance to insects should be measured on a relative scale, by comparing the damage to susceptible control plants.

Plant resistance to Hemipteran insects have been found and utilized in many crop plants [40-41]. From historical perspective, HPR to manage Hemipteran insect-pests of soybean has been highly successful. The resistance to potato leaf hopper (*Empoasca fabae*), a serious pest of soybean in the past, occurs due to the presence of pubescence on plant leaves [42-43]. The subsequent incorporation of pubescence trait into commercial varieties of soybean consigned the potato leaf hopper to a non-pest status [44]. Specifically for aphids, resistant genes have been identified in several crops, including cereals, vegetables, fruits and forages [40, 41, 44]. Identification and deployment of aphid resistance genes against Russian wheat aphid (*Diuraphis noxia*) in wheat [46, 47] and in barley [48] are very good examples of host plant resistance to Hemipertan pests. The Russian wheat aphid resistant wheat cultivars are commercially grown throughout USA and South Africa.

Plant resistance to important pests and pathogens is an integral component of soybean IPM and development of soybean cultivars with resistance to insects has been a long time priority for soybean breeders. However, one major drawback of employing HPR as a control measure against insect pests is the potential lack of its durability. Principally, the HPR based on major genes has not proved to be a long term solution for pest management because insect populations emerge which can overcome plant defenses resulting from those genes [40, 45], often referred to as insect biotypes. Smith [45] defines biotypes as "…populations within an arthropod species that differ in their ability to utilize a particular trait in a particular plant genotype". The success of HPR against Hemipteran pests is particularly compromised by the presence and emergence of insect biotypes. Variable number of biotypes have been reported

in many Hemipteran insects like brown plant hopper (*Nilaparvata lugens*), White fly (*Bemisia tabaci*), and in a number of aphid species. A total of 14 aphid species with known biotypes are listed in [45], and this does not include the soybean aphid. Managing the emergence and increase of insect biotypes will be critically important to extend the durability of HPR. The topic of biotypes will be discussed in detail in the subsequent sections of this chapter.

3.1. Soybean HPR to hemipteran pests

3.1.1. Soybean aphid

A limited number of studies were conducted in China on soybean host plant resistance to the soybean aphid before its invasion into North America and few aphid resistant soybean lines were identified [49-51]. None of the aphid resistant genes in these lines were reported to be genetically characterized. Several soybean lines with resistance to the soybean aphid have been recently identified by researchers in US [52-56]. Most of these studies used locally collected soybean aphid biotypes for greenhouse and field screenings of soybeans in early growth stages and estimating the number of soybean aphids on each seedling after 2 to 4 weeks of infestation with a known number of aphids. However, there are currently 3 soybean aphid biotypes recognized, biotype 1, 2, and 3 [57]. Biotype 1 is unable to survive (*i.e.* avirulent) on any known HPR soybean, whereas biotype 2 and biotype 3 can survive (*i.e. virulent*) on *Rag1* and *Rag2*, respectively. More recent soybean aphid HPR characterizations have included some or all biotypes.

Three lines with resistance to three soybean lines – Dowling, Jackson and a plant introduction (PI) 71506 were reported in [52]. These lines have resistance to biotype 1 but not to biotype 2 of the soybean aphid. The resistance in Dowling, Jackson, and PI71506 exhibited both antixenosis [58,59] and antibiosis [52]. Three PIs – PI243540, PI567301B and PI567324 – were identified to have resistance to soybean aphid biotypes 1 and 2 [56]. PI243540 showed antibiosis whereas PI567301B and PI567324 showed antixenosis type of resistance in no-choice tests. A total of 2147 soybean germplasm accessions were evaluated in choice tests and four PIs - PI 567598B, PI 567541B, PI567543C, and PI 567597C- with resistance to soybean aphid were identified [55]. The soybean aphids used in this study were collected from Michigan fields that comprised of unknown biotypes. In a no-choice test, PI 567598B and PI567541B were found to possess antibiosis resistance and PI567543C, and PI 567597C showed antixenosis resistance. Diaz- Montano [58] reported 11 soybean genotypes with resistance to soybean aphid of unknown biotype. Among the 11 genotypes, nine showed moderate antibiotic effects and the other two showed not only a strong antibiotic effect but also exhibited antixenosis as a category of resistance to the aphid. Pierson et al. [60] documented moderate resistance to the soybean aphid in soybean lines KS4202, K1639 and K1621 during the reproductive stages of development.

