A New Soybean Aphid (Hemiptera: Aphididae) Biotype Identified

CURTIS B. HILL,[1,2] LAURA CRULL,[1] THERESA K. HERMAN,[1,3]
DAVID J. VOEGTLIN,[4] AND GLEN L. HARTMAN[1]

J. Econ. Entomol. 103(2): 509–515 (2010); DOI: 10.1603/EC09179

ABSTRACT Shortly after its arrival, the soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), became established as the most important insect pest of soybean, *Glycine max* L. (Merr.), in the northern part of the North American soybean production region. Soybean resistance is an environmentally sustainable method to manage the pest and new soybean aphid resistant cultivars are beginning to be deployed into production. However, an earlier study identifying a soybean aphid biotype that could colonize plants with the *Rag1* resistance gene has raised concerns about the durability of soybean aphid resistance genes. Choice and nonchoice tests conducted in this study characterized the colonization of a soybean aphid isolate, collected from the overwintering host *Frangula alnus* P. Mill in Springfield Fen, IN, on different aphid resistant soybean genotypes. This isolate readily colonized plants with the *Rag2* resistance gene, distinguishing it from the two biotypes previously characterized and indicating that it represented a new biotype named biotype 3. The identification of soybean aphid biotypes that can overcome *Rag1* and *Rag2* resistance, even before soybean cultivars with the resistance genes have been deployed in production, suggests that there is high variability in virulence within soybean aphid populations present in North America. Such variability in virulence gives the pest a high potential to adapt to and reduce the effective life of resistance genes deployed in production. The search for new soybean aphid resistance genes must, therefore, continue, along with the development of alternative sustainable strategies to manage the pest.

KEY WORDS soybean, soybean aphid, biotype, aphid, aphid resistance

One of the greatest threats to soybean, *Glycine max* L. (Merr.), production in the main North American soybean production region continues to be the soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae). The most serious soybean aphid invasion to date occurred in 2003 throughout much of the North Central soybean production region. An estimated 1.6 million ha of soybean was reported damaged in Minnesota, resulting in a loss of US$80 million (Associated Press 2003). In Illinois, ≈0.5 million ha was damaged with an estimated loss of US$45 million (Steffey 2004). A recent economic analysis of the impact of the soybean aphid on soybean production predicted that without effective plant resistance $3.6 to $4.9 billion in soybean production could be lost annually, depending upon the cost of insecticide applications, the size of the aphid outbreak, and the price elasticity of soybean supply (Kim et al. 2008a).

The soybean aphid, native to eastern Asia (Ragsdale et al. 2004), was first reported in North America in 2000 (Hartman et al. 2001) and has since spread throughout the midwestern United States and southern Canada (Venette and Ragsdale 2004). The aphid has a heteroecious, holocyclic life cycle pattern (Hartman et al. 2001, Wu et al. 2004). *Rhamnus cathartica* L. (buckthorn) is the primary or overwintering host of *A. glycines* on which sexual reproduction occurs; however, eggs can be laid on *Rhamnus alnifolia* L. (Voegtlin et al. 2004, 2005). Gynoparae and oviparous nymphs have also been observed on glossy buckthorn, *Frangula alnus* (syn. *Rhamnus frangula*), but it is not clear whether this species is a true primary host of *A. glycines.* Soybean is the most important secondary or summer host of *A. glycines* (Hill et al. 2004a).

High soybean aphid populations reduce soybean yield directly when their feeding causes stunting, leaf distortion, and reduced pod set (Hill et al. 2004b). Furthermore, the soybean aphid has the ability to transmit plant viruses to soybean such as Alfalfa mosaic virus, Soybean dwarf virus, and Soybean mosaic virus (Hartman et al. 2001, Hill et al. 2001, Clark and Perry 2002, Wang and Ghabrial 2002, Domier et al. 2003). Honeydew excreted by soybean aphids onto leaves leads to the development of sooty mold, which inhibits photosynthesis (Hartman et al. 2001).

Plant resistance can provide an effective, economical, and sustainable method of insect control. Plant

[1] Department of Crop Sciences, National Soybean Research Center, University of Illinois, Urbana, IL 61801.
[2] Corresponding author, e-mail: curthill@illinois.edu.
[3] USDA–ARS, 1101 West Peabody, University of Illinois, Urbana, IL 61801.
[4] Center for Economic Entomology, Illinois Natural History Survey, Champaign, IL 61820.

