CHARACTERIZING SOYBEAN RUST RESISTANCE WITHIN POPULATIONS OF *GLYCINE TOMENTELLA* AND THE INHERITANCE AND CHARACTERIZATION OF SOYBEAN APHID RESISTANCE IN PI 587663, PI 587677, PI 587685, AND PI 594592

BY

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THESIS

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Abstract

Soybean rust, caused by *Phakopsora pachyrhizi* Syd., is known to cause significant damage to soybean yields in many production areas worldwide. Most soybean cultivars are susceptible to the fungus and even though sources of resistance have been discovered within the USDA soybean germplasm bank, isolates of soybean rust can overcome that resistance, so other sources of rust resistance are needed to combat rust. The wild perennial *Glycine* species may contain unique rust resistance genes and many of the accessions evaluated to date were reported to have resistance to many diseases including rust. Although the wild perennial *Glycine* species may represent promising sources of rust resistance, producing hybrids between soybean and the perennial accessions has been problematic. Among the wild perennials, *Glycine tomentella* germplasm has been reported to contain rust resistance and has been the only perennial to successfully hybridize with soybeans to produce fertile offspring. In this study, four *G. tomentella* rust resistant germplasm accessions (PI 441008, PI 483218, PI 509501, and PI 583970) were crossed, two being reciprocal crosses (PI 441008 and PI 583970), with one rust susceptible accession (PI 441011) to generate four F_2 populations that were evaluated for rust resistance. A $F_{2:3}$ population was generated from the PI 441011 x PI 441008 for further evaluation. F_2 and $F_{2:3}$ individuals and parents were inoculated with *P. pachyrhizi* under controlled greenhouse conditions. Resistance was evaluated using a qualitative scale based upon lesion color and sporulation of uredinia. Segregation analysis of F_2 and $F_{2:3}$ populations suggested that the inheritance of resistance fit models of a single dominant or two dominant genes. The rust resistance genes may be distinctive and uniquely different from those found in soybean.

Soybean aphids (*Aphis glycines* Matsumura) are a significant soybean [*Glycine max* (L.) Merr.] pest and pose a constant threat to the production of soybeans in the Midwest. Native throughout eastern Asia, soybean aphids first spread to Australia and then into North America where it has been found in 21 U.S. states and three Canadian provinces. Screening of the USDA germplasm collection has yielded several sources of aphid resistance. However, the identification of soybean aphid biotype 3 that colonizes plants with the *Rag1* and *Rag2* resistance genes has made the interaction between the aphid biotypes and resistance genes in soybeans more complex. Thus the continual assessment for aphid resistance in the USDA germplasm remains vital. Preliminary testing of 3000 accessions resulted in identifying new germplasm accessions PI 587663, PI 587677, PI 587685, and PI 594592, which demonstrate strong resistance to soybean aphid biotypes 1 to 3. The objectives of this study were to determine the inheritance of resistance, characterize the expression of resistance, and compare resistance of the four accessions with other sources against the three biotypes. F_2 populations developed from crosses between the accessions and three susceptible genotypes (LD02-5320, LD03-6566, and LD03-10504) were tested for resistance. F_2 plants from the crosses, when tested for resistance to biotype 3, fit a 3:1 ratio (single dominant genetic model). Segregation among $F_{2:3}$ families from the crosses supported the single dominant resistance gene hypothesis. The four accessions, when compared to other known sources of aphid resistance, showed a strong resistance to biotypes 1 and 2 while also displaying a resistance to biotype 3.

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Chapter 1

Characterizing soybean rust resistance within populations of *Glycine tomentella*

Abstract

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suggested that the inheritance of resistance fit models of a single dominant or two dominant genes. The rust resistance genes may be distinctive and uniquely different from those found in soybean.

1.1 Introduction

Leaf rust of soybean [*Glycine max* (L.) Merr.] is caused by the fungal pathogen, *Phakopsora pachyrhizi* Syd., which infects soybean leaves and often causes leaf chlorosis and premature defoliation (Sinclair and Hartman, 1999). The disease was first observed in Japan in 1902 (Ono et al., 1992) and has since been reported in other countries (Miles et al., 2003). South America suffered its first outbreak in 2001 (Yorinori et al., 2005) and the disease was first seen in the USA in Hawaii on cultivated soybeans in 1994 (Killgore and Heu, 1994). *P. pachyrhizi* was confirmed in the continental USA in 2004 when it was observed in Louisiana (Schneider et al., 2005) and has since occurred throughout the southeast region every year since 2004 and has occurred to some extent in the northern production area late in the season (USDA, 2012).

Reported yield losses caused by soybean rust include up to 80% in experimental plots in Taiwan (Hartman et al., 1991), and up to 55% in Africa, South America and the USA compared with control plots (Miles et al., 2007; Mueller et al., 2009). Yield losses from soybean rust are caused by a reduction in canopy green leaf area through the formation of rust lesions, and premature defoliation resulting in less dry matter accumulation and reduced harvest index (seed mass/plant mass) (Kumidini et al., 2008).

Soybean reactions to rust were independently described as three major infection types (Bromfield and Hartwig, 1980; Mclean and Byth, 1981). Two types of resistance reactions were described (Bromfield and Hartwig, 1980) as Type 0 (lacking macroscopically visible symptoms) or called an “immune” reaction and Type RB, a low infection type characterized by the

development of reddish brown (RB) lesions with two or fewer uredinia per lesion. Susceptible plants that contain two or more uredinia per lesion and profuse sporulation were described as a "TAN" infection type. It was later proposed that three subcategories of RB lesions (infection types 1 to 3) and two subcategories of TAN lesions (infection types 4 to 5) defined by the number of uredinia per lesion and sporulation intensity be added to the respective infection types (Bromfield, 1984). Identification of rust resistant soybean germplasm and studies on the inheritance of resistance has used the three main infection types (Hartman et al., 2011).

The five loci controlling resistance to specific isolates of *P. pachyrhizi* were recently reviewed (Hartman et al., 2011) and five single dominant genes (*Rpp1-Rpp5*) have been named. More recently *Rpp6* was described from PI 567102B (Li et al., 2012). The effectiveness of some of these resistance genes varied depending on the *P. pachyrhizi* isolate (Miles et al., 2011). For example, *Rpp1* has been referred to as the immune resistance gene because its expression provides a resistance that prevents any symptoms from developing from specific soybean rust isolates including Australia 79-1, India 73-1, Hawaii 94-1, and Hawaii 98-1 (Bromfield et al., 1980; Pham et al., 2009; Miles et al., 2011). The susceptibility of these genes to varying rust isolates would suggest that the durability of the single gene resistance might be short lived. The lack of broad-spectrum resistance and the problems with durability among the known resistance genes has made it increasingly important to discover new sources of resistance. Over 16,000 soybean accessions in the USDA Germplasm Collection were evaluated for resistance in order to find potentially new sources of resistance that may be more durable than the known genes (Miles, 2006). While new sources of resistance have been sought within the soybean germplasm, perennial *Glycine* species and other legumes also have been evaluated for soybean rust resistance although not nearly characterized to the extent of soybean.

The fungus, *P. pachyrhizi*, infects many legumes including 156 different species of Papilionodieae (Ono et al., 1992; Slaminko et al., 2008) which includes kudzu (Harmon et al., 2005), Florida beggarweed (Sconyers et al., 2006), dry bean, lima bean, and scarlet bean (Lynch et al., 2006). The 26 wild perennial *Glycine* species (Chung and Singh, 2008) are still of interest because many have been evaluated for soybean rust resistance (Burdon and Marshall, 1981; Hartman et al., 1992) and their potential to hybridize with soybean. Evaluation of 294 accessions from 12 perennial species of *Glycine* showed that 23% were resistant toward lesion formation and/or sporulation and that 18% were moderately resistant (Hartman et al., 1992). These accessions could have a broad spectrum of resistance to soybean rust, although they have not been challenged to all known isolates so their durability has not specifically been tested. In Australia, evaluation of 189 accessions from six native *Glycine* species found that 13%, 15%, 32%, and 33% of *G. canescens*, *G. clandestina*, *G. tabacina*, and *G. tomentella*, respectively, were resistant and that 22%, 13%, 100%, 100%, 22%, and 27% of *G. canescens*, *G. clandestina*, *G. falcata*, *G. latrobeana*, *G. tabacina*, and *G. tomentella*, respectively, were moderately resistant (Burdon and Marshall, 1981).