3.1.2. Stink bugs

Based on field and laboratory trials, Jones and Sullivan [61] found that 3 Mexican bean beetle-resistant PIs i.e. PI 171451, PI 227687, and PI 229358 [62] were also effective against southern

green stink bug. Subsequently these PIs were found to possess resistance to multiple pests including Lepidoptera, and thus were used as donor parents for major breeding programs across US [44]. These PIs exhibited antixenosis and antibiosis type of resistance against different pests. Amongst these, PI229358 appeared to be most resistant to stink bugs. Gilman et al. [63] evaluated 894 PIs and 26 cultivars (maturity groups V-VIII) for their resistance to the southern green stink bug. The resistance to stink bugs was, in general, associated with plant maturity as early maturing genotypes showed lesser damage compared to late maturing ones. They identified PI 171444 (MG VI) to be the highly resistant against southern green stink bug. The stink bug resistance in PI 171444 is exhibited as antixenosis, antibiosis and temporal separation [64].

Most research on soybean HPR against stink bugs has been conducted in Brazil, where the damage is often the heaviest [65]. The cultivar IAC-100 having PI 229358 and PI 274454 in its genealogy was officially released in Brazil, and it carries resistance to stink bug complex [66, 67]. In order to develop soybean lines adaptable to southeastern US, McPherson et al. [68] developed 65 breeding lines carrying IAC-100 in their genealogy. During 5 years of field testing, all these lines showed variable amount of resistance against stink bugs primarily the southern green stink bug. Among these, four breeding lines with either Hutcheson x IAC-100 or IAC-100 x V71-370 in their genealogy were identified as promising material for future development of stink bug resistant soybean. Recently, Campos et al [69] screened 16 genotypes for resistance against southern green stink bug by caging adult bugs on pods. Based on number of feeding punctures, three genotypes V00-0742, V00-0842, and V99-1685 were identified as resistant. Based on reduced seed weight loss, two genotypes (PI 558040 and V00-0870) were further identified as resistant.

3.2. Genetics of soybean resistance against Hemipterans

3.2.1. Soybean aphid

Inheritance of several major soybean genes (R-genes or single gene) for resistance to soybean aphids has been reported [53, 54, 70]. The aphid resistance in each of the two soybean cultivars Dowling and Jackson is controlled by a single dominant gene. The gene in Dowling was designated as *Rag*1 and the *Rag* gene in Jackson remained unnamed. The aphid resistance in PI 243540 is controlled by a single dominant gene [70]. In contrast, the aphid resistance in PI 567541B is controlled by quantitative trait loci (QTL) and resistance in PI 567598B is controlled by two recessive genes [71, 72] A total of six major genes for resistance to the soybean aphids have been reported by 2012 (Table 1).

Li et al. [78] have mapped *Rag*1 and the unnamed *Rag* gene from Jackson to the same genomic region on soybean chromosome 7 (LG M), indicating that these two resistance genes may be allelic. PI 243540 was resistant to both biotypes 1 and 2 and the resistance in this soybean accession from Japan was controlled by a single dominant gene [70]. Mian et al. [75] mapped the *Rag2* gene on soybean chromosome 13 (LG F) between SSR markers Satt334 and Sct_033. Zhang et al. [79] mapped two recessive loci in PI 567541B controlling soybean aphid resistance.

Gene name	Source PI (s)	Resistance category	Biotype response*		
			B1	B2	B3
Rag1	PI 548663 [53]; PI 548657[54]	Antibiosis [52,73] and antixenosis [58,59]	A	V	A/V[†]
Rag2	PI 200538 [74], PI 543540 [70-75]	Antibiosis [56,74]	A	A	V
Rag3	PI 567543C [72]	Antixenosis [72]	A	A	V
rag4	PI 567541B [72]	Antibiosis [72]	A	A	V
Rag5	PI 567301B [76]	Antixenosis [56]	A	A	V

*A-Avirulent; V-Virulent

†Virulent in choice tests, Avirulent in no-choice tests [77]

Table 1. Major HPR genes identified in soybean response to soybean aphid biotypes