0022-0493/10/0509–0515$04.00/0

resistance to the soybean aphid was discovered in soybean germplasm in 2004 (Hill et al. 2004b). Resistance in 'Dowling' had strong antibiosis that limited aphid colonization on plants in nonchoice tests. Detailed analysis of the effects of antibiosis on aphid biology indicated that the resistance in Dowling significantly reduced aphid survival, longevity, fecundity, and nymphal development (Li et al. 2004). The aphid resistance in Dowling was shown to be controlled by a single dominant gene named *Rag1* (Hill et al. 2006a) and was mapped to soybean linkage group (LG) M (Li et al. 2007). An unnamed gene with a similar soybean aphid resistance phenotype was found in the cultivar Jackson mapping to the same location and may be allelic with *Rag1* (Hill et al. 2006b). *Rag2*, identified independently in two different soybean germplasm accessions, PI 200538 and PI 243540, maps to soybean LG F (Kang et al. 2008, Mian et al. 2008, Hill et al. 2009). Two resistance genes were identified in PI 567541B (Zhang et al. 2009). One of these genes maps near *Rag1* on LG M and the other gene maps to LG F at a position different from *Rag2.*

Biotypes of the soybean aphid were unknown in North America until recently. While soybean breeding lines possessing *Rag1* were being tested in the field in Ohio in 2006, dense colonies unexpectedly developed on the plants, similar to levels of colonization observed on previously known susceptible germplasm. The aphid isolate collected in Ohio was tested on soybean aphid resistant lines, including lines with *Rag1*, and was distinguishable from an Illinois isolate by its ability to colonize plants with *Rag1* (Kim et al. 2008b). The morphology of the Ohio aphids was similar to the soybean aphids collected in Illinois. The Ohio isolate developed large colonies on plants with *Rag1.* In contrast, the Illinois isolate did not colonize plants with *Rag1.* 'Jackson' soybean was also susceptible to the Ohio isolate but not the Illinois isolate. Both isolates were virulent on the soybean 'Williams 82′ and a few other lines tested that were known to be susceptible to the Illinois isolate. A few germplasm sources previously found to be resistant to the Illinois isolate (Hill et al. 2004b) were also resistant to the Ohio isolate, including the soybean germplasm accession PI 200538. With the possibility that additional soybean aphid biotypes may be found in Illinois and Ohio, the Illinois aphid isolate was referred to as biotype 1 and the Ohio isolate biotype 2 (Hill et al. 2009). The frequencies and distribution of the biotypes are unknown.

Discovery of at least two soybean aphid biotypes in North America indicated that plant resistance controlled by major genes such as *Rag1* could be vulnerable to aphid adaptation. This finding is a major concern to soybean breeders engaged in developing new soybean aphid resistant cultivars. New sources of resistance will need to continually be sought and introduced into soybean in anticipation of soybean aphid adaptation to host resistance genes.

In 2007, soybean aphids were collected from *F. alnus* in Springfield Fen, IN. Preliminary testing on soybean plants with *Rag1* or *Rag2* indicated that the isolate could be a new soybean aphid biotype because it readily colonized plants with *Rag2.* The objective of this study was to characterize colonization of the Springfield Fen isolate on several sources of resistance to the soybean aphid to confirm that it represented a new biotype.

Materials and Methods

Experiments. Five experiments were conducted to characterize the Springfield Fen soybean aphid isolate. A single soybean plant was an experimental unit in all experiments except experiment 2 where two plants represented an experimental unit (Hill et al. 2004b). Experimental units were replicated three times in all of the experiments.

Aphid Culture. Soybean aphid isolates tested in this study included an Illinois isolate originally collected in 2000, tested in several previous studies (Hill et al. 2004a,b, 2006a,b, 2009; Li et al. 2004, 2007; Kim et al. 2008b) and referred to as biotype 1; an Ohio isolate collected in 2005, distinguished from the Illinois isolate by its ability to colonize plants with *Rag1* (Kim et al. 2008b) and referred to as biotype 2; and SF-55, an isolate collected from *F. alnus* in Springfield Fen, IN, during spring 2007. Biotype 1 was maintained on a continuous supply of Williams 82 plants in growth chambers as described previously (Hill et al. 2004b). Biotype 2 was maintained on the soybean breeding line LD05-16611 that possesses *Rag1.* SF-55 was initially maintained on Williams 82 plants until preliminary tests indicated that it readily colonized PI 200538, after which was maintained on that accession to prevent contamination with biotypes 1 and 2, which cannot colonize PI 200538. All three soybean aphid isolates were periodically cloned from isolated nymphs and were maintained in different plant growth chambers to avoid mixing.

Plant Culture. Methods for plant culture were described previously (Hill et al. 2004b, 2006a, 2009). Seeds were planted at a rate of three seeds per pot. Plant growth medium was a soil-less mix (Sunshine Mix, LC1, Sun Gro Horticulture Inc., Bellevue, WA). Plants were fertilized at planting with slow-release pellets (Osmocote 19-6-12) placed on the surface of the soilless growth medium to a density of 1–2 pellets per cm^2. Seedlings were thinned to one plant per pot after emergence.