The inheritance of resistance to soybean rust in the perennial *Glycine* species was reported from research completed in Australia over 20 years ago. No other inheritance studies have been reported since. One report from Australia showed that six accessions of *G. canescens* had single, dominant rust resistance genes while another accession had two independent genes at more than four loci (Burdon, 1988). It was also reported that *G. argyrea* had one single dominant rust resistance gene (Jarosz and Burdon, 1990). In *G. tomentella*, one accession was reported to have a single dominant resistance gene and another accession to have two dominant resistance genes (Schoen et al., 1992). The specific isolates tested for these inheritance studies

may not duplicated since the isolates are unavailable. Some of the accessions of *G. argyrea* and *G. tomentella* were challenged with multiple isolates of *P. pachyrhizi* and were reported to have the same resistant phenotype indicating that these may have broad spectrum resistance to different isolates (Jarosz and Burdon, 1990; Schoen et al., 1992).

The limitation in using the perennial *Glycine* species as sources of resistance to *P. pachyrhizi* for improving soybeans with rust resistance has been the lack of successful interspecific hybridizations between *G. max* and the wild perennial species. Although hybridization has been reported through the use of embryo and ovule cultures to rescue the crosses including *G. tabacina*, *G. canescens*, *G. clandestina*, and *G. tomentella* (Hymowitz et al., 1998; Newell and Hymowitz 1982; Newell et al. 1987; Singh et al. 1987; Singh et al., 1998; Singh and Hymowitz 1987; Shoemaker et al. 1990), the progeny have been largely sterile due to inefficient hybridization efforts or genomic incompatibility (Hymowitz et al., 1998). Continued attempts have improved the process leading to more successful crosses as well as being able to generate non-sterile progeny. However, these successes have mainly come from crosses of *G. max* x *G. tomentella* (Chung and Singh, 2008; Singh et al., 1993; Singh et al., 1990). To date, there are no commercial soybean cultivars grown that have any genes from hybridized perennial *Glycine* species.

G. tomentella is native to Australia and can also be found in New Guinea, the Philippines, and Taiwan (Chung and Singh, 2008). These plants can generally survive in warm and dry/humid climates. Work has been accomplished hybridizing *G. tomentella* PI 483218 with *G. max* cv. Altona producing viable seed (Singh et al., 1990). The interspecific hybrids with enough backcrossing to another *G. max* cultivar have led to the development of fertile lines retaining the ploidy level and appearance of the *G. max* parent (Singh et al., 1993). However in

some cases this has led to the loss of resistance during chromosome reduction and stabilization while backcrossing (Patzoldt et al., 2007). Despite the difficulties in transferring resistance to *G. max*, the use of *G. tomentella* as a source of resistance still remains promising. For example, *G. tomentella* PI 483218 has displayed resistance with a similar expression to that of *Rpp1* (Patzoldt et al., 2007). Continued evaluations of current *G. tomentella* lines could lead to the identification of other sources of soybean rust resistance that could be used in *G. max* cultivars. In an earlier report, *G. tomentella* PI 441011 (2n=78) showed susceptibility to soybean rust where greater than 20% of the foliage was covered in lesions with well-developed pustules and heavy sporulation (Hartman et al., 1992), while PI 441008 (2n=78) demonstrated resistance to soybean rust where few rust lesions developed on the leaves (Hartman et al., 1992). These lines were successfully crossed several years ago as they have the same chromosome number (Hymowitz, pers. comm.).

The objectives of the research were to: (1) determine the inheritance of resistance in the four accessions of *G. tomentella* and develop genetic models to explain the inheritance patterns, and (2) evaluate the F_2 progeny of the PI 441011 x PI 441008 cross of *G. tomentella* to provide a confirmation evaluation of the inheritance and characterization of resistance to the F_2 results through a $F_{2:3}$ population of the same cross.

1.2 Materials & Methods

Plant materials. Four *G. tomentella* resistance accessions, PI 441008, PI 483218, PI 509501, and PI 583970, were crossed to PI 441011, a known susceptible *G. tomentella* accession. PI 441008 and PI 583970 were reciprocal crosses with PI 441011 making a total of six crosses. All crosses generated hybrids (F_1) that were selfed to produce six F_2 populations. One F_2 population, PI 441008 x PI 441011, was selfed and seed ($F_{2:3}$) was generated for progeny tests.

***P. pachyrhizi* isolate culture**. The *P. pachyrhizi* isolate FL07-1 (collected in Gadsden County, FL in 2007) was maintained on the first trifoliate stage of detached leaves of soybean cv. Williams 82 on water agar supplemented with 6-benzylaminopurine (BAP, Sigma, St. Louis, MO) in 10 cm diameter petri dishes following a procedure by Twizeyimana et al. (2007). Rust spores were also maintained on whole soybean plants (inoculated at the first trifoliolate) of the cv. Williams 82.

Inheritance of soybean rust resistance. Approximately 100 F_2 seeds per population and three seeds per parent were scarified individually using razor blades (Fisherbrand Razor Blades, Thermo Fisher Scientific Inc., Waltham, MA) to scarify the seed coat. Scarified seeds were placed in 10 cm diameter petri dishes containing moist filter paper for germination. After 3 days each seedling was transplanted into a 15 cm diameter plastic pots containing soilless potting medium (Sunshine Mix LC1, Sun Gro Horticulture Inc., Bellevue, WA) and placed into a BSL-2 containment greenhouse. Plants were fertilized with slow release pellets (Osmocote 19-6-12, Scott Miracle Co., Marysville, OH). All seedlings were grown in the greenhouse under supplemental lighting using sodium halide lights set at 12 h daylight with 800 µmol $m^{-2}s^{-1}$ light intensity at 28^{o}C day/25^{o}C night temperature regime.

For inoculating plants, the soybean isolate FL07-1, purified isolate from Gadsden County, FL in 2007, was increased using a procedure previously described by Twizeyimana et al. (2010). Briefly, detached leaflets of the soybean cultivar Williams 82 were individually sprayed with a FL07-1 urediniospore suspension using an airbrush (Paashe Airbrush Co., Lindenhurst, IL) and a small compressor (Badger Co., Franklin Park, IL) at 138 kPa. Leaflets were placed in an environment chamber (Percival Scientific, Perry, IA) at 22 ^{o}C in the dark for a 12 hour and then at a 14 h day and 10 h night photoperiod regime with 102 µmol $m^{-2}s^{-1}$ light intensity for 3 weeks.

Afterwards urediniospores were collected from infected leaflets with a custom-made mini-cyclone spore collector and a vacuum pump (Barnant Co., Barrington, IL).

Collected spores were suspended in 2 mL of 0.01% Tween-20 (Sigma, St. Louis, MO) solution, vortexed for 30 seconds, counted using a hemocytometer, and diluted to 50,000 spores per 1 mL. Plant inoculations included 160-day-old F_2 plants, their parents, and six 60-day-old plants of the cv. Williams 82 were inoculated with the spore suspension using an airbrush (Delta, Jackson, TN). Ten µL droplets of the same spore suspension were placed on water agar plates and incubated at 22^{o}C for 12 hours in a tissue chamber to determine spore germination. Spore germination was evaluated under a dissecting microscope (Nikon, Melville, NY) at x50 magnification. Plants were then placed in a mist chamber in a BSL-2 containment greenhouse room with a relative humidity of 90% 30 min after inoculation. Infected plants were then removed 12 hours later and rated 18 days after inoculation (DAI).

For rust evaluations, the rating scale used was adapted from a combination of evaluation scales from past studies on soybean rust resistance on soybean and wild perennial *Glycine* species (Burdon and Marshall, 1981; Miles et al., 2006). A 1 to 5 scale was used: 1 = no lesions, 2 = reddish-brown (RB) lesions with no uredinia, 3 = RB lesions with sporulating uredinia (to a small degree), 4 = RB lesions fully sporulating (at the same amount as TAN lesions), and 5 = TAN lesions with full sporulation. A dissecting microscope (Nikon, Melville, NY) at 50x magnification was used to evaluate sporulation for RB and TAN lesions.