One locus was mapped on chromosome 13 (LG F) and the other loci on chromosome 7 (LG M). The major locus on chromosome 7 in this study was only about 3 cM from Satt435, which was the closely linked marker to the *Rag*1 locus and designated as *rag*1_provisional [80]. Thus, the major locus identified in this study is located in the same genomic region as *Rag*1, which indicates that they are either allelic at the same locus or two different loci tightly linked to each other. The second aphid resistance locus in this PI was mapped on chromosome 13 nearly 50 cM away from the *Rag*2 locus and this recessive gene was designated as *rag*4. Zhang et al. [80] mapped single dominant locus in PI567543C for soybean aphid resistance on chromosome 16 (LG J). This locus provided a near complete resistance to the soybean aphid indicating a single gene resistance and the gene has been named as *Rag*3. Jun et al. [76] mapped a major soybean aphid resistance gene on chromosome 13 near the *Rag*2 locus in PI567301B. However, the resistance in PI 567301B is antixenosis type while *Rag*2 is a locus for antibiosis resistance and thus the locus in PI567301B has been tentatively named as *Rag*5, pending approval from the Soybean Genetics Committee. Jun et al. [82] recently mapped three QTL for oligogenic resistance to the soybean aphids in PI567324 (in review). The inheritance and genetic mapping studies on tolerant lines have not been conducted yet.

3.2.2. Stink bug

Research on genetics of soybean resistance to stink bugs has been limited and confined to only a few PIs. Multiple studies have confirmed that the pest resistance (including against stink bugs) in 4 PIs i.e. PI 171451, PI 227687, PI 229358, and PI 229321 is a quantitative trait and is controlled by two or three major genes (reviewed in [44]). Because of quantitative nature of both resistance and yield in these PIs, conventional breeding strategies were not successful in introgressing these traits into the locally adapted varieties. The stink bug resistance in tolerant cultivars like IAC-100, IAC-74 2832, IAC-78 2318 is a complex polygenic trait that is exhibited as additive, dominant and epistatic effects of multiple genes [82, 83].

3.3. Mechanisms of soybean resistance against hemipterans

3.3.1. Soybean aphid

Until now, a limited amount of information is available on the mechanism of resistance provided by *Rag* genes to soybean aphid. Using cDNA microarrays, the transcript profiles of cultivars Dowling (*Rag1*, soybean aphid resistant) and Williams 82 (soybean aphid susceptible) were compared after aphid infestation [84]. Out of ~18,000 soybean genes tested, only 140 showed differential expression between resistant and susceptible cultivars after 6 and 12hrs of aphid feeding. In the resistant cultivar, genes involved in the salicylic acid (SA) and jasmonic acid (JA) pathways were upregulated compared to their expression in susceptible cultivar. Both SA and JA are signaling molecules that mediate the stress response in a resistant plant upon being attacked by an insect. In the downstream of defense signaling pathway, both SA and JA lead to the production of defensive allelochemicals that are deterrent or lethal to the insect. In addition, SA signaling may result in the production of reactive oxygen species that kill the insect due to oxidative injury [41].

3.3.2. Stink bugs

Most of the research into the mechanism of soybean HPR to stink bugs has been in the distant past. As mentioned earlier, 3 PIs PI 171451, PI 227687, PI 229358 show resistance to multiple pests (including stink bugs) through antixenosis and antibiosis. The mechanism of resistance in these PIs has been elucidated in various studies that involved various lepidopteran and coleopteran pests of soybean but not stink bugs (reviewed in [44]). In general, antixenosis and antibiosis resistance in these PIs is manifested through plant allelochemicals, which are isoflavones (plaseol, aformosin), phenolic acids, phytoalexins. However, the mechanism of resistance in stink bug-tolerant cultivars (IAC-100) is better understood. It includes pest evasion by shorter pod filling period, rejection of young damaged pods and replacement with new pods, normal leaf senescence under stress, and higher number of seeds in pods [82].

3.4. Soybean traits as selection criteria for breeding

In breeding for insect resistance, selection is the key step. Since soybean aphids build up in huge numbers due to asexual reproduction, susceptible germplasm of soybean is not able to withstand early stage infestation. General vigor, chlorosis, curling, infestation levels (*e.g.* insect counts) will allow for selection of cultivars showing antixenosis and antibiosis. Since tolerant cultivars continue to withstand high infestation levels of soybean aphid, yield and seed quality are the most important selection criteria for evaluation of this trait. During vegetative stages, plant health and rate of growth of infested plants compared to the uninfested plants can be used as criteria of tolerance. While on mature plants, measurement of agronomic traits, including plant height, maturity, lodging, seed yield and quality (discoloration and wrinkling of seed coats, shriveled seeds) are some of the traits that can be measured for determination of soybean tolerance to the aphid. Evaluations at both vegetative and mature plant stages will be more desirable. For selection of stink bug resistance germplasm, insect counts may not be the best indicators because insects may evade monitoring. Again the best indicators of

tolerance are the seed yield and seed quality of infested plants in reference to the uninfested plants. As stink bugs mostly feed on developing and developed pods and seeds of soybean, there is no reliable way of evaluation of soybean tolerance to these insects during the vegetative stages.