Soybean Genotypes. Soybean genotypes representative of known soybean aphid resistance sources along with known susceptible genotypes were selected to characterize the virulence of soybean aphid isolate SF-55 (Table 1). Williams 82 was chosen because it was susceptible to both biotypes 1 and 2 (Hill et al. 2009). Several genotypes chosen had either *Rag1*, *Rag2*, or both resistance genes, confirmed after screening with linked simple sequence repeat (SSR) markers (Li et al. 2007, Hill et al. 2009). Line names starting with LD were advanced breeding lines provided by Brian Diers, University of Illinois. Dowling was the original germplasm source of *Rag1* (Hill et al. 2006a). PIs 200538 and 243540 are sources of *Rag2*

Table 1. Soybean genotypes tested in six experiments to characterize the virulence of soybean aphid isolate SF-55 collected from *F. alnus* in Springfield Fen, IN

Name	Type[a]	Soybean aphid resistance genes	Exp
Dowling	Germplasm accession	*Rag1*	1
LD02-4485	Breeding line		2, 5
LD05-16611	Breeding line	*Rag1*	1, 2, 3, 4, 5
LD05-16657	Breeding line	*Rag1*	2, 5
LD08-114003a	Breeding line	*Rag1*, *Rag2*	2, 5
LD08-114013a	Breeding line	*Rag1*, *Rag2*	2, 5
LD08-89109a	Breeding line	*Rag2*	2, 5
LD08-89032a	Breeding line	*Rag2*	2, 5
LDXG05241R-1-6	Breeding line	*Rag1*	2, 5
PI 200538	Germplasm accession	*Rag2*	1, 2, 3, 4, 5
PI 243540	Germplasm accession	*Rag2*	1, 3
PI 437696	Germplasm accession		1, 2, 4, 5
PI 567541B	Germplasm accession	Two resistance genes[b]	2, 5
PI 567597C	Germplasm accession		2, 5
PI 567598B	Germplasm accession		2, 5
PI 71506	Germplasm accession		2, 5
PI 567543C	Germplasm accession		2, 5
Williams 82	Public cultivar		1, 2, 3, 4, 5

[a] Germplasm accession was obtained from the USDA Soybean Germplasm Collection, Urbana, IL; breeding line was from B.W. Diers, University of Illinois.

[b] One gene maps to the same location as *Rag1* on soybean LG M and the other maps to a location on LG F different from *Rag2* (Zhang et al. 2009).

(Hill et al. 2009, Kang et al. 2008, Mian et al. 2008). PI 71506 was chosen because it expressed primarily antixenosis-type resistance to biotype 1 (Hill et al. 2004b). PIs 567541B, 567597C, 567598B, and 567543C were included in the tests because they represented sources of soybean aphid resistance that may differ from *Rag1* and *Rag2* (Mensah et al. 2005). PI 567541B was reported to have two resistance genes that were linked with but probably nonallelic with *Rag1* and *Rag2* (Zhang et al. 2009). PI 437696 was chosen because it was found to be highly resistant to biotypes 1 and 2 in our ongoing resistance screening program (unpublished data).

Choice Tests. Two kinds of choice tests were conducted in which aphid movement was unrestricted (Experiments 1 and 2). Both tests were conducted in a greenhouse at 18–24°C with a 16-h photoperiod with ambient sunlight supplemented by a mixture of high pressure sodium and metal halide lamps.

Experiment 1 compared colonization of the Springfield Fen soybean aphid isolate SF-55 with biotypes 1 and 2 on six of the 16 soybean genotypes used in this study: Dowling, LD05-16611, PI 200538, PI 243540, PI 437696, and Williams 82. A split-plot design was used with the three soybean aphid isolates, biotype 1, biotype 2, and SF-55, as the main plots and the six soybean genotypes as subplots, with three replications arranged in a completely randomized design. The soybean genotypes were randomly planted around the perimeter of 20.3-cm-diameter azalea pots ≈2–2.5 cm from the pot rim and evenly spaced from each other. After plant emergence, each plant in a pot was infested with one of the soybean aphid isolates by placing infested leaflets with an undetermined number of aphids in multiple developmental stages on top of each seedling. Flower pot cages (Arnold Johnson, Bron y Glyn, Bronwydd, Carmarthen, Carmarthenshire, United Kingdom), with a 20.3-cm-diameter by 30.5-cm-tall metal frame covered with a fine white-colored cloth netting with 0.35-mm irregular sized openings, was placed over each pot after infestation to restrict aphid movement to the test plants within each pot. Positions of pots in three replications of the six soybean genotypes for each soybean aphid isolate, a total of nine pots in the experiment, were randomized on the greenhouse bench.

Experiment 2 determined colonization of the Springfield Fen soybean aphid isolate SF-55 on 16 soybean genotypes used in this study. Test plants of these genotypes were randomly planted in two-pot experimental units contained in 48-pot plastic inserts (#1204, Hummert International, Earth City, MO). Each insert had 12 rows of four pots and was placed inside a flat without drainage holes (#F1020, Hummert International). Experimental units were arranged in a completely randomized design with three replications. At plant emergence, 24 PI 200538 plants (stems and leaves) infested with an undetermined number of aphids in multiple developmental stages were spaced evenly over the seedlings in the experiment.

