

# Resistance to the Soybean Aphid in Soybean Germplasm

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## ABSTRACT

With an efficient greenhouse screening method, the first resistance to the soybean aphid (*Aphis glycines* Matsumura) was found in cultivated soybean [*Glycine max* (L.) Merr.] germplasm. No resistance was found in 1425 current North American soybean cultivars, 106 Maturity Group (MG) 000 through VII Asian cultivars, and in a set of 11 'Clark' isolines possessing different pubescence traits. Dense pubescence did not provide protection against the soybean aphid. Resistance was discovered and established in three ancestors of North American genotypes: 'Dowling', 'Jackson', and PI 71506. Expression of resistance in those genotypes was characterized in choice and nonchoice tests. In choice tests, significantly fewer aphids occurred on Dowling, Jackson, and PI 71506 plants compared with susceptible cultivars ( $P = 0.05$ ). Aphid populations did not develop on Dowling and Jackson in nonchoice tests, indicating that there was a negative impact on aphid fecundity on those cultivars. That evidence combined with observations of aphid mortality on those cultivars suggested that antibiosis-type resistance contributed to the expression of resistance. Possible donors of resistance to Dowling and Jackson were identified. In nonchoice tests, population development on PI 71506 was not significantly different from development on susceptible cultivars, indicating that antixenosis was more important in that genotype. Resistance was expressed in all plant stages. Dowling provided season-long protection against aphids equal to the use of the systemic insecticide imidacloprid {1-[6-chloro-3-pyridinyl)methyl]-N-nitro-2-imidazolidinimine} in a field test. Four other germplasm accessions, 'Sugao Zarai', 'Sato', 'T260H', and PI 230977, had levels of resistance not significantly different from Dowling, Jackson, and PI 71506 in a choice test ( $P = 0.05$ ).

SOYBEAN IS A MAJOR CROP in the USA, with >75 million Mg of grain produced in 2000 (USDA, 2002). A new threat to soybean production in the USA, the soybean aphid, recently arrived.

A native of Asia, the soybean aphid was first found in the Midwest in 2000 (Hartman et al., 2001). It rapidly spread throughout the region and into other parts of North America (Patterson and Ragsdale, 2002). High aphid populations can reduce crop production directly when their feeding causes severe damage such as stunting, leaf distortion, and reduced pod set (Sun et al., 1990). Yield losses attributed to the aphid in some fields in Minnesota during 2001, where several thousand aphids occurred on individual soybean plants, were >50% (Ostlie, 2002) with an average loss of 101 to 202 kg ha<sup>-1</sup> in those fields (Patterson and Ragsdale, 2002). In earlier reports from China, soybean yields were reduced up to 52% when there was an average of about 220 aphids per plant (Wang et al., 1994) and plant height was decreased by about 210 mm after

severe aphid infestation (Wang et al., 1996). An additional threat posed by the aphid is its ability to transmit certain plant viruses to soybean such as *Alfalfa mosaic virus*, *Soybean dwarf virus*, and *Soybean mosaic virus* (Sama et al., 1974; Iwaki et al., 1980; Hartman et al., 2001; Hill et al., 2001; Clark and Perry, 2002).

*Aphis glycines* and close relative *A. gossypii*, the cotton or melon aphid, are the only aphid species found colonizing soybean in the USA. In other parts of the world, *A. craccivora*, *Aulacorthum solani*, and other species have been found colonizing soybeans (D. Voegtlin, personal communication, 2003).

*Aphis glycines* has a heteroecious, holocyclic life-cycle pattern (Guang-xue and Tie-sen, 1982). *Rhamnus cathartica* (buckthorn) is the primary host of *A. glycines* (Hartman et al., 2001) and soybean is a secondary host. In autumn, when the soybean crop matures, the aphid moves to *R. cathartica*, where mating and oviposition occurs. The egg stage overwinters on *R. cathartica*. During the following spring, the eggs hatch and a few generations are produced before alatae (winged females) fly to soybean.

Because *A. glycines* is a recent pest in the USA, a comprehensive integrated management approach to control the aphid has yet to be developed. Research to evaluate the efficacy of currently available insecticides and other control measures has just begun. Researchers in Minnesota have developed recommendations for the use of insecticides to control the aphid (Ostlie, 2002). Until other components of integrated pest management (IPM) are developed, soybean producers will need to rely on the use of insecticides to control the aphid.