However, due to difficulties with seed germination, plant vigor, and seed production none of the F_2 populations progressed to progeny testing. In order to produce a F_3, seed amounts for the four populations were reassessed. The PI 441011 x PI 441008 reciprocal cross was the only population to have enough seed to start over at the F_2. Three hundred F_2 seeds (150 seeds from

PI 441008 x PI 441011 and 150 seeds from PI 441011 x PI 441008) along with five seeds of each parent, all scarified in the same manner as previously described, were planted in 3.8 cm diameter by 21 cm depth plastic cones (Ray Leach low density white cone-tainers, Hummert Intl.). Each cone was filled with soilless potting medium (Sunshine Mix LC1) and seeds were planted at a planting depth of 0.6 cm. The plastic cones were labeled to identify each seed individually and establish the pedigree. Each cone was fertilized with slow release pellets (Osomocote 19-6-12, Scott Miracle Co., Marysville, OH) and then spaced evenly among 61 cm by 30.5 cm, 98 cell trays (Ray Leach single cell cone-tainer system, Hummert Intl.) with each tray containing fifty-six plants. All seedlings were grown in a BSL-2 containment greenhouse room at the temperature and daylight regime mentioned previously.

The inheritance of resistance test was repeated to affirm the previous results. Younger, healthy, and fully expanded trifoliolate leaves were collected from each individual plant of the new F_2 population and placed within a clear plastic clam box on top of moistened paper towel. Each clam box contained ten leaf samples and labeled to identify from which plant the sample was taken from. There were 261 plant samples from the F_2 population and five samples from each parent that were inoculated using the soybean rust isolate FL07-1. Samples were checked periodically for maintenance and rust symptoms. Eighteen days after the second inoculation, the samples were removed, inspected for lesion formation and sporulation, and evaluated.

For rust evaluations; lesion color, the number of lesions formed, and the number of sporulating uredinia were counted and evaluated under a microscope. Lesions with a large number of uredinia sporulating (greater than or equal to fifteen spores) were counted as a susceptible sample. Lesions with a small number of uredinia (less than fifteen spores) with little

to no sporulation and samples that displayed no lesion formation were counted as resistant. Figure 1.1 was used as the basis in the rust resistance assessment for the F_2 tests.

Progeny testing. All F_2 plants, of the PI 441011 x PI 441008 reciprocal cross from inheritance of resistance testing, were allowed to self-pollinate to generate 256 $F_{2:3}$ lines. Seed generated from each individual plant was designated a specific $F_{2:3}$ identifier associated with each F_2 plant (or family) and identifiers with at least eight seeds produced were scarified and used for progeny testing. The experiment was designed as a completely randomized design with two replications of each F_2 family, four replications of the resistant parent, and six replications of the susceptible parent divided among each section. There were four plants per replication. Plants were grown in a BSL-2 containment greenhouse room at 28^{o}C day/25^{o}C night temperature schedule, in cones placed inside 98 cell trays (Ray Leach single cell cone-tainer system, Hummert Intl.) and fertilized with slow release pellets (Scott Miracle Gro Co., Marysville, OH) as previously mentioned. Plants were grown for 14 to 21 days (around the second and third trifoliolate) before being inoculated.

Inoculum was increased in the same manner as previously described. Infected leaves with urediniospores sporulating from lesions were taken from increased inoculum and suspended in 25 ml of 0.01% Tween-20 and mixed for approximately 10 to 20 seconds. Whole plants were inoculated manually using a hand-held spray bottle (Do It Best Corp., Fort Wayne, IN). After the initial inoculation, the samples were inoculated again the following day using the same method. After each inoculation the samples were placed in the dark in a controlled environmental chamber (Percival Scientific, Perry, IA) at 22^{o}C for a 12 hour dark photoperiod and then at a 14 hour day and 10 hour night photoperiod regime. Plants were removed from the chamber at 12 hours after inoculation and rated 16 DAI. Evaluations were based upon visual

assessment of leaf response to inoculation and divided into three sections; immune response (no lesion formation), RB lesions (taking notes to differentiate between non-sporulating and sporulating RB lesions), and TAN lesions. Figure 1.1 was also used as the basis in the rust resistance assessment for the $F_{2:3}$ tests.

Statistical analyses. Analysis was done by Chi Square (χ^2) testing two genetic models. For inheritance of resistance testing (detached leaves), since rust evaluations were done based upon a 1 to 5 disease scale, ratings were consolidated into two groupings for the first analysis with ratings 4 to 5 classified as susceptible. The second analysis grouped ratings 1 to 4 as resistant with a rating of 5 being susceptible. For the new F_2 population (repeat of the PI 441011 x PI 441008 reciprocal), partially resistant (lesions without sporulation or a small amount of sporulation) and resistant samples (no lesions) were classified as resistant while lesions with a large number of sporulating uredinia were classified as susceptible. For progeny testing of the $F_{2:3}$ evaluations (whole plants), immune and RB (regardless of sporulation) ratings were classified as resistant while TAN ratings were classified as susceptible.

1.3 Results

Inheritance of soybean rust resistance. The germination rate of soybean rust isolate FL07-1 was 95% (averaged over ten samples). Assessment of the susceptible checks and resistant germplasm accessions showed that Williams 82 and PI 441011 (susceptible checks) developed TAN lesions and were given a disease rating of 5. PI 441008, PI 509501, and PI 583970 all had a disease rating of 1 while PI 483218 had a disease rating of 3.

Rust resistance phenotyping of the F_2 progeny (Table 1.1) showed that the F_2 progeny derived from parents PI 441008 and PI 483218 displayed mainly immune (disease rating 1) type

of resistance (61 out of 117 and 54 out of 102 respectively) to rust with some plants showing susceptibility. F_2 progeny derived from PI 509501 and PI 583970, on the other hand, displayed split resistance reactions where the majority of plants either showed an immune or RB (disease rating 2) type of resistance (30/24 out of 74 and 30/23 out of 69, respectively) with some plants exhibiting susceptibility. Results of the phenotyping data were used to test the single gene dominant (3:1) and two gene dominant (15:1) genetic inheritance models (Table 1.2). Chi-square calculations suggest that the F_2 progeny from all crosses fit either a 3:1 or 15:1 ratio. This indicated that the soybean rust resistance in each *G. tomentella* germplasm accession is controlled by either a single dominant gene or two dominant genes.

Progeny testing. Results from the detached leaf assay of the PI 441008 x PI 441011 F_2 progeny (Table 1.2) indicated that soybean rust resistance was controlled by two dominant genes. Analysis of the $F_{2:3}$ family segregation (Table 1.3) also suggested that the soybean rust resistance in PI 441008 was controlled by two dominant genes. Tests of other genetic models (12:3:1 and 3:1) were also conducted for both the F_2 and $F_{2:3}$ generations but failed to fit those models. 24 of the 256 $F_{2:3}$ families that were tested were misevaluated for resistance in the F_2 generation based upon the test results. This gave the study a F_2 phenotyping error rate of 9.38% for the PI 441008 x PI 441011 reciprocal cross.

1.4 Discussion

In this study, it was shown that all the rust resistant accessions of *G. tomentella* tested contained one or possible two single dominant genes for resistance to *P. pachyrhizi*. None of these accessions had previously been genetically analyzed for this trait, but the results are similar to a previous report that showed two *G. tomentella* accessions had a single dominant resistance

gene (G 1408 and G 1468) and another had two dominant resistance genes (G 1188) (Schoen et al., 1992). Complementation studies have not been completed to determine if each of these genes discovered in *G. tomentella* accessions are unique or not. The accessions used in this study and those studied by Schoen et al. (1992) originated from nearby areas in Australia. G 1188 (PI 441005) and G 1408 (PI number not known) were collected in north Queensland, G 1468 (PI 563876) was collected in south Queensland (Schoen et al., 1992), PI 441008 and PI 509501 were collected in New South Wales, and PI 483218 and PI 583970 were collected in Queensland (USDA-ARS, 2012). Schoen et al. (1992) reported that the trend for susceptibility in the studied accessions was on the western portion of their ranges. However whether the origins of the accessions have any influence on the genes found is not known. In addition, future studies will need to determine the relationship between the *G. max* rust resistance loci and the *G. tomentella* resistance genes. Since various NBS-LRR regions with rust resistance have already been identified within the soybean genome, this may be one approach to determine if these genes occur in *G. tomentella*. If an association between the two could be found, it could expedite the discovery of resistance genes.