Aphid colonization was visually rated 3 wk after aphid infestation in both choice tests. A 1-4 nonparametric, nominal rating scale was used to estimate the degree of aphid colonization and plant damage caused by aphid feeding, with 1, few solitary live aphids, often with dead aphids; 2, several transient aphids present along with some viviparous aptera surrounded by a few nymphs, but without established colonies; 3, dense aphid colonies; and 4, dense colonies accompanied by plant damage, including leaf distortion and stunting (Hill et al. 2006a,b, 2009). This rating scale was developed from observations of the responses of thousands of soybean germplasm accessions to aphid infestation in a large soybean resistance-screening program. Plants devoid of aphids were rare in choice

tests because the aphids were free to roam throughout the test. Aphids that seemed to be transient were often observed on resistant plants in choice tests, along with dead aphids. Sometimes several viviparous aptera, surrounded by a few nymphs, were observed on resistant plants, but failed to develop established colonies.

Nonchoice Tests. Experiments 3, 4, and 5 were nonchoice tests with a similar experimental setup but with different soybean genotypes and aphid isolates. Seed of each soybean genotype tested in an experiment was planted in a 12.7 cm diameter azalea pot filled with soil-less mix and thinned to one plant per pot after emergence. Ten second to third instar soybean aphid nymphs were placed together on the adaxial side of one of the unifoliolate leaves at V1 stage (Fehr et al. 1971) test plants with a moistened synthetic sable hair paint brush (3050SP, Princeton Art & Brush Co., Princeton, NJ). After aphid infestation, individual plants were isolated in 100- by 300-mm clear plastic cylindrical cages with 4-mm-thick walls and two 80- by 180-mm side windows plus the top covered with a white silk fabric with irregular-shaped 0.1-mm openings (Rosebrand East, Secaucus, NJ). The open bottom of each cage was pressed into the soil-less medium inside each pot ≈10 mm deep to prevent aphid escape. Total aphid populations on each plant were enumerated at periodic intervals up to 14 d after infestation. All three nonchoice experiments were conducted in the same plant growth chamber (Conviron PGR15, Winnipeg, MB, Canada) with 500 μmol m^{-2} s^{-1} PAR irradiation set to a 14-h photoperiod.

Experiment 3 tested the response of four of the 16 soybean genotypes used in this study, LD05-16611, PI 200538, PI 243540, and Williams 82, to infestation by SF-55. In this test, the apical meristem of each test plant was removed after aphid infestation. The diurnal temperature range was 18–22°C. Experiment 4 was a factorial test with the three soybean aphid isolates, biotype 1, biotype 2, and SF-55, tested on the four soybean genotypes, LD05-16611, PI 200538, PI 437696, and Williams 82, with a diurnal temperature range of 20–24°C and was repeated once. Experiment 5 tested soybean aphid isolate SF-55 on all 16 soybean genotypes and was repeated once. The diurnal temperature was 18–22°C for the first test and 20–24°C for the repeat test. All tests were arranged in a randomized complete block design with three replications.

Statistical Analysis. All statistical analyses were performed with the aid of JMP version seven (SAS Institute, Cary, NC). Nonparametric analysis using the nominal logistics test was conducted on the choice test data collected by applying the 1–4 nominal scale to assess aphid colonization and plant damage. Standard least squares analysis was performed on the nonchoice population enumeration data after transformation by adding one to the population count and then taking the log to the base 10 of the sum to correct for heterogeneity of variance among the soybean genotype and soybean aphid isolate treatments. Least square means from the analyses were de-transformed before presentation in Tables 4 and 5, and Fig. 1.

Table 2. Colonization of six soybean genotypes infested with three soybean aphid isolates in a choice test at 3 wk after infestation

Aphid isolate	Soybean genotype	Resistance gene	No. plants aphid colonization class[a]			
			1	2	3	4
Biotype 1	Dowling	*Rag1*	2	1	0	0
	LD05-16611	*Rag1*	2	1	0	0
	PI 200538	*Rag2*	3	0	0	0
	PI 243540	*Rag2*	3	0	0	0
	PI 437696		3	0	0	0
	Williams 82[b]		0	0	1	2
Biotype 2	Dowling	*Rag1*	0	0	1	2
	LD05-16611	*Rag1*	0	0	0	3
	PI 200538	*Rag2*	1	2	0	0
	PI 243540	*Rag2*	0	3	0	0
	PI 437696		2	1	0	0
	Williams 82[b]		0	0	0	3
SF-55	Dowling	*Rag1*	0	3	0	0
	LD05-16611	*Rag1*	0	0	1	2
	PI 200538	*Rag2*	0	0	2	1
	PI 243540	*Rag2*	0	0	0	3
	PI 437696		3	0	0	0
	Williams 82[b]		0	0	0	3

[a] Aphid colonization classes: 1, few solitary live aphids, often with dead aphids; 2, several transient aphids present along with some viviparous aptera surrounded by a few nymphs, but without established colonies; 3, dense aphid colonies; and 4, dense colonies accompanied by plant damage, including leaf distortion and stunting.
[b] No known resistance gene.