An integral component of an IPM program to control aphids is plant resistance (Auclair, 1989; Harrewijn and Minks, 1989). Insect resistance can significantly reduce input costs for producers (Luginbill, 1969). Resistance was reported in *G. soja* (Sun et al., 1990), a close relative of *G. max* (Hymowitz, 1970), and other wild relatives (Zhuang et al., 1996). There are no reports of resistance in *G. max*. A report from Indonesia indicated that there was no resistance in a test of 201 soybean cultivars and breeding lines (Sama et al., 1974).

There are three basic kinds of resistance: tolerance, antixenosis, and antibiosis (Smith, 1989). Knowledge of the mechanism of resistance is necessary to develop effective screens to identify resistant plants. Choice tests, where aphids are allowed to choose their preferred hosts, help identify resistance, but do not distinguish between the types of resistance. Nonchoice tests, where aphid movement is restricted to a single host, help distinguish antibiosis, the effect of resistance on the biology of the aphid, from antixenosis, nonhost preference. Field tests to measure yield in the presence of aphid infestation

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**Abbreviations:** IPM, integrated pest management; MG, maturity group; RH, relative humidity; VIPS, Variety Information Program for Soybeans.

**Table 1.** Description of screening and replicated experiments conducted to evaluate soybean germplasm for resistance to the soybean aphid.

Exp.	Evaluation	No. of entries	Exp. design <sup>†</sup>	Type of test <sup>‡</sup>	Data recorded <sup>§</sup>
1	2001 MG <sup>¶</sup> II-IV commercial cultivars entered into the Illinois VIPS <sup>††</sup> testing program	798	screen	choice 1	index
2, 3	Short lists of VIPS commercial cultivars with putative resistance	24, 12	RCB-4	choice 1	index
4	2002 MG II-IV commercial cultivars entered into the Illinois VIPS testing program	644	screen	choice 1	index
5	Commercial cultivars adapted to the SE USA (obtained from P. Raymer, Univ. of Georgia)	79	RCB-2	choice 1	index
6	Asian PI accessions (assembled by R. Nelson, USDA Soybean Germplasm Curator)	109	RCB-3	choice 1	index
7, 8	11 'Clark' near-isogenic lines having different pubescence (Bernard and Weiss, 1973; Bernard et al., 1991; Specht et al., 1985) genes	12	RCB-4	choice 2	population
9	North American soybean ancestors (Gizlice et al., 1994)	87	RCB-3	choice 1	index
10	North American ancestors not previously screened and 'Stonewall'	24	RCB-2	choice 1	index
11, 12	Test of resistant germplasm I & II	7	RCB-3	choice 2	population
13	Origin of resistance in ancestral germplasm	24	RCB-4	choice 1	index
14, 15	Test of resistant germplasm III & IV	8	CR-4	nonchoice	population
16	Clonal variability on resistant germplasm	8	RCB split plot-3	choice 1	index
17	Comparison of resistance with insecticide use	8	split block-4	choice 1	index

<sup>†</sup> Screen (nonreplicated); RCB = randomized complete block, number after dash indicates the number of replicates; and CR = completely randomized, number after dash indicates the number of replicates.

<sup>‡</sup> Choice 1 = infested leaves transferred to test plants during the  $V_c$ -stage; Choice 2 = migration of alatae during the  $V_1$ -stage; nonchoice = single viviparous alate (Exp. 13) and two first instar nymphs (Exp. 14) transferred to test plants.

<sup>§</sup> Index = aphid population density (0, no live aphids, to 3, high aphid density)  $\times$  aphid damage (0, no damage, to 3, severe damage) 3 wk after infestation; and population counts 5, 7 d (Exp. 7), 9 d (Exp. 10, 11), 13 d (Exp. 14), and daily (Exp. 13) after transfer of aphids.

<sup>¶</sup> MG, maturity group.

<sup>††</sup> VIPS = Variety Information Program for Soybeans.

help identify tolerance, the ability to produce similar yield in the presence or absence of aphids.

Resistance can be eroded by the rise of biotypes that can overcome resistance (Auclair, 1989; Smith, 1989). There is no information on the existence of biotypes in *A. glycines*.