3.5. Sustainable HPR against soybean aphid biotypes

Smith [40] has reviewed the occurrence of biotypes in various insect species. Among insects, 'biotypes' is the most abundant phenomenon in aphids. Smith [40] argued that aphids will continue to produce biotypes because of their parthenogenic reproduction, high reproductive potential and clonal diversity. There is no clear evidence to suggest the cause behind occurrence of biotypes in insects. In some insects like Hessian fly and Russian wheat aphid, biotypes emerged as these were exposed to resistant cultivars. These biotypes developed probably due to selection pressure placed by agronomic production, recombination or mutation to overcome the defense due to resistance genes. However in insects like soybean aphid and green bug, virulent biotypes have been discovered in field populations *before* the deployment of resistant cultivars. Three biotypes of soybean aphid are known so far that can defeat resistance genes identified in several PI's (Table 1). Thus, the success of resistance genes has been greatly hindered by the occurrence of virulent biotypes of soybean aphid. Based on successful examples in other plant-insect systems and resistance-management approaches, here we discuss strategies and questions that need to be answered to sustain the success of HPR in soybean against soybean aphid.

1.   Gene deployment based on biotype distribution:

The knowledge on biotype distribution is extremely important for the success of resistance gene deployment. To characterize soybean aphid populations from various geographical locations, there is a need to perform regular and systematic sampling to monitor the soybean aphid populations from various geographical locations. Aphids that are collected from the field can be tested and characterized under laboratory conditions to investigate levels of virulence. There are two possible ways to detect for the presence of novel biotypes. First, collected populations can be exposed to a set of plant differentials containing known major genes for resistance, and their response can be compared with the known biotypes. Second, PCR based strategy using biotype-specific markers can be helpful (see [85] for detection of *Orseolia oryzae* biotypes). Recent work has focused on expanding the molecular resources for the soybean aphid [86-88] but to date, no biotype diagnostic marker for field populations exist. Both of these methods will provide data to develop biotype distribution maps. This could be extremely important for resistant gene deployment in that growers may avoid planting a resistant variety if it is unlikely to control soybean aphid populations. A geographic based approach will also help to avoid growing of a particular resistance cultivar over a wide area, which has hastened the development of virulent biotypes in insects such as in case of early wheat cultivars and Hessian fly biotype adaptation [40]. However, more research is needed in understanding soybean aphid migration and how virulent biotypes may spread. While the overwintering host of buckthorn is restricted to more northern latitudes (above 40°N, [15]), dispersal of winged aphids across much of the US soybean growing region occurs late in the

growing season [89]. Not only does this spread clonal and genetic diversity immediately prior to sexual reproduction, it may also allow virulent biotypes to rapidly move across the environment.

2. Gene pyramiding:

This involves the release of a variety containing more than one major resistance gene. Pyramided varieties are likely to have extended durability as survival and subsequent multiplication of virulent individuals appearing in a susceptible pest population are highly reduced because of multiple resistance genes. Another advantage is that the pyramided varieties may yield more compared to single gene varieties due to higher reduction in pest population. Cultivars with both *Rag1* and *Rag2* had less aphid numbers and less yield reduction than soybeans with only one resistant gene [90].