Results

Preliminary choice and nonchoice testing of the original isolate collected from *F. alnus* in Springfield Fen indicated that the isolate could readily colonize PI 200538 and PI 243540 that contain the soybean resistance gene *Rag2*. Colonization on plants with *Rag1* was unclear in the preliminary tests and was highly variable between different plants of the same soybean genotype, although there seemed to be a clear trend of colonization on plants with *Rag2*. To eliminate the possibility that the isolate was an admixture of different isolates due to contamination, the isolate was recloned from a single nymph and the new cloned isolate named SF-55 was maintained on PI 200538 for subsequent testing.

Experiment 1. There were significant differences among the soybean genotypes ($P < 0.01$), soybean aphid isolates ($P < 0.01$), and the interaction between soybean genotypes and soybean aphid isolates ($P < 0.01$) in the Experiment 1 choice test. The number of plants in the three replications of the soybean genotype x aphid isolate combination placed into each of the four aphid colonization rating classes are presented in Table 2. Results indicated that SF-55 readily colonized Williams 82, and PI 200538 and PI 243540 that contain the soybean resistance gene *Rag2*, and also LD05–16611 that contains *Rag1*, but it did not develop dense, well-established colonies on Dowling, also with *Rag1*. SF-55 did not colonize PI 437696. Soybean aphid biotype 1 colonized only Williams 82, whereas biotype 2 colonized Williams 82 plus Dowling and LD05-16611 with *Rag1*, as found previously (Kim et al. 2008b), but it did not colonize PI 200538 and PI 243540 with *Rag2*, or PI 437696.

Table 3. Colonization of 16 soybean genotypes with soybean aphid isolate SF-55 in a choice test at 3 wk after infestation

Soybean genotype	Resistance gene	No. plants aphid colonization class[a]			
		1	2	3	4
LD02-4485		0	0	2	1
LD05-16611	*Rag1*	0	1	2	0
LD05-16657	*Rag1*	0	0	1	2
LD08-114003a	*Rag1*, *Rag2*	0	0	3	0
LD08-114013a	*Rag1*, *Rag2*	0	0	3	0
LD08-89032a	*Rag2*	0	0	3	0
LD08-89109a	*Rag2*	0	1	2	0
LDXG05241R-1-6	*Rag1*	0	0	3	0
PI 200538	*Rag2*	0	0	2	1
PI 437696		2	1	0	0
PI 567541B		0	1	2	0
PI 567543C		0	3	0	0
PI 567597C		0	2	1	0
PI 567598B		0	1	1	1
PI 71506		0	2	1	0
Williams 82[b]		0	0	1	2

[a] Aphid colonization classes: 1, few solitary live aphids, often with dead aphids; 2, several transient aphids present along with some viviparous aptera surrounded by a few nymphs, but without established colonies; 3, dense aphid colonies; and 4, dense colonies accompanied by plant damage, including leaf distortion and stunting.
[b] No known resistance gene.

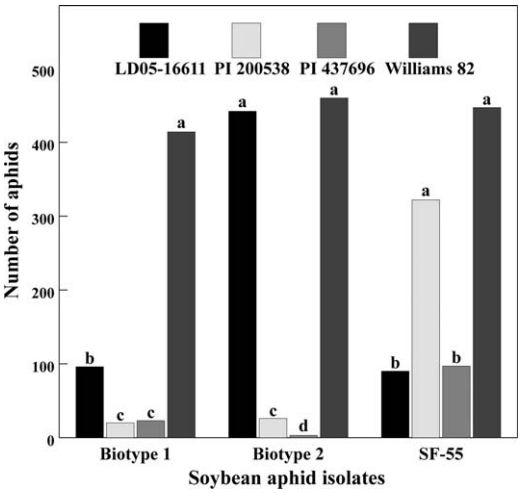

Fig. 1. Colonization of four soybean genotypes by three soybean aphid isolates in a nonchoice test at 10 d after infestation with 10 second- to third-instar nymphs. LD05-16611 (*Rag1*) resistant to biotype 1; PI 200538 (*Rag2*) and PI 437696 resistant to biotypes 1 and 2; Williams 82 susceptible to biotypes 1 and 2. Biotype 1 from soybean (Urbana, IL, 2001); Biotype 2 from soybean (Wooster, OH, 2006); SF-55 from *F. alnus* (Springfield Fen, IN, 2007). Means followed by a different letter differ significantly, least significant difference ($\alpha = 0.05$).