The objectives in this study were to identify resistance to the soybean aphid in soybean, determine type of action of resistance (antibiosis, antixenosis, tolerance), examine variation among aphid clones toward plant resistance, and compare the agronomic performance of resistant and susceptible cultivars under severe aphid infestation and when protected by a systemic insecticide. The studies were conducted in the greenhouse and in the field.

## MATERIALS AND METHODS

### Experiments

Table 1 lists the experiments that were conducted to evaluate soybean germplasm for resistance to the soybean aphid and provides information on number of entries, experimental design, type of test, and the parameters measured. A systematic approach was used to screen the following soybean germplasm for resistance: (i) commercial cultivars entered into the Variety Information Program for Soybeans (VIPS) at the University of Illinois, (ii) commercial cultivars adapted to the Southern USA obtained from P. Raymer, University of Georgia (Exp. 1-5), (iii) a set of Asian cultivars assembled by R. Nelson, USDA Soybean Germplasm Curator (Exp. 6), (iv) a set of Clark isolines containing different pubescence genes (Bernard and Weiss, 1973; Specht et al., 1985; Bernard et al., 1991) (Exp. 7, 8), and (v) a collection of North American ancestral germplasm (Gizlice et al., 1994) (Exp. 9, 10).

Experiments 11, 12, 14, and 15 were conducted to confirm and characterize resistance that was discovered in the screening experiments. Possible origins of resistance in three ancestral lines were tested in Exp. 13. Experiment 16 was designed to test potential variability among aphid clones toward colonization on resistant germplasm. A field experiment (Exp. 17) was conducted to compare the effects of resistance with the

effects of a systemic insecticide on aphid colonization, plant height, dry mass, number of pods, number of seeds, seed yield, and 100-seed weight of resistant and susceptible germplasm.

### Aphid Culture

Three clones of soybean aphids were established from single first-instar nymphs isolated from different aphid collections. One clone was from Urbana, IL, one from Brown County, Illinois, and the third clone came from Oxford County, Iowa. David Voegtlin (Illinois Natural History Survey, Urbana, IL 61801) confirmed the identification of the aphids as *A. glycines*. Aphids were reared on a continuous supply of seedlings of the soybean cultivar Williams 82 grown inside plant growth chambers at 22°C, the optimum temperature for population development (Hirano et al., 1996) and under continuous 300  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PAR irradiation, and 70% relative humidity (RH). The Urbana clone was used in all resistance-screening experiments described below.

### Plant Culture

Plants in greenhouse experiments were grown in soilless potting medium (Sunshine Mix, LC1, Sun Gro Horticulture Inc., Bellevue, WA),<sup>1</sup> in plastic multi-pot inserts (Hummert Intl., Earth City, MO) (pot sizes ranged from 30  $\times$  40  $\times$  60 mm to 60  $\times$  60  $\times$  60 mm), contained inside plastic trays without holes (Hummert Intl., #F1020) (Exp. 1-13, 16), or in 125- $\times$  87.5-mm plastic azalea pots (Hummert Intl.) (Exp. 14, 15). Size and number of multi-pots per insert used depended on the number of test entries and the experimental design. Before planting, multi-pots were filled to capacity with the potting medium and then the medium was premoistened to field capacity. Two seeds of each test entry were placed in a shallow depression ( $\approx$ 10 mm deep) made by lightly pressing a finger into the potting medium, and were covered with  $\approx$ 5-mm layer of course-textured vermiculite (Strong-Lite, Sun Gro Horticulture Inc.). Slow release nutrient pellets (Nutricote 18-6-6,

<sup>1</sup> Trade and manufacturers' names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

Hummert Intl.) were evenly spread over the surface of the vermiculite to a density of 3 to 5 pellets per pot. Seedlings were thinned to one plant per pot after emergence.

### Choice Tests

Choice tests, where aphids were allowed to move to preferred host plants, were conducted in an air-conditioned, insecticide-free greenhouse, dedicated to aphid testing, and maintained at 22 to 25°C with supplemental continuous illumination provided by a mixture of 1000-W high-intensity discharge and high-pressure sodium lamps.

