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NOTE

Soybean Aphid Biotype 4 Identified

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ABSTRACT

The soybean aphid [*Aphis glycines* Matsumura (Hemiptera: Aphididae)] is an invasive pest of soybean [*Glycine max* (L.) Merr.]. Since the first report in the United States in 2000 it has become one of the most damaging soybean pests. Three soybean aphid biotypes have been reported to date. The objective of this research was to determine whether an *A. glycines* field isolate collected near Lomira, WI, was unique from those previously reported. The response of the Lomira isolate was compared to existing soybean aphid Biotypes 1, 2, and 3 by conducting caged and noncaged assays using 10 soybean genotypes. In both the caged and noncaged assays, there were significant effects ($P < 0.0001$) of soybean aphid isolate, genotype, and soybean aphid isolate × genotype interaction. The Lomira isolate reaction profile was different than those of previously reported biotypes, therefore identifying a new biotype to use in characterization of soybean aphid resistant germplasm.

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Abbreviations: LG, linkage group.

The soybean aphid is native to eastern Asia (Ragsdale et al., 2004). It was found in the United States in 2000 (Hartman et al., 2001) and has since spread throughout much of the Midwest and southern Canada (Venette and Ragsdale, 2004). When soybean aphid populations build to a high level their feeding negatively impacts soybean yield through reduced pod set, stunting, and leaf distortion (Hill et al., 2004b). Yield losses of 50% have been reported (Ostlie, 2002) making soybean aphids an important pest of interest for breeders.

Host plant resistance has been pursued as an economical, effective, and environmentally friendly tool to combat aphids. Antibiosis, antixenosis, and tolerance are three types of plant resistance that have been reported (Painter, 1951; Kogan and Ortman, 1978). Antibiosis describes the ability of the plant to reduce growth, reproduction, and survival of an insect on the plant. Antibiosis may be measured through insect size, rate of growth, survival, or fecundity comparisons. Antixenosis is a nonpreference mode of action. It is used to describe a plant's ability to repel insects leading to decreased feeding and oviposition. Tolerance allows the plant to withstand colonization without impacting plant health and fitness. Both antibiosis and antixenosis have proven to be effective against soybean aphids (Hill et al., 2004a).

Several sources of host plant resistance have been identified. Hill et al. (2004a) reported the first set of soybean genotypes, including Dowling, with host plant resistance for soybean aphids. The resistance

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in Dowling has strong antibiosis that restricted the aphid colonization on plants in nonchoice assays. The resistance in Dowling is controlled by a single dominant gene, *Rag1* (Hill et al., 2006a), mapped to chromosome 7 (linkage group [LG] M) (Li et al., 2007). Another genotype, Jackson, also has a resistance gene mapping to the same area of chromosome 7 (LG M) and has a similar phenotype as Dowling (Hill et al., 2006b). Two different sources of *Rag2* have been identified, PI 200538 (Hill et al., 2009) and PI 243540 (Mian et al., 2008a). Both *Rag2* sources map to chromosome 13 (LG F) and have a similar phenotype (Mian et al., 2008b; Hill et al., 2009). Zhang et al. (2009) identified two resistance genes in PI 567541B, which mapped to chromosome 7 (LG M) and chromosome 13 (LG F). The two genes were later named by Zhang et al. (2010) (*rag1c_provisional*) and (*rag4*). *Rag3* was reported by Zhang et al. (2010) on chromosome 16 (LG J). *Rag3* in PI 567597C and *rag1b_provisional/rag3* in PI 567598B were described by Wang (2012).

Soybean aphid biotypes were unknown in North America until Kim et al. (2008) reported soybean aphids readily reproducing on *Rag1* genotypes in Ohio. The Ohio isolate was tested on lines containing *Rag1* and was differentiated from the Illinois isolate (Biotype 1) by its ability to colonize lines with *Rag1*. The Illinois isolate was not able to colonize plants with *Rag1* resistance. *Rag2* genotypes provided host plant protection to both the Ohio and Illinois isolates. The aphid isolate, originally collected from Ohio, was named Biotype 2.

Biotype 3 was reported by Hill et al. (2010). Collected from Springfield Fen, IN, Biotype 3 colonizes *Rag2* but not *Rag1* genotypes. The geographical distribution and occurrence of the three biotypes are unknown. The *Rag1/Rag2* stack had efficacy to all three biotypes.

Studies have begun to differentiate soybean aphid biotypes on a molecular level. Michel et al. (2009) tested 18 microsatellite markers from cotton aphid (*Aphis gossypii* Glover) and black bean aphid (*Aphis fabae* Scopoli) to identify polymorphism between Korean and North American soybean aphid populations. They found nine polymorphic loci in four soybean aphid populations. Jun et al. (2011) tested simple sequence repeat markers developed from genomic resource specific to the soybean aphid. They were able to differentiate between soybean aphid collected from Ohio and South Dakota.