Experiment 2. Significant differences in aphid colonization ($P < 0.05$) were found among the soybean genotypes in Experiment 2. Numbers of plants in the three replications of each soybean genotype placed into each of the four aphid colonization rating classes are presented in Table 3. As in experiment 1, most genotypes with *Rag1* or *Rag2* had established colonies. In this test, well-established colonies were not observed on PI 437696 plants or on PI 567543C plants. Well-established colonies were found on at least one experimental unit of LD02-4485, PI 567541B, PI 567597C, PI 567598B, PI 71506, and Williams 82.

Experiment 3. Highly significant differences were found among the soybean genotypes in the Experiment 3 nonchoice test ($P < 0.01$) for numbers of aphids 8 d after infestation. SF-55 colonization on Williams 82, PI 243540, and PI 200538 was not significantly different but was significantly higher than colonization on LD05–16611 (Table 4). These results indicated that LD05-16611 had antibiosis-type resistance.

Experiment 4. Differences between tests and the interactions between tests with soybean aphid isolates and soybean genotypes for number of aphids per plant 10 d after infestation were nonsignificant ($P > 0.05$) in this nonchoice test. Therefore, the mean number of aphids for each genotype after combining the data from both tests is presented in Fig. 1. Highly significant differences ($P < 0.01$) were found among soybean aphid isolates, soybean genotypes, and their interaction. Significantly higher numbers of SF-55 aphids were found on PI 200538 and Williams 82 than on LD05-16611 and PI 437696 (Fig. 1). Numbers of biotype 1 aphids on Williams 82 were significantly higher than those on LD05-16611, which were significantly higher than those on PI 200538 and PI 437696. The number of biotype 1 aphids on PI 200538 and PI 437696 was not significantly different. The number of biotype 2 aphids on LD05-16611 and Williams 82 was not significantly different, but was significantly greater than populations on PI 200538, which harbored significantly more biotype 2 than PI 437696. Although the number of SF-55 aphids was significantly lower on LD05–16611 and PI437696 than on PI 200538 and Williams 82, the number was not significantly different from the number of biotype 1 aphids on LD05-16611. The numbers of biotype 1 and biotype 2 aphids on PI200538 and PI 437696 were significantly lower than the number of SF-55 aphids on those soybean germplasm accessions. These results indicated that the three soybean aphid isolates colonized the four soybean genotypes differentially, demonstrating that each represented a different soybean aphid biotype.

Table 4. Colonization of four soybean genotypes with nymphs[a] of soybean aphid isolate SF-55 in a nonchoice test at 8 d after infestation

Soybean genotype	Resistance gene	No. aphids
Williams 82[b]		354a
PI 243540	*Rag2*	312a
PI 200538	*Rag2*	233a
LD05-16611	*Rag1*	15b

Means not followed by the same letter were significantly different by the least significant difference at $P = 0.05$.
[a] Ten second- to third-instar nymphs.
[b] No known resistance gene.

Experiment 5. There were highly significant differences ($P < 0.01$) found between the tests, replications,

Table 5. Colonization of 16 soybean genotypes by soybean aphid isolate SF-55 in a nonchoice test at 14 d after infestation[a]

Soybean genotype	Resistance gene	No. aphids
Williams 82[b]		743a
PI 567541B		673a
LD02-4485		657a
PI 200538	*Rag2*	594a
PI 567543C		576a
LD08-89109a	*Rag2*	527ab
LD08-89032a	*Rag2*	494ab
PI 71506		284bc
PI 567598B		241cd
LD08-114013a	*Rag1*, *Rag2*	171cde
LD08-114003a	*Rag1*, *Rag2*	166cde
PI 567597C		139de
LD05-16611	*Rag1*	138de
LD05-16657	*Rag1*	123e
LDXG05241R-1-6	*Rag1*	95ef
PI 437696		61f

Means not followed by the same letter were significantly different by the least significant difference at $P = 0.05$.

[a] Ten second- to third-instar nymphs.

[b] No known resistance gene.

and among the soybean genotypes for numbers of SF-55 aphids 14 d after infestation in the nonchoice test. The interaction between tests with soybean genotypes was nonsignificant. Therefore, the mean number of aphids for each genotype after combining the data from both tests is presented in Table 5. Results of this experiment were in agreement with those from experiments 3 and 4, again indicating that SF-55 readily colonized Williams 82 and plants with *Rag2* but not plants with *Rag1* (Table 5). SF-55 aphid numbers on plants with both *Rag1* and *Rag2* were not significantly different from the numbers on plants with *Rag1* alone. Numbers of SF-55 aphids on PI 437696 were significantly lower than those on all other soybean genotypes except for LDXG05241R-1-6. Soybean germplasm accessions PI 567541B and PI 567543C had numbers of SF-55 aphids that were not significantly different from Williams 82. Germplasm accessions PI 71506 and PI 567597C had SF-55 numbers significantly lower than Williams 82 and not significantly different from the soybean breeding lines with both *Rag1* and *Rag2*. Numbers of SF-55 aphids on PI 567597C were also not significantly different from the numbers found on the breeding lines with *Rag1* alone. These results confirmed the ability of SF-55 to colonize plants with *Rag2*, which distinguishes it from biotypes 1 and 2.