In most of the choice tests (Exp. 1–6, 9, 10, 13, 16), leaves from Williams 82 plants infested with the Urbana clone were placed on top of  $V_E$ -stage seedlings (Fehr and Caviness, 1977) arranged in randomized complete blocks with three or four replications and with a row of two to four plants per experimental unit. There were 50 to 200 aphids of all stages on each infested leaf used to transfer aphids to the test plants. The aphids moved from the infested leaves to the test seedlings within the first day after transfer, and after about 7 to 14 d, they distributed themselves on preferred hosts and developed colonies. To avoid disturbing the aphids, plants were bottom watered by flooding the trays containing the plants as needed.

Resistance was evaluated at periodic intervals after infestation with estimates of aphid colonization and plant damage or by actual counts of aphid populations on each experimental unit that consisted of a row of two to four plants. An aphid index was calculated by multiplying the estimate of aphid population size, 0 to 3, where 0 = no aphids observed to 3 = high aphid density (>100 aphids per plant), by the rating of plant damage, 0 to 3, where 0 = no perceptible damage to 3 = severe leaf distortion and stunting, or plant death, giving a range of possible index values from 0 to 9 (Hill et al., 2002).

Indirect aphid infestation was done during the  $V_1$ -stage (Fehr and Caviness, 1977) in Exp. 7, 8, and 11 and during the  $V_E$ -stage (Fehr and Caviness, 1977) plants in Exp. 12 by exposing test plants to viviparous alatae from neighboring infested plants in the greenhouse test plants. Aphid populations were counted 5 (Exp. 8), 7 (Exp. 7), and 9 d (Exp. 11, 12) after infestation.

### Nonchoice Tests

Characterization of the type of resistance in resistant germplasm was studied in nonchoice tests conducted in a plant growth chamber with environmental conditions as described above. Two methods to apply and confine aphids to test plants were used. Experiments were arranged in completely randomized design with four replications and one plant per replication.

In the first method (Exp. 14), a single viviparous alate was placed on the abaxial side of the lamina of the center leaflet of a new, fully expanded trifoliolate leaf of individual  $V_1$ - to  $V_2$ -stage plants (Fehr and Caviness, 1977) with the aid of a moist camel's hair paint brush. Aphids were isolated on the leaves by attaching leaf cages over the aphids to restrict their movement. The cages were made with 1-mm-thick plastic tubing with a 10-mm i.d., cut 12 mm long, and covered with plastic mesh with 100- $\mu$ m openings (Sterling Net Co.) glued on one end. On the opposite end of the cage tubing, a 4-mm-wide  $\times$  4-mm-thick foam ring with a 8-mm i.d. and 12-mm o.d. was centered and glued on to provide a seal between the cage and leaf surface when attached to the leaf. Cages were placed over the aphids with the foam end down on the leaf surface and were fastened to the leaf with a metal clip held closed by spring tension. Alates were placed on four individual plants

of each test entry. New aphid offspring from each alate placed on each plant were counted and removed daily. After 12 d, the cumulative number of aphid offspring on each plant was determined.

For the second method (Exp. 15), two first instar nymphs were placed together on the abaxial side of a unifoliolate leaf of four different  $V_1$ -stage test plants of each test entry with a moist camel's hair paint brush. Individual plants were isolated in 100-  $\times$  300-mm clear plastic cylindrical cages, with 4-mm-thick walls, and with 80-  $\times$  180-mm side windows plus the top covered with a plastic mesh with 100- $\mu$ m openings (Sterling Net Co.). The open bottom of a cage was pressed into the soilless medium about 10 mm deep to prevent aphid escape. Total aphid populations on each plant were counted at 3- to 4-d intervals, up until 13 d after placing the nymphs on the test plants.

### Test of Variability of Aphid Clones

Resistant germplasm was challenged with the three aphid clones that were established, as described above, in a choice test to evaluate the effect of resistance on different soybean aphid clones (Table 1, Exp. 16). Single plants of five resistant and three susceptible cultivars were arranged in a split-plot design, with clones organized as main plots and cultivars as subplots, with three replications. The clones were kept isolated by placing plants in 32-pot inserts inside 360-  $\times$  510-  $\times$  380-mm cages made with 4-mm-thick clear Plexiglas. A 250-  $\times$  380-mm window was cut in the top of each cage for aeration and was covered with a plastic mesh with 100- $\mu$ m openings (Sterling Net Co.). Aphids were transferred onto  $V_E$ -stage seedlings and aphid population and damage ratings were recorded weekly for 3 wk.