3. Variety mixture:

Using seed mixtures of resistant and susceptible plants may extend durability of soybean aphid-HPR; this system would be analogous to the refuge requirements for transgenic insect resistance [91-93]. In most cases, 80-95% of a field would be resistant, with 5-20% of the plants being susceptible to provide a population of insects unexposed to the resistance genes. For maize, modeling predicts that using refuge plants extends durability, particularly if resistant plants have multiple genes (*i.e.* pyramids) [94]. However, for any HPR strategy that involves insect resistance management (IRM), several questions remain regarding soybean aphid biology that differs for models developed for corn pests. For example, mating, and therefore the transfer of genetic variation for virulence, is dependent on the overwintering host buckthorn, which, as stated previously, is not randomly distributed across the environment. In most corn pests, mating occurs in the field and therefore may allow a more random assortment of virulent (*i.e.* resistant) and avirulent individuals. Additionally, the inheritance of resistance is still unknown. Most importantly, soybean aphids asexually reproduce in the presence of the HPR selection pressure. In these cases of asexual reproduction, resistance can be delayed when 1) refuges are large, 2) resistance genotypes are low in frequency, 3) resistant individuals are less fit than susceptible individuals on refuge plants (i.e. fitness among biotypes, fitness costs), and 4) resistant individuals are less fit on resistant plants than susceptible plants (fitness of virulent biotypes on different plants, i.e. incomplete resistance) [95]. Fitness costs can be due to both physiological mechanisms and direct competition among susceptible and resistant individuals on the same plant. Few studies have investigated the impact of differing reproductive strategies such as parthenogenesis, but in the most basic sense, the genotype (virulent or avirulent) with the highest fitness has the highest reproductive output and becomes the most common [95]. Through simulation modeling, Crowder and Carrière [95] determined that the key parameters for delaying the evolution of resistance in parthenogenetic organisms were the presence of fitness costs and incomplete resistance. Fitness costs had the least effect, but "…incomplete resistance delayed resistance evolution more than fitness costs, and in some cases, resistance was prevented with incomplete resistance and fitness costs." [95]. While recessive resistance can delay resistance, "…such delays [in resistance] are not possible in haplodiploid or parthenogenetic pests without additional factors such as fitness costs and

incomplete resistance." [95]. These parameters have not been experimentally estimated for soybean aphid in an IRM framework, but must be understood to develop and evaluate appropriate IRM strategies for soybean aphids

3.6. Integration of soybean HPR with other IPM tactics

IPM is loosely defined as the integration of multiple tactics to control insect pests [96]. These tactics include chemical, biological and cultural (agronomic), and are decided based on economic, environmental and societal impacts [96]. Much has changed in the past 15 years regarding IPM in soybean [97]. In the past, less than <1% of soybean acreage in the Midwestern USA was treated with insecticides [5], and insect pests of interest centered on defoliators such as bean leaf beetle (*Cerotoma trifurcata* (Forster)), and Mexican bean beetle (*Epilachna varivestis* Mulsant), as well as various Lepidopteran and Gastropodan (*e.g.* slugs) pests [6, 98]. Apart from various native stink bugs such as the brown and green stink bugs, very little attention was given to Hemipteran pests of soybean. Within the past 10 years, focus of insect pests of soybean has shifted toward the invasive Hemipterans such as the soybean aphid and BMSB. In fact, Ragsdale et al. [4] report that in less than ten years, insecticide use in soybean has increased 130-fold, in large part due to soybean aphid infestations. As these invasive Hemipterans expand their distribution, soybean researchers and producers will look to implement various methods of control.

3.6.1. Chemical control

The use of chemical insecticides remains the most widely used option for control of Hemipteran soybean pests because of mainly 2 reasons. First, most exotic pests invade new environments lacking natural enemies, resistant plants, or even basic biological information; often research is published in different languages and can be difficult or time-consuming to translate. Additionally, as invasive pests adapt to their new environment, previous data from native environments may lose relevancy. Secondly, most commercial insecticides are widely available and broad-spectrum which can act quickly and effectively to control economic populations. Therefore, in most cases, insecticides are the only short-term options.

There are several insecticide classes that have proven effective against Hemipteran soybean pests [5, 99-101]. While the wide availability of effective insecticides provides soybean producers with choices, responsible use of these chemicals requires timely applications based on field scouting and economic analysis. Economic injury levels (EIL, the pest population at which plant injury occurs) and economic or action thresholds (ET, the pest population at which treatment is recommended to prevent plant injury) have been estimated for a few Hemipteran soybean pests such as the soybean aphid, brown and green stink bug [5,32, 102]. Although general recommendations based on field observations are known for the BMSB and kudzu bug, more research is necessary to determine the EIL and the ET. Finally, the use of neonicotinoid seed treatments appears to be increasing, however these chemicals are only active for 30-40 days. These seed treatments will control early season soybean aphids, they do not prevent late season aphid infestations [5]. Likewise, most stink bug feeding occurs in the reproductive stages when the activity of the seed treatment has decreased dramatically.