The assessment methods used to evaluate soybeans and perennial *Glycine* species for studying the genetics of resistance has been by reaction types. Previous publications have also used lesion color to evaluate and distinguish resistant and susceptible phenotypes with sporulation differentiating like colored lesions (Jarosz and Burdon, 1990; Schoen et al., 1992). PI 441008 appeared to have soybean rust resistance controlled by two dominant genes. Even though initial tests of the F_2 pointed to a single dominant gene controlling resistance, further tests of the F_2 and $F_{2:3}$ suggested that results derived from these tests failed to fit a single dominant gene model. The segregation patterns of the $F_{2:3}$ families would also indicate a two gene model

over a single gene model. The discrepancy between the earlier test and the later test could have been due to the phenotyping error seen in the F_2. Twenty families that were considered susceptible in the $F_{2:3}$ were classified as resistant in the F_2, this distribution of families could have easily contributed to the differing results. The 9.38% F_2 phenotyping error was a little high, this could have been attributed to using a detached leaf assay then switching to a whole plant assay. Especially considering that *G. tomentella* leaves are very small, symptom development may have been better on whole plants than on detached leaves. Another possibility was that during F_2 testing PI 441008 x PI 441011 (for the initial test and the repeat), there were many seeds that failed to germinate or prematurely died. Eighty-three out of 200 seeds and 49 out of 300 seeds, for both tests, failed to make it through F_2 testing. In addition, only one population was advanced to the $F_{2:3}$ due to difficulties with seed production. Only the PI 441008 x PI 441011 cross generated enough seed to be advanced to the next generation (F_3). All these possibilities during F_2 testing could have contributed to the differing results in genetic models. A test of heterogeneity was also conducted afterwards for both tests (Table 1.2), but only the PI 441008 x PI 441011 F_2 repeat for the 3:1 ratio showed considerable heterogeneity with 91%, while the others did not (0%).

At any rate, rust resistance displayed by particular accessions of *G. tomentella* does show a great deal of promise. But there still remains a possibility, that the identified resistant accessions could possess race-specific resistance and could be overcome by isolates from other geographic regions. In a previous study, portions of *G tomentella* accessions evaluated were resistant to some races of rust, while susceptible to others (Schoen et al., 1992). Eventually this perennial, as well as other *Glycine* perennials, may offer additional soybean rust resistance genes

that could be packaged with the other *Rpp* genes to potentially provide a more durable and broader type of resistance.

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1.6 Tables and Figures

Table 1.1. Distribution of the F_2 progeny, derived from crossing soybean rust-resistant *Glycine tomentella* PI accessions, in five phenotype classes.

	Rust evaluation				
Cross	1	2	3	4	5
PI 441008 x PI 441011* PI 441011 x PI 441008	61	24	13	13	6
PI 483218 x PI 441011	54	21	8	15	4
PI 441011 x PI 509501	30	24	12	5	3
PI 441011 x PI 583970* PI 583970 x PI 441011	30	23	6	6	4

*Reciprocal cross

Table 1.2. Analyses of the segregation of F_2 families derived from crosses between soybean rust resistant and susceptible *Glycine tomentella* PI accessions.*

Cross**	Plant Phenotype	No. of observed plants*	$\chi^2_{3:1}$	$P_{3:1}$	Hetero-geneity	No. of observed plants*	$\chi^2_{15:1}$	$P_{15:1}$	Hetero-geneity
441008	Resistant	85				111			
	Susceptible	32				6			
			0.35	0.56	0.00		0.25	0.62	0.00
441008***	Resistant	211				238			
	Susceptible	40				13			
			10.99	0.001	90.90		0.49	0.48	0.00
483218	Resistant	75				98			
	Susceptible	27				4			
			0.12	0.73	0.00		0.94	0.33	0.00
509501	Resistant	54				71			
	Susceptible	20				3			
			0.16	0.68	0.00		0.61	0.44	0.00
583970	Resistant	53				65			
	Susceptible	16				4			
			0.12	0.71	0.00		0.03	0.88	0.00

*3:1 genetic model was based on 1-2 ratings as resistant & 3-5 ratings as susceptible. 15:1 genetic model was based on 1-4 ratings a resistant & 5 rating as susceptible

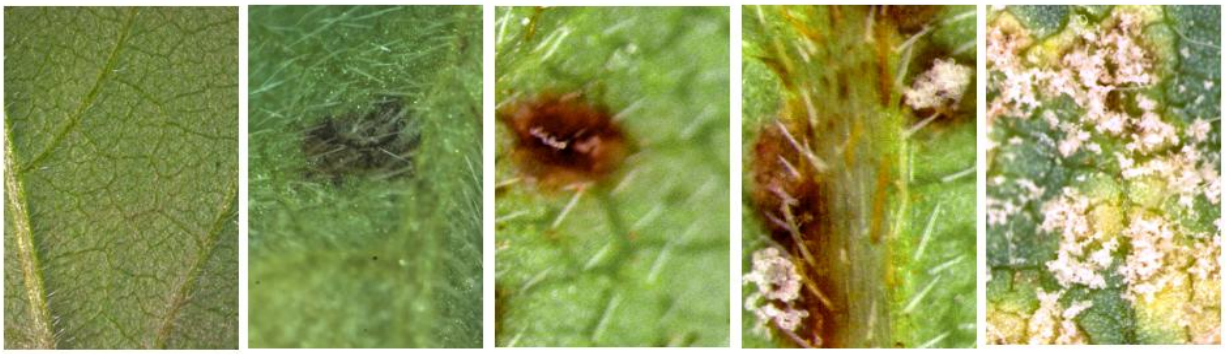
***G. tomentella* accessions were crossed with *G. tomentella* PI 441011

***Repeat of the PI 441008 x PI 441011 F_2 family

Table 1.3. Genetic analysis of the segregation of the PI 441008 $F_{2:3}$ family for rust resistance.

Cross	F_2 Plant Phenotype	$F_{2:3}$ Plant Genotype	No. of $F_{2:3}$ Families	$\chi^2_{55:9}$	*P*
PI 441011 x PI 441008	Resistant	R (all $F_{2:3}$ plants resistant)	83		
		H (resistant and susceptible $F_{2:3}$ plants)	140		
		r (all $F_{2:3}$ plants susceptible)	20		
	Susceptible	R (all $F_{2:3}$ plants resistant)	3		
		H (resistant and susceptible $F_{2:3}$ plants)	1		
		r (all $F_{2:3}$ plants susceptible)	9		
				1.584	0.208

Figure 1.1. Soybean rust rating scale used to assess resistance of *G. tomentella* progeny.*



1	2	3	4	5
Immune	RB lesion w/o uredinia	RB lesion w/ low sporulation	RB lesion w/ sporulation	TAN lesion w/ sporulation

*Rating scale of 1 to 5 was used to determine the inheritance of resistance

Chapter 2

Inheritance and characterization of soybean aphid resistance in PI 587663, PI 587677, PI 587685, and PI 594592

Abstract

Soybean aphids (*Aphis glycines* Matsumura) are a significant soybean [*Glycine max* (L.) Merr.] pest and pose a constant threat to the production of soybeans in the Midwest. Native throughout eastern Asia, soybean aphids first spread to Australia and then into North America where it has been found in most soybean producing areas in the United States and Canada. Screening of the USDA germplasm collection has yielded several sources of aphid resistance. However, the identification of soybean aphid biotype 3 that colonizes plants with the *Rag1* and *Rag2* resistance genes has made the interaction between the aphid biotypes and resistance genes in soybeans more complex. Thus the continual assessment for aphid resistance in the USDA germplasm remains vital. Preliminary testing of 3000 accessions resulted in identifying new germplasm accessions PI 587663, PI 587677, PI 587685, and PI 594592, which demonstrate strong resistance to soybean aphid biotypes 1 to 3. The objectives of this study were to determine the inheritance of resistance, characterize the expression of resistance, and compare resistance of the four accessions with other sources against the three biotypes. F_2 populations developed from crosses between the accessions and three susceptible genotypes (LD02-5320, LD03-6566, and LD03-10504) were tested for resistance. F_2 plants from the crosses, when tested for resistance to biotype 3, fit a 3:1 ratio (single dominant genetic model). Segregation among $F_{2:3}$ families from the crosses supported the single dominant resistance gene hypothesis.