Biotype differentiation is a common phenomenon in many insect pests and a major concern in development of host plant resistance genes in crop plants (Weng et al., 2010). In greenbug (*Schizaphis graminum*) the criterion used to identify biotypes is the assessment of virulence to a set of plant differentials. Molecular analysis based on mitochondrial DNA sequences found greenbug biotypes comprise genetically diverse individuals sharing similar virulence genes (Shufran et al., 2000; Anstead et al., 2002; Lopes-Da-Silva et al., 2004; Weng et al., 2010).

In 2011, soybean aphids were collected from a soybean field in Lomira, WI. A biotypic profile of the isolate was determined based on the ability to survive and reproduce on a set of host plant differentials in choice and nonchoice assays. As in greenbug, a soybean aphid isolate was considered a new biotope when the biotype profile was unique. The biotype profile of the Lomira isolate was determined by using previously established resistant and susceptible genotypes. Assays determined the Lomira isolate is a new biotype because it readily colonizes *Rag1* and *Rag2*. The objective of this experiment was to characterize the Lomira isolate and to confirm it as a new soybean aphid biotype.

MATERIALS AND METHODS

Soybean Aphid Field Collection

The Lomira isolate was field collected from a naturally infested soybean field near Lomira, WI, on 31 Aug. 2011 (Animal and Plant Health Inspection Service permit number P526P-11-02454). The isolate was sampled from an experimental *Rag1* genotype by pulling two infested trifoliates from within the 4.57 m (15 foot) row. The infested material was placed in a collection bag and shipped with the required documentation to the DuPont Pioneer Dallas Center, IA, containment facility where it was maintained. A colony was started by transferring five nymphs from each trifoliate onto Pioneer variety 90M60 (soybean aphid susceptible) plants using a moistened camel hair paint brush. The caged and noncaged assays of the Lomira isolate indicated that it could readily colonize *Rag1*, *Rag2*, and *Rag1/Rag2* stacked material. To eliminate the possibility that the isolate was a combination of Biotypes 2 and 3, a single nymph was used to restart the colony. The single clone colony was used in both the caged and noncaged experiments.

Biotypes used for comparison in this study included Biotypes 1, 2, and 3. Biotypes 1 and 2 were received from Brian Diers at the University of Illinois in March 2008. Biotype 3 was received from Curtis Hill at the University of Illinois in May 2011.

All isolates were maintained on a continuous supply of the susceptible variety. Each colony was maintained within individual 150 by 150 mesh (24.5 by 24.5 by 63.0 cm) BugDorm-4F insect cage (BugDorm Insect cages; Megaview) tents to avoid mixing or contamination. The colonies were maintained in an environmental growth chamber with a photoperiod of 16:8 (light:dark) at 27°C.

Host Plant Differentials

The resistant and susceptible soybean genotypes were selected to determine the biotype profile of each isolate. Nine soybean genotypes (Table 1) previously identified as resistant were used to determine the biotype profile. Dowling and PI 200538 were identified as resistant by Hill et al. (2004a). Mensah et al. (2005) identified PI 567597C, PI 567543C, PI 567541B, and PI 567598B as resistant to Michigan collected aphids. Pioneer variety 95B97 was identified as resistant by Diaz-Montano et al. (2006). Plant Introduction 243540 was identified as resistant by Mian et al. (2008a). Pioneer variety PE38211339 was developed

by crossing an experimental line containing *Rag1* from Pioneer variety 95B97 with an experimental line containing *Rag2* from PI 200538 (J. Alt, unpublished data, 2012). Seed of the resistant plant introductions were obtained from the USDA Soybean Germplasm Collection in Urbana, IL, through the Germplasm Resources Information Network. Pioneer variety 93B15 is highly susceptible to soybean aphids and was used as the susceptible check in both the noncaged and caged experiments.

Noncaged Assay

A noncaged assay was conducted to determine the resistance profile for each isolate. Seeds of each soybean genotype were planted in a 3.8 cm diameter by 21.0 cm deep plastic cone-tainer (Ray Leach Cone-tainer; Hummert International) containing soilless potting mix (3B Professional Mix; Farfard). The cone-tainers were filled to the top with potting mix and packed. Two seeds of each entry were pressed approximately 0.75 inches into the potting medium. Entries were thinned to one plant per cone-tainer at the VC stage (Fehr et al., 1971).