3.6.2. Cultural control

There are very few options for adapting crop production methods for controlling Hemipteran soybean pests. One of the most common practices is to alter planting dates. The heaviest damage from Hemipteran feeding occurs late in the season—by planting early, most of the yield potential has been already made. In practice, planting dates are often at the mercy of ideal weather conditions rather than based on managing insect pressure. Additionally, early emerged fields may act as trap crops for other soybean insect pests such as bean leaf beetle. Studies on variable planting dates with the soybean aphid have been unclear [5] but likely vary across geography based on the reliance of its overwintering host buckthorn (*Rhamnus* spp.). The presence of buckthorn has been shown to be the key predictor of aphid infestation through ecological modeling and was supported by population genetic evidence [89,103]. Virtually no research has been performed with the BMSB and kudzu bug on soybean planting date, but [104] reported that planting date impacted the presence of native stink bugs.

Cultural control also involves manipulation of the environment. As most Hemipteran pests migrate into soybean fields during the season, controlling these source populations could limit pest damage. For example, research has been directed on the impact of buckthorn on aphid movement and dispersal [103], including a citizen science project to map the distribution of buckthorn and detect the presence of aphids [105[. Similarly, the kudzu bug is also known to feed on kudzu before moving to soybean [27]. The impact of removing these host plants in preventing pest outbreaks is unknown; however, removing buckthorn and kudzu may be beneficial regardless due to their devastating impacts on ecosystems [106]. For BMSB, early observations from soybean suggest most damage is restricted to field edges, particularly along edges close to forest patches. Control may be achieved by either restricting spraying to these edges, or keeping the most susceptible soybean varieties away from edges.

3.6.3. Biological control

The importance of natural enemies for Hemipteran soybean control has been comprehensively documented in several studies, although to date, most research has focused on the soybean aphid (see [4] for a review of biological control for soybean aphid). Natural enemies of Hemipteran pests include parasitoids, predators and diseases [4, 15, 107-109]. General predators such as lady beetles, insidious flower bug and ground beetles are probably the most important natural enemies [4, 108] as most of invasive Hemipteran pests lack specific natural enemies that provide control in native regions [4, 110]. The indigenous parasitoids for most of the invasive Hemipteran soybean pests are either poorly adapted or are just beginning to attack these new hosts [4, 27]. Foreign exploration for natural enemies has resulted in candidates for classical biological control, with at least one species, *Binodoxys communis*, undergoing field evaluations for persistent control of soybean aphid. Preliminary exploration for parasitoids of BMSB has revealed several candidates of Scelionid wasps (*Trissolcus* and *Telenomus*) [111], and at least 1 egg parasitoid for kudzu bug [112]. While the role of natural enemies has extensively been researched for soybean aphid, more research is needed for their role in controlling other invasive Hemipterans such as BMSB and kudzu bug.

3.6.4. Integrating HPR for hemipteran pest control

Host-plant resistance offers many benefits to soybean producers in controlling insect pests, but also must fit within production practices. HPR varieties need to be in the proper maturity group, high-yielding, and without any increased susceptibility to other pests and diseases. For example, an interaction among soybean aphids, soybean cyst nematode, and brown stem rot was noticed [113]. Indeed, as other invasive Hemipterans spread into new areas, HPR with resistance to multiple pests would be desirable.

Similar to the rise of insecticide resistance, insects have also shown adaptation potential to overcome HPR. If HPR is to be a successful component in IPM of Hemipteran pests, more research is needed to develop strategies that preserve the utility of these traits. For example, the durability of HPR could be predicted through ecological modeling, similar to research for transgenic maize [91-94]. In addition to modeling, more basic biological research is needed for all Hemipteran pests including migration and gene flow estimates, virulent biotype frequencies, and competitive interactions between biotypes. Results from this research would also help to estimate the accuracy of modeling and improve any strategy for managing insect virulence and preserving HPR traits. Early research [90] with soybean aphid suggests that stacking resistance genes (e.g. *Rag1/Rag2* stack) offers better and more sustainable protection from soybean aphid than single gene resistance. In addition, more research needs to be studied in terms of how using HPR may alter efficiency of natural enemies [114-117]). The cues to which aphid or stink bug parasitoids use to locate prey are unknown, and might be either from the prey or plant host. Any breeding for host-plant resistance should also be careful not to disrupt volatile signaling [117]. Plant nutrients and resistance influence growth rate and size of herbivores which, in turn, could influence natural enemy biology [45]. Indeed, the parasitoid *Binodoxys communis* had lower fitness when attacking aphids on *Rag1* plants compared to aphids on susceptible plants [117].