The four accessions, when compared to other known sources of aphid resistance, showed a strong resistance to biotypes 1 and 2 while also displaying a resistance to biotype 3.

2.1 Introduction

Aphis glycines Matsumura, the soybean aphid, is a native throughout eastern Asia including northern China, Indonesia, Japan, Korea, Malaysia, and Philippines (Ragsdale, 2004). The soybean aphid was only known in Asia until 1999-2000 when it was reported in Australia (Venette and Ragsdale, 2004). The insect also spread into North America in 2000 (Hartman et al., 2001a), and since was reported in 21 U.S. states and three Canadian provinces (Venette, 2004). Currently, the soybean aphid continues to infest the Midwest region in the U.S. and poses a constant threat to the production of soybeans [*Glycine max* (L.) Merr.]. Soybean aphids damage a soybean plant by leaching photosynthates from the host. Plant injury resulting from excessive aphid feeding includes stunting, yellowing, leaf distortion, and reduced pod set (Sun et al., 1990). Aphids can also further damage their soybean hosts by transmitting certain plant viruses during feeding which includes; bean yellow mosaic, alfalfa mosaic, and soybean mosaic (Clark and Perry, 2002; Hartman et al., 2001b; Wang et al., 2006). Additionally, honeydew produced during aphid feeding promotes the development of black sooty mold fungus, which inhibits plant photosynthesis (Hartman et al., 2001b).

The agronomic and economic impact of aphid damage can be considerable, and aphid numbers can quickly exceed the economic injury level of 700 aphids per plant within six days (Catangui et al., 2009). Unchecked aphid populations have led to significant damage, and it has been estimated to cause losses of $3.6 to $4.9 billion annually in North America (Kim et al., 2008a). Minnesota reported approximately 1.6 million hectares of damaged soybeans resulting

in $80 million in estimated losses in 2003 (North Central Soybean Research Program, 2004), and Illinois had approximately 0.5 million hectares of damaged soybeans resulting in $45 million in estimated losses (Steffey, 2004).

Plant resistance to insects provides a sustainable option toward pest control for soybean producers. Screening for resistance to the soybean aphid began soon after the aphid migrated from Asia. Several sources of resistance were identified in the USDA germplasm collection. To date, inheritance and linkage map locations of six resistance genes, called *Rag* genes, named for resistance to *A. glycines*, have been determined (Hill et al., 2006a; Hill et al., 2006b; Hill et al., 2009; Jun et al., 2012; Li et al., 2007; Mian et al., 2008; Zhang et al., 2009; Zhang et al., 2010). Current knowledge on soybean resistance to soybean aphids has been extensively reviewed (Hill, et al., 2012).

Since the identification and characterization of the first known soybean aphid resistance gene in soybean, *Rag1*, was completed, the existence of a biotype that can colonize plants with *Rag1* was discovered (Kim et al., 2008b). Another biotype was later discovered that could overcome the *Rag2* resistance gene (Hill et al., 2010). Three biotypes have now been documented that are distinguished by differential virulence on *Rag1* and *Rag2*. Based on these discoveries, it is likely additional biotypes exist. The interaction of the three biotypes with soybean aphid resistance genes has been reviewed (Hill et al., 2012).

Preliminary testing of PI accessions, PI 587663, PI 587677, PI 587685, and PI 594592, in a collection of 3000 accessions, assembled by R. Nelson, USDA Soybean Germplasm Collection Curator, that represented maximum soybean genetic diversity, appeared to have resistance against soybean aphid biotypes 1, 2, and 3 (unpublished results). The objectives of this research were to: (1) determine the inheritance of resistance to the soybean aphid in each of the four

multi-biotype-resistant PI accessions, and (2) to characterize the expression of resistance and compare the resistance of the four accessions with other sources of resistance against the three biotypes.

2.2 Materials and Methods

Aphid culture. Soybean aphid isolates used in this study included biotype 1, an Illinois isolate originally collected in 2000 (Kim et al., 2008b); biotype 2, an Ohio isolate with the ability to infest plants with *Rag1* collected in 2005 (Kim et al., 2008b); and biotype 3, an isolate originally labeled as SF-55 collected from *F. alnus* at Springfield Fen, IN in 2007 (Hill et al., 2010). Methods to rear and maintain the aphids were previously described (Hill et al., 2004; Hill et al., 2010; Kim et al., 2008b). The biotypes were maintained in separate growth chambers and on different soybean genotypes to prevent contamination between the aphids. Growth chambers were kept at 22 to 25^{o}C. The soybean cultivar Williams 82 was used to maintain biotype 1. Biotype 2 was maintained on soybean breeding line LD05-16611 with *Rag1*. Biotype 3 was maintained on soybean breeding line LD08-12597a with *Rag2*. Each aphid biotype was periodically parthogenically propogated from isolated nymphs and maintained in separate growth chambers.

Plant culture. Plants used in greenhouse experiments were planted in soilless potting medium (Sunshine Mix, LC1, Sun Gro Horticulture Inc., Bellevue, WA) in plastic multi-pot inserts (Hummert Intl., Earth City, MO) with each pot insert size being 30 x 40 x 60 mm, contained inside plastic trays with holes (Hummert Intl.). The number of multi-pot inserts used depended on the experimental design and number of test entries. The inserts were filled with the soilless potting medium and then moistened to field capacity. Two seeds of each entry were

placed in a shallow indent constructed by using a finger to press into the potting medium. Slow release pellets were then spread evenly on top of the soilless growth medium to approximately a density of 2 to 3 pellets per cm^2. Seedlings were thinned to one plant per pot after emergence.

Resistance inheritance determination. Known soybean aphid resistance and susceptible genotypes were selected to use as checks in the phenotyping tests to determine the inheritance of resistance in the four PI accessions and in tests to compare resistance among resistance sources (Table 2.1). Populations were generated, from crosses made between the aphid-resistant soybean germplasm accessions and aphid-susceptible soybean breeding lines, and obtained from Brian Diers, University of Illinois: LD02-5320 x PI 587663, LD03-6566 x PI 587677, LD03-10504 x PI 587685, and LD02-5320 x PI 594592. Soybean germplasm accessions were the male parents in all crosses. PI 587663, PI 587677, PI 587685, and PI 594592 belong to maturity group VII germplasm accessions originating from China (USDA-ARS National Genetic Resources Program, 2010). LD02-5320, LD03-6566, and LD03-10504 were soybean breeding lines susceptible to biotypes 1, 2, and 3. F_1, F_2, and $F_{2:3}$ development and seed production from crosses were planted, maintained, and harvested as previously described (Hill et al., 2009).

F_2 progeny testing was conducted to determine the soybean aphid resistance genotypes of each F_2 plant using choice tests. F_2 plants, parents, and resistant and susceptible checks were planted in four-pot rows, with each plant counted as an experimental unit, that were randomized throughout all flats in each test outlined in a previous publication (Hill et al., 2010). The genotypes and number of plants used in F_2 progeny testing (and $F_{2:3}$ tests) are listed in Table 2.2. After completion of F_2 tests, plants were transplanted to produce $F_{2:3}$ seed for progeny testing and genotyping, using previously described methods (Hill et al., 2006a, Hill et al., 2006b). $F_{2:3}$ lines that had at least 12-16 seeds, regardless of F_2 aphid resistance phenotype, were used in

progeny tests. At least 12, with a maximum of 16, F_3 seeds were planted. Test entries from each of the $F_{2:3}$ lines were replicated three or four times, with four seeds per replication, and randomized with susceptible checks throughout the test with four replications of each resistant check in a similar fashion to the experimental design previously mentioned (Hill et al., 2009). Choice tests (Figure 2.1) were infested by biotype 3 aphids and conducted in the greenhouse under environmental conditions and methods previously described (Hill et al., 2009; Hill et al., 2010). These tests were conducted in an air conditioned, insecticide free greenhouse at 18-24^{o}C with a 16 hour photoperiod. Leaves from infested plants were spread evenly and placed on top of seedlings between the VE and VC stages (Fehr and Caviness, 1977). There were an undetermined number of aphids of all stages on each infested leaf used to transfer aphids to the plants. Eventually the aphids, where aphid movement was unrestricted, would migrate onto preferred hosts and develop colonies.