### Field Cage Experiment

A field experiment was conducted at Urbana, IL, in 2002 to measure the agronomic performance of resistant and susceptible cultivars under severe aphid infestation and when protected by a systemic insecticide (Exp. 17). Plots of eight cultivars, three resistant and five susceptible, were arranged in a split block design with four replicates arranged in a RCB. Main plots were cultivars and subplots received either no insecticide treatment or a granular formulation of the systemic insecticide imidacloprid (1% G, Marathon, Olympic Horticultural Products, Mainland, PA) by applying a top-dress band at a rate of 4.2 mL  $m^{-1}$  of row when plants were transplanted into the cage. Plants of each cultivar were grown in a growth chamber maintained at 28°C, 70% RH, and 300  $\mu$ mol  $m^{-2} s^{-1}$  irradiation controlled by a timer programmed to give a 12-h photoperiod for 4 wk. The short photoperiod was required to synchronize the floral development of the plants of cultivars from several MGs. Six  $R_1$ -stage (first bloom) (Fehr and Caviness, 1977) plants were transplanted on 5 June 2002 into 0.3-m single-row main plots with 5-cm spacing between each plant. There were eight main plots in a range and eight ranges, with two ranges per replication. One of the ranges in each replication comprised the subplot that was treated with insecticide and the other range in a replication was the subplot that was not treated with the insecticide. Plots within a range were separated by 0.6 m and ranges were separated by 0.3 m. The experiment was contained inside a 6-  $\times$  6-  $\times$  2.1-m field cage with a 52  $\times$  52 threads per linear inch mesh polypropylene covering supported by a galvanized steel frame (Redwood Empire Awning and Furniture Co., Santa Rosa, CA). The distance from the cage covering and the plots was from 0.6 to 0.9 m. Controlled-release nutrient pellets (Nutricote 14-14-

14 N-P-K, Hummert Intl.) were top-dressed around the plants at a rate of 5 g per plant. The soil type at the Urbana, IL, location was a Parr silt loam (fine-loamy, mixed, active, mesic Oxyaquic Argiudolls). Plants were irrigated with an overhead sprinkler as needed. Immediately after transplanting, leaves infested with aphids from the Urbana clone were evenly distributed among the plots. One month later, aphid indices were recorded for each plot. At this point, plants reached the R<sub>2</sub> to R<sub>3</sub> stage (Fehr and Caviness, 1977) depending on the entry, MG, and the length of time needed to recover from transplanting. Reproductive development of Dowling (MG VIII) and Jackson (MG VII) was slower than the entries adapted to the location (MGs II–IV). At the R<sub>6</sub> stage (Fehr and Caviness, 1977), early to mid-September for adapted entries, the average plant height for each plot was measured. Height of Dowling and Jackson plants was measured very late in the season, 8 November, just before the first killing frost event, because they had not reached the R<sub>6</sub> stage. At the R<sub>8</sub> stage (Fehr and Caviness, 1977) for the adapted entries, late September to late October, physiological maturity date for each plot was recorded and plots were hand harvested by cutting each plant at the soil line. Plants of Dowling and Jackson did not mature before they were killed by frost. All surviving plants of each plot were bulked and dried. After drying, the total dry plant mass, seed mass, number of pods, number of seeds, and 100-seed mass were measured. Dry mass of Dowling and Jackson plants included leaves because they were gathered after being killed by frost and before they matured. For each parameter, means per plant were calculated for each plot. An analysis of variance was calculated for each parameter. Comparisons (single degree of freedom contrasts) of the means in the imidacloprid treatment vs. no insecticide treatment for each parameter were calculated for each cultivar.

### Statistical Analyses

All statistical data analyses were performed with the aid of JMP Version 5 (SAS Institute Inc., Cary, NC). Aphid population counts were first transformed to  $\log_{10} + 1$  before performing analysis of variance and least squared means were detransformed. Mean separation was done by calculating the LSD at  $P = 0.05$  when treatment means were significantly different ( $P < 0.05$ ) in the ANOVA.