In the US and Canada, soybean HPR for soybean aphid have been available since 2010, and are often combined with insecticidal seed treatments. Combining seed treatments with HPR may allow a greater opportunity for natural enemies to maintain populations below the economic threshold and therefore prevent a chemical application. However, the full benefit of seed treatments may not be fulfilled where soybean aphid infestations mainly occur late in the season, and may unnecessarily increase selection pressure for resistance.

4. Future directions in breeding for Hemipteran resistance in soybean

While much of current research has focused on traditional and classical breeding and screening methods for host-plant resistance, research is emerging which incorporates newer genomic and molecular technology. Likely, a combined approach will be necessary to improve the durability and decrease development time of Hemiptera resistant soybean. Here we list several considerations for the future of HPR in soybean:

1. Tolerant cultivars must be emphasized in breeding programs. As mentioned earlier, tolerant cultivars have the ability to withstand or recover from damage caused by insect

populations equal to those on susceptible cultivars. Thus, unlike antixenosis and antibiosis, tolerance comprises of plant features which are not involved in plant-insect interaction. Though breeding for tolerant cultivars is more difficult and time consuming (as the crop has to be grown till maturity in the infested condition) tolerance based resistant cultivars nonetheless offer two major advantages. 1) New virulent biotypes are less likely to emerge in a cropping system based on tolerant cultivars. While feeding upon tolerant plants, infesting insect populations are not reduced as they are on plants exhibiting antibiosis and antixenosis. As a result, there is no selection pressure and frequency of novel virulence trait remains lower. This is directly in contrast to what is observed in insects feeding on plants showing antixenosis and antibiosis, as various physical and chemical factors in these plants allows for selection of virulent individuals. Thus, the chances for development of biotypes that can overcome resistance genes are significantly reduced through the use of tolerant cultivars. Russian wheat aphid populations were not able to overcome tolerant plants but can break antibiosis based resistance in wheat [46]. 2) Tolerant cultivars are highly compatible with biological control measures, thus can be combined in IPM program. The allelochemical based toxins in plants exhibiting antibiosis and antixenosis could be detrimental for natural enemies of insect pests [45]. On the other hand, tolerance based cultivars do not have any known adverse impact on beneficial insects. In North-America, biological control employing natural enemies makes up an important component for IPM of soybean aphid. Thus, tolerant cultivars provide an excellent opportunity for integrating HPR and biological control in IPM.

2. Marker assisted breeding will facilitate faster and more efficient development of resistant cultivars. Markers will also be useful for pyramiding major genes as well as quantitative trait loci (QTL) for multigeneic defense against the insect as found in tolerant soybeans. Development of closely linked markers for known resistant genes in soybean will enhance the selection efficiency.

3. Exploration for new sources of HPR: In South America especially Brazil, extensive research on HPR against stink bugs has been conducted. But in US especially, the southern states, lepidopteran foliage feeders have been the focus of HPR research [68]. Further, due to the preference for insecticide based control, soybean germplasm has not been extensively explored for resistance against stink bugs. No cultivar of soybean showing resistance against stink bugs has been released so far in US. There is a need to identify native sources of resistance against stink bugs. Though the selection for resistance against stink bugs is relatively time consuming and labor intensive, novel sources that offer wider pool of traits such as pest resistance, yield, etc. should be incorporated into breeding programs.

    Traditionally, lepidopteran foliage feeders and 3 species of stink bugs (*N. viridula*, *A. hilare*, *E servus*) were major insect pests of soybean in southern us. However, as mentioned earlier, recent expansions of red-banded stink bug in southern US, brown-marmorated stink bugs in eastern, central and southern US, and soybean aphid in north central us have

significantly changed the pest scenario in these regions. Thus, for effective HPR based IPM program, there is a need to identify novel germplasm sources that are resistant against more than one insect. Further, the known resistant sources against a single pest can be explored for their response to other insect pests e.g. in breeding programs for pest management in north central US, soybean aphid resistant (*Rag* containing soybean) PIs can be screened for response against brown marmorated stink bug. Previously, several soybean PIs have shown resistance against multiple insect pests e.g. PI171444 which originally identified for resistance against stink bug complex also showed resistance against bean leaf beetle and banded cucumber beetle [44,118]. Thus, there is strong potential for soybean PIs having multiple pest resistance and to be incorporated into the breeding programs.