Resistance was visually assessed by aphid colonization at 10 and 21 days after infestation for all choice tests. A 1 to 4 non-parametric nominal rating scale was used to approximate aphid colonization and plant damage caused by aphid infestation. 1 = few solitary live aphids, 2 = viviparous aptera surrounded by few nymphs, 3 = multiple dense aphid colonies, and 4 = dense aphid colonies with plant damage (Hill et al., 2006a; Hill et al., 2006b; Hill et al., 2009). The scale was also applied to choice tests while characterizing resistance expression.

Resistance expression characterization. For non-choice tests, plants grown in greenhouse experiments were planted in 125 x 87.5 mm plastic azalea pots (Hummert Intl.). Upon planting, 102 mm diameter heavy wall, clear plastic tubes of variable height (ClearTec Packaging) were used to isolate the plants. Planting, maintenance, and thinning methods for both choice and non-choice tests were the same as previously mentioned in the plant culture sub-

section (Hill et al., 2009; Hill et al., 2010). Non-choice tests (Figure 2.2) were designed to compare antibiosis-type resistance performance between multiple soybean genotypes. A randomized complete block design was used with sixteen different soybean genotypes tested, which are specified in Table 2.1, and each soybean genotype replicated three times. Once plants were between the VC and V1 growth stages (Fehr et al., 1971), 10 soybean aphids between the second and third instar stages were infested as previously described (Hill et al., 2010). Aphid populations on each plant were counted at 7-day intervals with the final count at 14 days after infestation. Biotype 3 non-choice tests were conducted in a plant growth chamber (Conviron PGR15) with 500 µE $m^{-2}s^{-1}$ PAR irradiation set to a 14 hour photoperiod. Biotype 1 and 2 non-choice tests were conducted under greenhouse environmental conditions as previously reported (Hill et al., 2009; Hill et al., 2010).

For the choice test, plants were infested and conducted under environmental conditions and methods as previously described (Hill et al., 2009; Hill et al., 2010). The experiment was designed to compare the overall antixenosis and antibiosis-type resistance performance of multiple soybean genotypes. A randomized complete block design was used with 24 different soybean genotypes tested and there were four replications of each genotype. Genotypes used are specified in Table 2.1.

Statistical analyses. All statistical analyses were performed with SAS software version 9.2 (SAS Institute, Cary, NC). Least squares analysis of the non-choice aphid population numbers was performed after the original data was transformed by adding 1 to the count and then taking the $\log_{10}$ of the total to correct for the heterogeneity of variance among the soybean genotype treatments. Mean separation was done by calculating the LSD at a significance of 0.05 upon confirmation of significant differences among the soybean genotypes in the analysis of

variance. Chi-square analyses were performed for the inheritance of resistance experiments based upon classes determined by the non-parametric nominal scale. The F_2 population was separated into two classes: resistant (phenotype ratings of 1 or 2) and susceptible (phenotype ratings of 3 or 4) (Hill et al., 2009). Segregation among $F_{2:3}$ families were analyzed after classification of each family with at least 11 plants into homozygous resistant (all plants with ratings of 1 and/or 2), homozygous susceptible (all plants with ratings of 3 and/or 4), or segregating (plants with a mixture of 1 to 4 ratings) (Hill et al., 2009).

2.3 Results

Inheritance of aphid resistance. R:S segregation ratios were 200:77, 147:55, 99:25, and 226:60 for the PI 587663, PI 587677, PI 587685, and PI 594592 F_2 populations, respectively (Table 2.3). Analysis of these ratios indicated that aphid resistance to biotype 3 was controlled by single, dominant genes with *P*-values of 0.28, 0.46, 0.21, and 0.12, respectively (Table2.3). Analysis of the $F_{2:3}$ family segregation (Table 2.3) supported this conclusion in all four PI accessions. Ninety-two out of the 409 $F_{2:3}$ families that were tested were misevaluated for resistance in the F_2 generation based upon the progeny test results. This gave the study a F_2 phenotyping error rate of 33%, 13%, 27%, and 3% for the crosses of LD02-5320 x PI 587663, LD03-6566 x PI 587677, LD03-10504 x PI 587685, and LD02-5320 x PI 594592, respectively (with a 22% overall F_2 phenotyping error rate).

Resistance expression characterization. Results of the choice tests showed that there were differences in the distribution of plants falling into each of the four aphid colonization rating classes among the test genotypes, indicating level of colonization of each of the biotypes on different genotypes (Table 2.4). Most plants of PI 587663, PI 587677, and PI 587685 had

relatively low aphid colonization by each biotype. Colonization of biotype 1 and 2 was relatively low on PI 594592, but biotype 3 colonization appeared to be higher on this PI accession than the other three accessions.

PIs 567541B and 567543C (*rag1c* with *rag4* and *Rag3* respectively), did display a moderate resistance to biotype 3. Colonies were able to form, but the density wasn't as great compared to susceptible checks. Both accessions did display resistance to biotype 1. However 567541B demonstrated susceptibility to biotype 2 while 567543C was only moderately resistant. Biotype 3 colonization on the accessions with primarily antixenosis-type resistance, PIs 567301B, 567597C, and 71506, varied greatly. All three accessions had low biotype 1 colonization. Biotype 2 colonization on PIs 567597C and 71506 was higher than on PI 567301B. PIs 567597C and 71506 had moderate levels of biotype 3 colonization, whereas biotype 3 colonization on PI 567301B was low compared to the other genotypes with antixenosis. It was observed that at 10 days after infestation, PIs 567597C and 71506 had low colonization, but by 21 days, biotype 2 colonization had increased noticeably.

There were significant differences ($P<0.05$) in aphid colonization among the soybean genotypes when infested by all three biotypes in the non-choice tests (Table 2.5). PI 587663, PI 587677, PI 587685, and PI 594592 had significantly lower colonization of each biotype than susceptible Williams 82. Numbers of biotype 3 aphids on PI 594592 was not significantly different than LD05-16611 with *Rag1* 14 days after infestation (DAI). PIs 587663, 587677, and 587685 had significantly lower numbers of aphids than LD05-16611 and LD08-12597a with *Rag2* 14 DAI. Numbers of biotype 3 aphids on PIs 587663, 587677, 587685, and 594592 were significantly lower than on PI 567541B with *rag1c* plus *rag4* and PI 567301B with *rag5*, but were not significantly different than the numbers on PI 567543C with *Rag3* and PI 567598B.

Differences also could not be seen with PIs 71506 and 567597C (antixenosis resistance) with the four accessions of interest. Number of aphids on PI 567592 was significantly higher than on PIs 587663, 587685, and 587677, but not significantly different than PI 594592. PI 587972 had significantly higher numbers of aphids than PIs 587663 and 587677, but not PIs 587685 and 594592. PI 437696 had significantly lower numbers of aphids than PIs 587685 and 594592 but not PIs 587663 and 587677.

Of the three accessions with antixenosis-type resistance, PIs 567597C and 71506 had significantly lower numbers of biotype 3 aphids than Williams 82 and LD08-12597a 14 DAI. However PI 71506 was not significantly different than LD05-16611 while PI 567597C had significantly lower numbers. Numbers of biotype 3 aphids on PI 567301B were significantly lower than on Williams 82, but were not significantly different than numbers on LD08-12597a. PI 567597C had significantly lower aphid colonization than PI 567301B while PI 71506 was not significantly different than either one. PIs 567598B and 567543C had significantly lower numbers of biotype 3 aphid colonization than Williams 82, LD05-16611, and LD08-12597a. PI 567541B was not significantly different from LD08-12597a, and PIs 567592 and 587972 were not significantly different than the *Rag1* check, but did fare better than the susceptible and *Rag2* checks. PI 437696 had significantly lower numbers of biotype 3 aphids than the majority of soybean genotypes, but was not significantly different than PIs 587663, 587677, and 567598B.

Numbers of biotype 2 aphids in the non-choice test on PIs; 587663, 587677, 587685, 594592, 437696, 567543C, 567597C, 587972, and 567598B were not significantly different from numbers on LD08-12597a with *Rag2*. PIs 71506, 567592, and 567301B represent a handful of accessions that minimized aphid colonization to be considered significantly different

than the *Rag1* and susceptible checks, but high enough numbers to be considerably different than rest of the resistant PIs and the *Rag2* check.