## RESULTS

### Screening for Soybean Aphid Resistance

No resistance to the soybean aphid was found among the set of 798 commercial MG II through IV cultivars

tested in 2001, nor in subsequent retests (Table 1, Exp. 1–3). Similarly, no resistance was found in the set of 644 commercial MG II through IV cultivars tested in 2002 (94 cultivars were also included in the 2001 set) (Table 1, Exp. 4). All cultivars had aphid indices  $>5$  (0–9 possible), indicating that relatively high aphid densities occurred, accompanied by plant damage, including leaf discoloration (yellowing), leaf distortion, stunting, desiccation, and death. The susceptible checks had average aphid indices  $>7$ . Aphid indices for the tested commercial cultivars in 2001 can be found at the VIPS web site (<http://www.vipsoybeans.org>; verified 8 Oct. 2003).

There were no significant ( $P = 0.37$ ) differences among the entries in the test of 79 cultivars adapted to the southern USA (Table 1, Exp. 5). All but three of the cultivars tested had aphid indices  $>5$ . The cultivar Stonewall had an index of 4.0, and Asgrow AG6202 and SS RT7499N both had indices of 4.5. The susceptible checks had indices  $>6$ . Stonewall had an aphid index of 8.5 when retested in Exp. 10.

Significant ( $P < 0.001$ ) differences in aphid indices were found in the test of 106 Asian soybean cultivars 21 d after aphid infestation (Table 1, Exp. 6). However, all had indices  $>5$ , indicating a lack of resistance to the soybean aphid in this collection of cultivars. The susceptible checks had indices  $>8$ .

In tests of aphid population development on the Clark isolines with various types and densities of pubescence (Table 1; Exp. 7, 8), significant differences were found in both experiments (Table 2;  $P < 0.001$  in the first test and  $P = 0.009$  in the second test). Population development on all isolines was significantly higher or not significantly different from the susceptible check Williams 82 in both choice tests of those lines ( $P = 0.05$ ). Two isolines with *Pd* genes conditioning dense pubescence had the highest populations in the first experiment, and all three dense pubescent isolines with *Pd* genes had the highest populations in the second experiment. A glabrous isolate, L62-1385, had significantly lower populations than the dense pubescent isolines L62-1686 and L76-1815 in the first experiment and significantly lower than L62-1686 and L77-1040 in the second experiment ( $P = 0.05$ ).

**Table 2. Populations of soybean aphids on V<sub>1</sub>-stage plants of isolines of ‘Clark’ with various types and densities of pubescence in two choice tests (Table 1, Exp. 7 and 8).**

Genotype	PI†	Genotype	Phenotype	Aphid population per plant	
				Exp. 7‡	Exp. 8§
L62-1686	547415	<i>Pd1</i>	dense	81a¶	97a
L73-1046	547577	<i>Pd1</i>	dense	41bc	68ab
L76-1815	547649	<i>Pd1, Pd2</i>	dense	73a	67abc
L67-497	547481	<i>pa1, pa2</i>	appressed	43b	55bcd
L64-326	547457	<i>pa1, Ps-s</i>	semispars	29bcd	50bcd
L63-2999	547422	<i>Ps</i>	sparse	28bcd	49bcd
L62-1385	547412	<i>P1</i>	glabrous	24bcd	38cd
L76-1291	547634	<i>pa1</i>	semiappressed	30bcd	51bcd
‘Clark’	548533		normal density, erect, blunt	27bcd	45bcd
L73-1034	547576	<i>Pb</i>	sharp	23cd	37d
L63-2435	547421	<i>pc</i>	curly	20d	44bcd
‘Williams 82’				14d	48bcd

† Plant introduction accessions obtained from the USDA Soybean Germplasm Collection, University of Illinois, Urbana, IL 61801.

‡ Mean of three replications of four plants 7 d after being exposed to alate migrants.

§ Mean of three replications of four plants 5 d after being exposed to alate migrants.

¶ Means followed by the same letters are not significantly different by the least significant difference test ( $P = 0.05$ ).