Discussion

Results of the experiments conducted in this study indicated SF-55 readily colonized plants with *Rag2* in both choice and nonchoice tests, which distinguished the isolate from biotypes 1 and 2 that do not have the ability to colonize plants with the *Rag2* resistance gene (Kim et al. 2008b, Hill et al. 2009). Therefore, SF-55 represents a new biotype apart from biotypes 1 and 2 and is named biotype 3.

Level of SF-55 colonization on plants with *Rag1* was similar to the levels observed on Williams 82 in the choice tests (Tables 2 and 3) with the exception of Dowling in experiment 1, in which colonization was lower than on LD05-16611 (Table 2). However, colonization on soybean genotypes with *Rag1* was significantly lower than on Williams 82 or on genotypes with *Rag2* in nonchoice tests (Fig. 1, Tables 4 and 5). These results seemed to indicate that some soybean genotypes with *Rag1* expressed antibiosis-type resistance with little, if any, antixenosis-type resistance. Although SF-55 colonization was reduced on LD05-16611 in nonchoice tests (Fig. 1; Table 5), there were still ≈100 or more aphids observed on the plants 10–14 d after infestation. Therefore, the antibiosis effect on SF-55 colonization expressed by *Rag1* in LD05-16611 did not seem to be as strong as the effect of *Rag2* on biotypes 1 and 2 found in this study and in an earlier study of those two biotypes (Kim et al. 2008b). This could help explain why plants with *Rag1* did not seem to have discernible resistance to SF-55 in the choice tests.

The major implication in the identification of biotype 3 is the potential of soybean aphid populations to adapt to *Rag2*. The previous identification of biotype 2 (Kim et al. 2008b) indicated the potential threat of adaptation on *Rag1*. Results of this study suggested that biotype 3 could also colonize soybean cultivars with *Rag1* deployed in production. The first soybean cultivars with *Rag1* were marketed to growers for production in 2009 (Caspers-Simmet 2008). The fact that soybean aphid biotypes with the ability to overcome *Rag1* and *Rag2* resistance were identified before soybean cultivars with those resistance genes were deployed into production suggests that there is high variability in virulence in soybean aphid populations present in North America, posing a significant challenge to soybean breeders developing soybean aphid resistant cultivars. Resistance gene stacking is a method used by breeders to improve the durability of plant disease resistance (Boyd 2006); however, results of this study suggested that stacking *Rag1* and *Rag2* together may not provide long-term resistance to the soybean aphid. The search for new resistance genes will need to continue. As with other aphid pests (Haley et al. 2004, Wang et al. 2004), new soybean aphid biotypes probably will be identified concomitantly with the identification of new resistance genes.

The distributions of the soybean aphid biotypes are unknown. However, with the ability of soybean aphid alates to fly over long distances, they may be widespread. Evidence of biotype diversity present in North America in the absence of selection pressure by resistance genes suggests that there may not be significant fitness costs experienced by soybean aphids with unnecessary virulence genes that could limit their distribution and persistence. Virulence of the potato aphid, *Macrosiphum euphorbiae* (Thomas), was shown to be persistent in the absence of selection pressure by the *Mi* resistance gene in tomato, *Lycopersicon esculentum* Mill. (Goggin et al. 2001).

Future work to identify markers closely associated with soybean aphid virulence would aid in studies of the distribution of biotypes. Knowledge on the occur-

rence and distribution of soybean aphid biotypes in different geographic areas could be used by the soybean seed industry to determine where to market soybean cultivars with particular soybean aphid resistance genes and would help soybean producers to select appropriate resistant cultivars based on the virulence potential in their area.

Acknowledgments

We are grateful to the United Soybean Board for providing the majority of the funding for this work.

References Cited

Associated Press. 2003. Aphids whittling soybean farmers' profits. (http://www.highbeam.com/doc/1P2-11104969.html).

Boyd, L. A. 2006. Can the durability of resistance be predicted? J. Sci. Food Agric. 86: 2523–2526.

Caspers-Simmet, J. 2008. Soybean-aphid resistant varieties will come with a difference. Agri News. (http://webstar.agrinews.com/agrinews/295310252644869.bsp).

Clark, A. J., and K. L. Perry. 2002. Transmissibility of field isolates of soybean viruses by *Aphis glycines.* Plant Dis. 86: 1219–1222.

Domier, L. L., I. J. Latorre, T. A. Steinlage, N. McCoppin, and G. L. Hartman. 2003. Variability and transmission by *Aphis glycines* of North American and Asian *Soybean mosaic virus* isolates. Arch. Virol. 148: 1925–1941.