The plants were infested with seven adult apterous females at the V1 stage (Fehr et al., 1971). At 10 d after planting, seven adults were placed on the new trifoliate of each plant, using a moistened camel hair paint brush. Each entry had three replicates in individual cone-tainers. The assays were conducted in a growth chamber set at 27°C and a 16:8 (light:dark) photoperiod. According to McCornack et al. (2004), the optimum temperature for soybean aphid development is 27.8°C. After infestation, the plants were placed in a cone-tainer tray (61 by 30 by 18 cm) (Ray Leach Cone-tainer; Hummert International) in a randomized complete block design. The cone-tainer tray was enclosed within a 150 by 150 cm Bugdorm-4F series mesh tent. To avoid disrupting the aphid feeding, the racks were placed in a water bath and water filled as needed. The aphids were free to stay on the plant where they were originally infested or move off to other plants within the container tray. At 7 and 14 d, the number of aphids found on the entire plant was counted and ratings assigned. Rating of 1 represents greater than 200 aphids per plant, 2 represents 150 to 200 aphid per plant, 3 represents 101 to 150 aphids per plant, 4 represents 76 to 100 aphids per plant, 5 represents 41 to 75 aphids per plant, 6 represents 26 to 40 aphids per plant, 7 represents 10 to 25 aphids per plant, 8 represents less than 10 aphids per plant, and 9 represents no aphids on the plant. The 1 to 9 scale was used to further characterize differences between plants that may have been overlooked using a 0 to 4 scale. Plants with antixenosis properties rated as resistant in this assay.

Caged Assay

Antibiosis was evaluated on the same genotypes used in the noncaged assay. The lines were screened in a similar experimental setup with three replicates of each entry. Two single viviparous apterae adult females were placed within a double sided sticky cage (Converters, Inc.), on the abaxial side of a unifoliate for each entry. A piece of organdy fabric (Hancock Fabrics) slightly larger than the cage was placed over the sticky cage to isolate the aphids on the unifoliates. The plants were placed in cone-tainer trays within Bugdorm-4F series mesh tents. After 7 d, the total aphid population within the cage was counted. According to McCornack et al. (2004), nymphs turn into adults between 5 to 6 d at the temperature used in this experiment. All plants were arranged in randomized complete block design with three replications.

Table 1. Host plant differentials used to determine soybean aphid biotypes.

Soybean genotype†	Resistance type	*Rag* gene or genes
Pioneer variety 93B15	None	
Pioneer variety 95B97	Antibiosis and antixenosis	*Rag1* (J. Alt, unpublished data, 2012)
Dowling	Antibiosis and antixenosis	*Rag1*
PI 243540	Antibiosis	*Rag2*
PI 200538	Antibiosis	*Rag2*
PE38211339	Antibiosis	*Rag1/Rag2*
PI 567541B	Antibiosis	*rag1c_provisional/rag4*
PI 567543C	Antixenosis	*Rag3*
PI 567597C	Antixenosis	*Rag3*
PI 567598B	Antibiosis	*rag1b_provisional/rag3*

†Plant introductions obtained from USDA Germplasm working collection.

Analysis of Variance

A split-plot experimental design with three replications was used in both the antibiosis and antixenosis experiments. Soybean aphid isolates were the main effect and soybean genotype were the subeffect. Statistical analyses were performed using PROC GLM in SAS (SAS Institute, 2001). Replications were nonsignificant; therefore, the averages were reported. Appropriate error terms were calculated. Means were separated using the LSD at $P = 0.05$ if their effects were found to be significant in the ANOVA.

RESULTS

Results showed the isolate collected from Lomira colonized both *Rag1* and *Rag2* material. The isolate could readily colonize both *Rag1* genotypes Pioneer variety 95B97 and Dowling. This isolate could also readily colonize both *Rag2* lines PI 200538 and PI 243540. The *Rag1/Rag2* stacked material was also colonized with dense aphid populations in both the caged and noncaged assays.

Caged Assay

There were significant ($P < 0.001$) effects of aphid isolate, genotype, and aphid isolate × genotype interaction for the number of aphids per plant after 7 d of infestation. The mean number of aphids produced on each genotype was calculated and is presented in Table 2. The Lomira isolate colonized all *Rag1*, *Rag2*, and *Rag1/Rag2* stacked genotypes.

Noncaged Assay

There were significant ($P < 0.0001$) effects of aphid isolate, genotype, and aphid isolate × genotype interaction at both 7 and 14 d after infestation. The results are presented in Table 3. The noncaged results indicate that Lomira readily colonizes Pioneer variety 93B15, Pioneer variety 95B97, Dowling, PI 243540, PI 200538, *Rag1/Rag2* stacked material, PI

Table 2. The average number of soybean aphids per plant after 7 d of infestation in the caged assay.