**Table 3. Soybean aphid indices on V<sub>1</sub>-stage plants of 87 ancestral soybean lines or first progeny of ancestors of North American cultivars and three susceptible cultivars in a choice test 21 d after aphid infestation (Table 1, Exp. 9).**

PI†	Genotype	Aphid index‡	PI	Genotype	Aphid index
548663	'Dowling'	1.0	597387	'Pana'	6.3
71506		1.9	548318	'Dunfield'	6.5
548657	'Jackson'	2.8	548382	'Manitoba Brown'	6.8
548938	'Tracy'	3.1	548411	'Seneca'	6.8
548623	'Vansoy'	3.1	360955 B	'Fiskeby V'	6.9
548633	'Wye'	3.4	84946-2	'Kandokon'	6.9
548559	'Emerald'	4.0	548307	'Blackeye'	6.9
548624	'Verde'	4.0	542402	'Chico'	6.9
567790	'Curtis'	4.3	548379	'Mandarin (Ottawa)'	6.9
80837	'Mejiro'	4.5	548383	'Mansoy'	6.9
548402	'Peking'	4.5	548477	'Ogden'	6.9
548302	'Bansei'	4.8	548528	'Protana'	6.9
548561	'Hodgson'	5.0	548407	'Sac'	6.9
548195	'T204'	5.0	53048	'Vance'	6.9
96983		5.0	548626	'Wabash'	6.9
548603	'Perry'	5.1	438477	'Fiskeby 840-7-3'	7.0
548178	'T145'	5.1	548356	'Kanro'	7.0
548604	'Pershing'	5.3	548469	'Mammoth Yellow'	7.0
88811	'Pakute'	5.3	548391	'Mukden'	7.0
548311	'Capital'	5.5	248404	'Novosadska Bela'	7.0
548485	'Roanoke'	5.5	548488	'S-100'	7.0
84637		5.5	84631	'S-56'	7.0
88788		5.5	614088	'Loda'	7.0
33243	'Anderson'	5.6	518671	'Williams 82'	7.4
548456	'Haberlandt'	5.6	535807	'Crockett'	7.5
548463	'Laredo'	5.6	548548	'Delmar'	7.5
548595	'Maple Isle'	5.6	548325	'Flambeau'	7.5
548400	'Patoka'	5.6	548461	'Improved Pelican'	7.5
508269	'Stafford'	5.6	548360	'Korean'	7.5
548298	'A.K. (Harrow)'	5.8	548362	'Lincoln'	7.5
438471	'Fiskeby III'	5.8	548406	'Richland'	7.5
159925	'Glycine H'	6.0	240664	'Bilomi #3'	7.6
548336	'Habaro'	6.0	548352	'Jogun'	7.6
548493	'Tokyo'	6.0	548599	'Monroe'	7.6
548301	'Aoda'	6.3	180501	'Strain No. 18'	7.6
360955 A	'Fiskeby V'	6.3	54615-1		7.6
548342	'Higan'	6.3	513382	'Glenwood'	8.3
548348	'Illini'	6.3	548587	'Kim'	8.3
171450	'Kisaya'	6.3	548359	'Kingwa'	8.3
200492	'Komata'	6.3	317335	'Koganejiro'	8.3
171451	'Kosamame'	6.3	91110-1		8.3
548414	'Sioux'	6.3	548438	'Arksoy'	9.0
548169	'T117'	6.3	548457	'Hahto'	9.0
548193	'T201'	6.3	548484	'Ralsoy'	9.0
548494	'Volstate'	6.3	65338		9.0
Mean		6.2			
LSD(0.05)		3.1			

† Plant introduction accessions obtained from the USDA Soybean Germplasm Collection, University of Illinois, Urbana, IL 61801.

‡ Aphid index = aphid population (0, no live aphids, to 3, high aphid density) × aphid damage (0, no damage, to 3, severe damage). Mean of two replications of three plants each.

There were significant ( $P < 0.001$ ) differences in aphid indices (Table 3) among the set of 87 ancestral lines or first progeny of ancestors of North American soybean cultivars and susceptible checks tested in a choice experiment 21 d after aphid infestation (Table 1, Exp. 9). Three ancestors or first progeny, Dowling, PI 71506, and Jackson, had indices  $<3$ . Very few and often no live aphids were observed on Dowling. Fewer than 20 aphids were observed on PI 71506 and Jackson. There was no plant damage observed on Dowling, whereas on PI 71506 and Jackson, minor plant damage may have occurred on some plants, but it was often not clearly distinguishable. Nine other lines had aphid indices  $<5$ . They had moderate aphid densities accompanied with minor leaf discoloration and leaf distortion. Four of these, Tracy, Verde, Mejiro, and Peking, were retested (Exp. 8) and had aphid indices significantly ( $P = 0.05$ ) higher than Jackson, Dowling, 'Palmetto', and 'CNS'. The 75 remaining lines had moderate to high aphid densities accompanied with moderate to severe damage.