Numbers of biotype 1 aphids on Williams 82 were significantly higher than on all of the genotypes tested. The remaining genotypes formed two distinct groups. The first group had no significant differences between PIs 567301B, 567541B, 567592, 71506, 567543C, 567598B, and 567597C. This group had a significantly larger number of biotype 1 aphids than LD05-16611 and LD08-12597a. The second group had differences that were mainly non-significant between PIs 587663, 587677, 587685, 594592, 437696, and 587972. This group was generally not significantly different than LD08-12597a, but had significantly lower aphid colonization than LD05-16611. PI 594592 was the lone exception as there was no difference with LD05-16611.

2.4 Discussion

Results of this study clearly indicated that resistance in PI 587663, PI 587677, PI 587685, and PI 594592 was controlled by single, dominant aphid resistance genes. Each gene appeared to be a new gene different than *Rag1* and *Rag2* because they provided resistance to biotype 3, whereas *Rag1* and *Rag2* do not. However, PI 594592 showed susceptibility to biotype 3 in the resistance characterization test as approximately half of the plants were densely colonized. This was not the case as the accession, in choice (F_2 and $F_{2:3}$ phenotyping tests) and non-choice tests, had aphid colonization no different than the other resistant accessions. Surprisingly, when looking at the choice and non-choice tests, PI 594592 did not demonstrate a resistance to biotype 3 better than that conferred by *Rag1*. This would suggest that the resistance gene found within the accession would most likely be *Rag1*, but biotype 2 non-choice tests would indicate otherwise since this PI displayed resistance on par with the *Rag2* check. At any rate, PI 594592

could be characterized as moderately resistant to biotype 3 aphids. This conclusion and taking into account the experimental design between choice tests could explain the discrepancy. Progeny tests were designed with a very large number of susceptible check replications compared to resistance characterization choice tests. Given more opportunity and considering aphid preference, colonization of a moderately resistant genotype would be lower in progeny tests versus resistance characterization tests.

These types of incongruities highlighted the troubles in making consistent qualitative assessments. The root of the problem could be seen in the high overall F_2 phenotyping error rate of 22%. This was most likely caused by the difficulty in learning the scoring system where initial assessments were inconsistent, but became more consistent over time as rater error was being corrected.

Resistance expression of PIs 587663, 587677, 587685, and 594592 was not only distinguishable from *Rag1* and *Rag2*, but also from *rag1c*, *rag4*, and *Rag3*. Therefore the resistance in these sources will provide a different spectrum of resistance against soybean aphid populations.

Furthermore, when examining the other accessions, PIs 567541B and 567543C showed different results to the biotype 3 isolate than what was previous reported. It was reported that both accessions had aphid numbers not significantly different than Williams 82 (Hill et al., 2010). However current results show that both accessions were not as susceptible to biotype 3 as Williams 82 and in this case PI 567543C expressed resistance on par with the four resistant accessions evaluated. Whether this was due to contaminated seed or a mutation occurring within the biotype 3 isolate used is unknown. However further tests conducted with PI 567541B and PI

567543C to biotype 3 have matched prior results (unpublished) and prior tests with PI 567597C, PI 567598B, and PI 71506 (Hill et al., 2010) have generally matched the current results.

Results of the expression of aphid resistance to biotypes 1 and 2 in PI 71506, PI 567543C, PI 567301B, PI 567597C, and PI 567541B were consistent with previous reports (Kim et al., 2008b; Mian et al., 2008; Van Nurden et al., 2010; Zhang et al., 2010).

The sources of resistance and genes reported in this study will be useful to soybean breeders developing new soybean aphid-resistant soybean cultivars. New multi-biotype resistant genes can be stacked with other aphid resistance genes to potentially provide a broader spectrum of aphid resistance and reduce the likelihood of aphid populations adapting to discovered aphid resistance genes.

2.5 References

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2.6 Tables and Figures

Table 2.1. Soybean genotypes tested in experiments to determine the inheritance and characterize the expression of resistance in four multi-biotype-resistance PI accessions.

Soybean genotype	Type*	Aphid resistance gene	Experiment**
Dowling	Germplasm accession	*Rag1*	Inheritance
Dwight	Public cultivar		Inheritance/Characterization(choice test)
Ina	Public cultivar		Inheritance/Characterization(choice test)
LD02-5320	Breeding line		Inheritance/Characterization(choice test)
LD03-10504	Breeding line		Inheritance/Characterization(choice test)
LD03-6566	Breeding line		Inheritance/Characterization(choice test)
LD05-16611	Breeding line	*Rag1*	Inheritance/Characterization(choice test & non-choice test)
LD05-16675	Breeding line	*Rag1*	Inheritance/Characterization(choice test)
LD08-12422a	Breeding line	*Rag2*	Inheritance/Characterization(choice test)
LD08-12597a	Breeding line	*Rag2*	Inheritance/Characterization(choice test & non-choice test)
Loda	Public cultivar		Inheritance/Characterization(choice test)
Pana	Public cultivar		Inheritance/Characterization(choice test)
PI 200538	Germplasm accession	*Rag2*	Inheritance
PI 437696	Germplasm accession		Characterization (choice test & non-choice test)
PI 567301B	Germplasm accession	*Rag5*	Inheritance/Characterization(choice test & non-choice test)
PI 567541B	Germplasm accession	*rag1c, rag4*	Inheritance/Characterization(choice test & non-choice test)
PI 567543C	Germplasm accession	*Rag3*	Characterization (choice test & non-choice test)
PI 567592	Germplasm accession		Characterization (choice test & non-choice test)
PI 567597C	Germplasm accession		Characterization (choice test & non-choice test)
PI 567598B	Germplasm accession	*Putative Rag1/Rag3*	Inheritance/Characterization(non-choice test)
PI 587663	Germplasm accession		Inheritance/Characterization(choice test & non-choice test)
PI 587677	Germplasm accession		Inheritance/Characterization(choice test & non-choice test)
PI 587685	Germplasm accession		Inheritance/Characterization(choice test & non-choice test)
PI 587972	Germplasm accession		Characterization (choice test & non-choice test)
PI 594592	Germplasm accession		Inheritance/Characterization(choice test & non-choice test)
PI 71506	Germplasm accession		Inheritance/Characterization(choice test & non-choice test)
Williams 82	Public cultivar		Inheritance/Characterization(choice test & non-choice test)

*Germplasm accessions obtained from the USDA Soybean Germplasm Collection, Urbana, IL; Breeding line from B.W. Diers, University of Illinois

**Genotypes used in; Inheritance – Resistance Inheritance Determination or Characterization - Resistance Expression Characterization (choice and/or non-choice test) experiments

Table 2.2. Soybean genotypes evaluated in F_2 and $F_{2:3}$ progeny tests.

		F_2 Progeny Test*/***				$F_{2:3}$ Progeny Test**/***			
Soybean genotype	Type	1	2	3	4	1	2	3	4
LD02-5320 x PI 587663	F_2 plants		300				200		
LD03-6566 x PI 587677	F_2 plants			320				148	
LD03-10504 x PI 587685	F_2 plants	152				26			
LD02-5320 x PI 594592	F_2 plants				308				36
PI 587663	Resistant parent		32				16		
PI 587677	Resistant parent			40				16	
PI 587685	Resistant parent	20				16			
PI 594592	Resistant parent				40				16
LD02-5320	Susceptible parent		32		60	16			28
LD03-10504	Susceptible parent	20							
LD03-6566	Susceptible parent			60				16	
Dowling	*Rag1* check	32	32	40	40			16	16
LD05-16611	*Rag1* check	32	32				80	16	16
LD05-16675	*Rag1* check							16	16
LD08-12422a	*Rag2* check							16	16
LD08-12597a	*Rag2* check							16	16
PI 200538	*Rag2* check	32	24	60	60		80		
PI 567301B	*Rag5*							16	16
PI 567541B	*Rag1c, rag4*							16	16
PI 567598B	*Putative Rag1*/*Rag3*							16	16
PI 437696	Antibiosis	32		40	40				
PI 71506	Antixenosis							16	16
Dwight	Susceptible check							480	144
Ina	Susceptible check		24					480	144
Loda	Susceptible check	32						480	144
Pana	Susceptible check		24					480	144
Williams 82	Susceptible check	32	28	160	172	360	944	480	148

*Total number of plants tested (four plants per row)

**Total number of plants tested (four plants per replication with three to four replications per family derived from each F_2 plant)

***Four separate progeny tests (one to four) were conducted with different soybean genotypes allotted to each test

Table 2.3. Genetic analyses of the segregation of $F_{2:3}$ families for soybean aphid resistance tested against Biotype 3.