Soybean genotype	Soybean aphid biotype				
	1	2	3	Lomira	LSD(0.05)†
Pioneer variety 93B15	28	30	28	34	17
Pioneer variety 95B97	2	26	1	21	9
Dowling	3	33	3	39	11
PI 243540	6	9	42	24	8
PI 200538	1	2	25	39	7
PE38211339	2	1	2	29	12
PI 567541B	9	17	13	18	18
PI 567543C	13	13	22	9	18
PI 567597C	13	6	10	11	7
PI 567598B	11	4	18	22	5

†Least significant different at the 0.05 probability level between biotypes within a genotype.

567541B, and PI 567598B. Lomira did not develop dense established populations on PI 567543C or PI 567597C. After 14 d, the Lomira isolate colonized all *Rag1*, *Rag2*, and *Rag1/Rag2* stacked material. Soybean aphid Biotype 1 only densely colonized Pioneer variety 93B15. Biotype 2 colonized *Rag1* lines Pioneer variety 95B97 and Dowling and moderately infested PI 567541B. Biotype 3 readily colonized *Rag2* lines PI 243540 and PI 200538 and moderately colonized PI 567541B, PI 567543C, PI 567597C, and PI 567598B. The aphid rating 14 d after infestation showed higher aphid numbers and less clear differentiation among genotypes than 7 d after infestation. The ratings for most genotype–biotype combinations decreased between 7 and 14 d.

DISCUSSION

Multiple biotypes occur in other aphid species such as Russian wheat aphid (*Diuraphis noxia*) and greenbug (Burd and Porter, 2006; Haley et al., 2004). Understanding the diversity within the soybean aphid population is important to soybean breeders when developing resistant cultivars. Since the first reports of soybean aphids in North America in July 2000, four soybean aphid biotypes have now been identified.

Results of these experiments indicate a new soybean aphid biotype was discovered. The Lomira isolate readily colonized all *Rag1* and *Rag2* material, which is different from previously reported biotypes. Therefore, the Lomira isolate is named Biotype 4. Results suggest material containing *Rag1*, *Rag2*, or *Rag1/Rag2* stacked material may not work in areas inhabited by this biotype.

Further experiments need to be conducted to understand Biotype 4. At this time, the geographic range of Biotype 4 is unknown. Field collections need to be done on a wide scale across the soybean aphid region to assess Biotype 4 distribution. Experiments could be conducted manipulating environmental factors such as heat and starvation to assess the stability of the Biotype 4 phenotype. Genotyping Biotype 4 will provide data on the genetic relation to Biotypes 1, 2, and 3.

Management options for growing areas infested with Biotype 4 need to be evaluated. At this time, the level of Biotype 4 within a soybean field infested with aphids is unknown. Insecticide applications may be warranted in areas heavily infested with Biotype 4. Further research needs to be conducted to find soybean genes that confer resistance to Biotype 4.

Having fully characterized biotypes is an important phenotyping tool. This allows breeders to target appropriate resistance genes and correctly place genotypes. Until the discovery of Biotype 4, *Rag1/Rag2* stacked material appeared to have wide adaptation. Each time a new biotype is characterized, additional information is learned about existing resistance genes.

Table 3. The average rating of soybean aphids per plant 7 and 14 d after infestation in the noncaged assay.

Soybean genotype	Soybean aphid biotype									
	1	2	3	Lomira	LSD(0.05)†	1	2	3	Lomira	LSD(0.05)††
	7 d					14 d				
Pioneer variety 93B15	3§	3	3	3	0.0	1	1	1	1	0.0
Pioneer variety 95B97	8	2	7	5	1.0	8	1	7	1	0.6
Dowling	8	3	7	4	1.5	6	1	7	1	1.1
PI 243540	8	7	3	4	1.0	6	4	1	1	1.5
PI 200538	7	7	5	4	1.4	6	1	1	1	1.0
PE38211339	8	7	8	4	1.4	7	7	6	1	1.0
PI 567541B	6	5	7	6	1.7	5	3	7	5	1.6
PI 567543C	7	7	6	8	1.3	7	5	6	7	1.9
PI 567597C	8	7	6	8	0.7	6	6	5	6	1.6
PI 567598B	6	7	6	7	0.7	5	4	3	5	0.6

†Least significant different at the 0.05 probability level between biotypes within a genotype 7 d after infestation.

††Least significant different at the 0.05 probability level between biotypes within a genotype 14 d after infestation.

§Rating of 1 represents greater than 200 aphids per plant, 2 represents 150 to 200 aphid per plant, 3 represents 101 to 150 aphids per plant, 4 represents 76 to 100 aphids per plant, 5 represents 41 to 75 aphids per plant, 6 represents 26 to 40 aphids per plant, 7 represents 10 to 25 aphids per plant, 8 represents less than 10 aphids per plant, and 9 represents no aphids on the plant.

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