Fehr, W. R., C. E. Caviness, D. T. Burmood, and J. S. Pennington. 1971. Stage of development descriptions for soybeans, *Glycine max* (L.) Merr. Crop Sci. 11: 929–931.

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

Haley, S. D., F. B. Peairs, C. B. Walker, J. B. Rudolph, and T. L. Randolph. 2004. Occurrence of a new Russian wheat aphid biotype in Colorado. Crop Sci. 44: 1589–1592.

Hartman, G. L., L. L. Domier, L. M. Wax, C. G. Helm, D. W. Onstad, J. T. Shaw, L. F. Solter, D. J. Voegtlin, C. J. D'Arcy, M. E. Gray, et al. 2001. Occurrence and distribution of *Aphis glycines* on soybeans in Illinois in 2000 and its potential control. (http://www.plantmanagementnetwork.org/pub/php/brief/aphisglycines/).

Hill, J. H., R. Alleman, D. B. Hogg, and C. R. Grau. 2001. First report of transmission of *Soybean mosiac virus* and *Alfalfa mosiac virus* by *Aphis glycines* in the new world. Plant Dis. 85: 561.

Hill, C. B., Y. Li, and G. L. Hartman. 2004a. Resistance of *Glycine* species and various cultivated legumes to the soybean aphid (Homoptera: Aphididae). J. Econ. Entomol. 97: 1071–1077.

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

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

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

Hill, C. B., K.-S. Kim, L. Crull, B. W. Diers, and G. L. Hartman. 2009. Inheritance of resistance to the soybean aphid in soybean PI 200538. Crop Sci. 49: 1193–1200.

Kang, S.-T., M. A. Rouf Mian, and R. B. Hammond. 2008. Soybean aphid resistance in PI 243540 is controlled by a single dominant gene. Crop Sci. 48: 1744–1748.

Kim, C., G. Schaible, L. Garrett, R. Lubowski, and D. Lee. 2008a. Economic impacts of the US soybean aphid infestation: a multi-regional competitive dynamic analysis. Agric. Res. Econ. Rev. 37: 227–242.

Kim, K.-S., C. B. Hill, G. L. Hartman, M.A.R. Mian, and B. W. Diers. 2008b. Discovery of soybean aphid biotypes. Crop Sci. 48: 923–928.

Li, Y., C. B. Hill, and G. L. Hartman. 2004. Effect of three resistant soybean genotypes on the fecundity, mortality, and maturation of soybean aphid (Homoptera: Aphididae). J. Econ. Entomol. 97: 1106–1111.

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

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

Mian, M.A.R., S. T. Kang, S. E. Beil, and R. B. Hammond. 2008. Genetic linkage mapping of the soybean aphid resistance gene in PI 243540. Theor. Appl. Genet. 117: 955–962.

Ragsdale, D. W., D. J. Voegtlin, and R. J. O'Neil. 2004. Soybean aphid biology in North America. Ann. Entomol. Soc. Am. 97: 204–208.

Steffey, K. L. 2004. Soybean aphids in Illinois. (http://www.ipm.uiuc.edu/fieldcrops/insects/soybean_aphids/workshop/SoybeanAphidsInIllinois_Steffey.ppt).

Venette, R. C., and D. W. Ragsdale. 2004. Assessing the invasion by soybean aphid (Homoptera: Aphididae): where will it end? Ann. Entomol. Soc. Am. 97: 219–226.

Voegtlin, D. J., R. J. O'Neil, and W. R. Graves. 2004. Tests of suitability of overwintering hosts of *Aphis glycines:* identification of a new host association with *Rhamnus alnifolia* L'Heritier. Ann. Entomol. Soc. Am. 97: 233–234.

Voegtlin, D. J., R. J. O'Neil, W. R. Graves, D. Lagos, and H.J.S. Yoo. 2005. Potential winter hosts of soybean aphid. Ann. Entomol. Soc. Am. 98: 690–693.

Wang, R. Y., and S. A. Ghabrial. 2002. Effect of aphid behavior on efficiency of transmission of Soybean mosaic virus by the soybean-colonizing aphid, *Aphis glycines.* Plant Dis. 86: 1260–1264.

Wang, Y.-M., P.-F. Zhang, and J.-Q. Chen. 2004. Host-preference biotypes of the cotton aphid, *Aphis gossypii* Glover and the behavioral mechanism in their formation. Acta Entomol. Sin. 47: 760–767.

Wu, Z. S., D. Schenk-Hamlin, W. Y. Zhan, D. W. Ragsdale, and G. E. Heimpel. 2004. The soybean aphid in China: a historical review. Ann. Entomol. Soc. Am. 97: 209–218.

Zhang, G., C. Gu, and D. Wang. 2009. Molecular mapping of soybean aphid resistance genes in PI 567541B. Theor. Appl. Genet. 118: 473–482.

Received 29 May 2009; accepted 18 November 2009.