4. Bt soybean potential against Hemipterans: The development of transgenic (e.g. Bt) resistance against the Hemipteran insects has not succeeded so far. Pyramiding of Bt genes with HPR genes may be an useful strategy. In Lepidoteran insects, the highest level of resistance to Lepidopteran insects obtained through MAS using the native soybean genes was 70% reduction in feeding [119]. However, when the soybean insect resistance loci were pyramided with a *cry*1Ac transgene from *Bacillus thuringiensis* (Bt) the level of feeding damage was reduced to 90% compared to susceptible checks [119]. Such native crop gene and transgene pyramids may be useful in several aspects of insect resistance. First, the Cry protein from a single Bt transgene may only protect the host plant from one or at best two classes of insects. For instance, the Cry1Ac toxin provides resistance against many Lepidopteran pests, but not to Coleopteran pests. A combination of native insect resistance gene with resistance to beetle (e.g, insect resistance loci on chromosome 7) with the Bt transgene could broaden resistance of plants to include Coleopteran pests that are insensitive to Cry1Ac toxins. Second, several insect pests have demonstrated the ability to develop resistance to Cry toxins, so effective strategies are needed to manage resistance to Bt [120]. Some populations of the diamondback moth [*Plutella xylostella* (L.)] have developed resistance to Bt toxins in different parts of the world where Bt are routinely used on cruciferous crops [121]. Soybean lines carrying the PI 229358 allele at the insect resistance locus on chromosome 7 in addition to a Cry1Ac transgene were more protected against defoliation by corn earworm and soybean looper than related transgenic lines lacking the PI 229358 allele [119]. Studies to investigate weight gain of tobacco budworm larvae from Cry1Ac-resistant and Cry1Ac-sensitive strains demonstrated that larvae fed on leaves of plants with both a Cry1Ac transgene and the native insect resistance allele on chromosome 7 gained weight more slowly than larvae fed on leaves from transgenic plants lacking the native resistance allele [119].

5. RNAi and other genomic approaches: Given issues with ineffectiveness of Bt on Hemipterans, RNA interference (RNAi)- mediated control presents an attractive avenue for management of these pests. RNAi results in sequence specific knockdown of gene expression at the post-transcriptional level as introduced dsRNA causes the degradation of identical mRNAs [122]. Crops based on RNAi-mediated pest protection are expected

to achieve the same level of success as Bt-based transgenic crops [123]. Though there are various categories of insect genes that could be silenced through RNAi to achieve the desired results, targeting of genes encoding for effector proteins in salivary glands of Hemipteran insects has been promising. At the start of feeding, Hemipteran insects inject the saliva produced by salivary glands into plant tissues. Hemipteran saliva contains various chemical substances such as digestive enzymes that facilitate feeding. Importantly, the saliva also contains the effector proteins that are determinants of virulence for these insects. RNAi knockdown of *coo2*, which is an effector protein of pea aphid secreted into the fava beans leaves during feeding, significantly reduced the survival of this insect [124,125]. In addition to pea aphid, successful RNAi studies in Hemipterans insects like peach aphid (*Myzus persicae*), Brown plant hopper (*Nilaparvata lugens*) have also been reported [126-129]. To develop soybean employing RNAi-based management of Hemipteran pests, there is a need to generate significant amount of molecular resources for these insects. To date, a whole genome sequence is only known for 1 Hemipteran insect, the pea aphid, *Acyrthosiphon pisum* [130]. Besides RNAi, there are other novel approaches such as the transgenic plant resistance against Hemipteran pests. The management of Hemipteran pests by use of transgenic plants expressing lectins and protease inhibitors has been recently reviewed [131], thus not discussed in this chapter.

5. Conclusions

Although there has been some success with HPR for Hemipteran pests, for example the glandular hairs for potato leafhopper, there are many opportunities for expanding this important pest management tool. Research has already resulted in the commerical availability of HPR against the soybean aphid, with many more varieties to come. However, HPR research for the other major Hemipteran pests of soybean continues to lag behind. More molecular and genomic techniques increase the feasibility of finding HPR loci and improve the ability to combine both traditional HPR approaches and newer RNAi methodologies. This includes not only developing resistance to multiple insect pests, but potentially other pathogens that they may interact with to impact soybean [113]. However, these new varieties will need to be studies and balanced in terms of the other aspects of integrated pest management (i.e. chemical and biological control) to both limit non-target impacts and extend durability in the face of insect adaptation.

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