Cross	F_2 plant phenotype	No. of F_2 plants	$\chi^2_{1:2:1}$	*P*	$F_{2:3}$ Plant Genotype	No. of $F_{2:3}$ Families	$\chi^2_{1:2:1}$	*P*
LD02-5320 x PI 587663	Resistant	200			RR (all $F_{2:3}$ plants resistant)	37		
					Rr (resistant and susceptible $F_{2:3}$ plants)	82		
					rr (all $F_{2:3}$ plants susceptible)	29		
	Susceptible	77			RR (all $F_{2:3}$ plants resistant)	10		
					Rr (resistant and susceptible $F_{2:3}$ plants)	26		
					rr (all $F_{2:3}$ plants susceptible)	16		
			1.15	0.28			1.32	0.52
LD03-6566 x PI 587677	Resistant	147			RR (all $F_{2:3}$ plants resistant)	33		
					Rr (resistant and susceptible $F_{2:3}$ plants)	71		
					rr (all $F_{2:3}$ plants susceptible)	12		
	Susceptible	55			RR (all $F_{2:3}$ plants resistant)	0		
					Rr (resistant and susceptible $F_{2:3}$ plants)	7		
					rr (all $F_{2:3}$ plants susceptible)	25		
			0.53	0.46			0.65	0.72
LD03-10504 x PI 587685	Resistant	99			RR (all $F_{2:3}$ plants resistant)	8		
					Rr (resistant and susceptible $F_{2:3}$ plants)	9		
					rr (all $F_{2:3}$ plants susceptible)	5		
	Susceptible	25			RR (all $F_{2:3}$ plants resistant)	0		
					Rr (resistant and susceptible $F_{2:3}$ plants)	2		
					rr (all $F_{2:3}$ plants susceptible)	2		
			1.55	0.21			0.69	0.71
LD02-5320 x PI 594592	Resistant	226			RR (all $F_{2:3}$ plants resistant)	7		
					Rr (resistant and susceptible $F_{2:3}$ plants)	23		
					rr (all $F_{2:3}$ plants susceptible)	1		
	Susceptible	60			RR (all $F_{2:3}$ plants resistant)	0		
					Rr (resistant and susceptible $F_{2:3}$ plants)	0		
					rr (all $F_{2:3}$ plants susceptible)	4		
			2.47	0.12			4.22	0.12

Table 2.4. Number of plants in four phenotypic classes of soybean aphid colonization on 15 soybean genotypes infested with three soybean aphid biotypes in a choice test at 21 days after infestation.

		Soybean aphid biotype								
		1			2			3		
Soybean genotype	Aphid resistance	n	μ	SE	n	μ	SE	n	μ	SE
LD05-16611	*Rag1*	16	2	0	16	2.88	0.13	26	2.54	0.1
LD08-12597a	*Rag2*	16	2.06	0.06	14	2.14	0.14	27	2.93	0.05
PI 437696	antibiosis	12	1.67	0.14	14	1.93	7.22	30	2.03	0.08
PI 567301B	*Rag5*	12	2.33	0.14	10	2.3	0.15	25	3.04	0.14
PI 567541B	*rag1c*, rag4	15	2.06	0.11	15	2.47	0.13	29	2.14	0.1
PI 567543C	*Rag3*	13	2.31	0.13	4	2.5	0.29	22	2.32	0.1
PI 567592	antibiosis	16	2.44	0.13	13	2.15	0.11	29	2.07	0.09
PI 567597C**	antixenosis	14	2.29	0.13	14	2.43	0.17			
PI 587663	antibiosis	9	1.89	0.11	15	1.8	0.11	29	1.93	0.11
PI 587677	antibiosis	14	1.86	0.1	13	2.23	0.17	31	2.03	0.07
PI 587685	antibiosis	14	1.93	0.07	10	2.2	0.33	29	2.03	0.09
PI 587972***	antibiosis	15	2	0.14	15	2	0.1			
PI 594592	antibiosis	13	1.92	0.14	15	1.93	0.12	15	2.68	0.12
PI 71506	antixenosis	12	2.33	0.14	15	2.4	0.13	23	2.13	0.07
Williams 82	susceptible	14	2.86	0.1	14	2.88	0.09	30	2.97	0.06

*n – number of plants, μ – mean rating, SE – standard error
**PI 567597C was not test with soybean aphid biotype 3
***PI 567972 was not tested with soybean biotype 3

Table 2.5. Number of soybean aphids on 16 soybean genotypes 14 days after infestation in non-choice tests.

Soybean genotype	Aphid resistance	Soybean aphid biotype																													
		1*							2*									3*														
Williams 82	susceptible	696	0.08	a					703	0.03	a							848	0.14	a											
PI 567301B	*Rag5*	218	0.11		b				180	0.24		b	c					272	0.24		b	c	d								
PI 567541B	*rag1c, rag4*	198	0.16		b				209	0.17		b						281	0.3		b	c									
PI 567592	antibiosis	165	0.39		b				103	0.25		b	c					186	0.14			c	d	e							
PI 71506	antixenosis	161	0.25		b				60.2	0.48			c					101	0.09				d	e	f	g					
PI 567543C	*Rag3*	111	0.33		b				12.7	0.57				d				64.9	0.32						f	g					
PI 567598B	*Put. Rag1/Rag3*	108	0.12		b				1.82	0.45					e	f		62.8	0.49						f	g	h				
PI 567597C	antixenosis	84.4	0.38		b				11.1	0.35				d				65.3	0.92						f	g					
LD05-16611	*Rag1*	12.5	0.62			c			856	0.05	a							189	0.12			c	d	e							
PI 594592	antibiosis	5.06	0.51			c	d		1.62	0.38					e	f		97.1	0.23					e	f	g					
PI 587972	antibiosis	3.69	0.78				d	e	1.76	0.47					e	f		165	0.42			c	d	e	f						
LD08-12597a	*Rag2*	2.82	0.5				d	e	f	4.29	0.47				d	e		629	0.31	a	b										
PI 587677	antibiosis	2.3	0.39				d	e	f	4.36	0.93				d	e		59	0.32							g	h				
PI 587685	antibiosis	1.64	0.34					e	f	1.98	0.36					e	f	66.9	0.48						f	g					
PI 437696	antibiosis	1.59	0.36					e	f	1.25	0.21						f	23	0.26								h				
PI 587663	antibiosis	1.15	0.13						f	2.53	0.85					e	f	55.7	0.5							g	h				

*Mean number of aphids of each soybean genotype to each tested biotype followed by the respective standard deviation

Means not followed by the same letter were by a LSD at $P = 0.05$.

Infestation of plants between the VE and VC growth stages

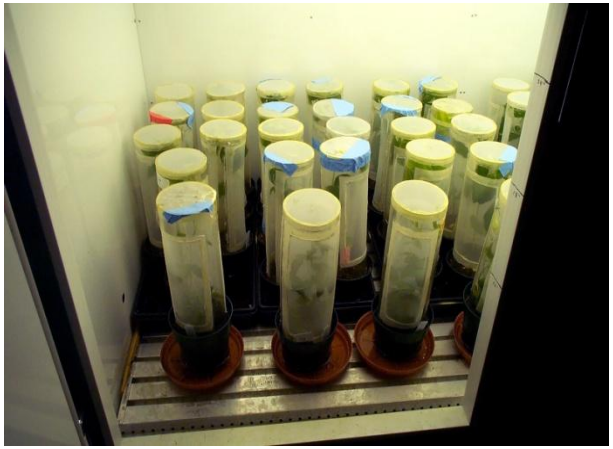
21 days after infestation

Evaluation of resistant and susceptible plants

1 to 2: Resistant

3 to 4: Susceptible

Figure 2.1. Overview of choice test procedure used to evaluate soybeans for resistance to the soybean aphid.



Infestation of plants between the VE and VC growth stages

14 days after infestation

Susceptible Resistant

Evaluation of resistant and susceptible plants

Count data taken on the number of aphids found on the plant

Figure 2.2. Overview of non-choice test procedure used to evaluate soybean for resistance to the soybean aphid.