In another choice test (Table 1, Exp. 10), four additional germplasm accessions: Sugao Zairai, Sato, T260H, and PI 230977 had aphid indices not significantly different from Jackson, Dowling, Palmetto, CNS, and PI 71506 ( $P = 0.05$ ).

### Confirmation of Resistance

Further evaluation of the resistance of Dowling, PI 71506, and Jackson in replicated choice tests (Table 1; Exp. 11, 12) indicated that plants of all three had significantly lower populations of aphids than susceptible check plants (Table 4). Populations on Jackson plants were significantly ( $P = 0.05$ ) lower than on Dowling and PI 71506 plants in the first choice test (Exp. 11). In the second choice test (Exp. 12), populations on Jackson and Dowling plants were significantly ( $P = 0.05$ ) lower than on PI 71506 plants. Higher aphid populations developed when plants were exposed to alatae at the V<sub>c</sub>-stage in the second test compared with exposure at the

**Table 4. Populations of soybean aphids on V<sub>1</sub>- to V<sub>2</sub>-stage plants of three resistant and four susceptible soybean genotypes in two choice tests 9 d after exposure to alatae at two different plant stages (Table 1; Exp. 11, 12).**

Genotype	Number of aphids per plant	
	V <sub>1</sub> -stage exposure (Exp. 11)	V <sub>2</sub> -stage exposure (Exp. 12)
'Jackson'	9a†	15a
'Dowling'	15b	11a
PI 71506	19b	53b
'Williams 82'	42c	186c
'Loda'	48c	187c
'Pana'	55cd	152c
'Ina'	71d	150c
Mean	27	61

† Mean of three replications of four plants each. Means followed by the same letters are not significantly different by the least significant difference test ( $P = 0.05$ ).

V<sub>1</sub>-stage in the first test, although resistance appeared to be expressed at either stage. A number of dead aphids were observed on Dowling and Jackson in both tests.

### Origin of Resistance in Two Ancestral Accessions

There were highly significant ( $P < 0.001$ ) differences (Table 5) among the entries in the test to determine the origin of resistance in Dowling and Jackson (Table 1, Exp. 13). The aphid index of the cultivar Palmetto, a parent of Jackson, was not significantly different from Jackson, while aphid indices of Jackson's other parent, Volstate, and its grandparents 'Tokyo' and PI 54610 were significantly ( $P = 0.05$ ) greater than Jackson. CNS, a grandparent of Dowling, had an aphid index not significantly different from Dowling, while the aphid indices of all other ancestors of Dowling: 'A.K. (Harrow)', 'Ogden', 'Illini', 'Clemson', Tokyo, 'Semmes', 'Komata', 'S-100', 'Arksoy', and 'Ralsoy', were significantly ( $P = 0.05$ ) greater than Dowling. Again, very few live aphids were observed on Dowling, Jackson, PI 71506, and similarly on Palmetto and CNS. Several dead aphids were observed on Dowling, Jackson, and Palmetto. All susceptible checks had aphid indices  $>6$  and were significantly ( $P = 0.05$ ) higher than Palmetto, Jackson, Dowling, and PI 71506.

### Characterization of Type of Resistance

Nonchoice tests (Table 1; Exp. 14, 15) of the resistant genotypes CNS, Dowling, Jackson, Palmetto, and PI 71506, indicated that aphid population development on Dowling, Jackson, and Palmetto was significantly ( $P = 0.05$ ) lower than on CNS and PI 71506 (Table 6). Alatae failed to survive on some of the plants of Dowling, Jackson, and Palmetto after a few days. All but one of the first instar nymphs transferred in Exp. 15 (Table 1) survived after 2 d on the test plants (one died on a PI 71506 plant), but mortality increased through the course of the experiment on Dowling, Jackson, and Palmetto and development of the surviving nymphs appeared to be retarded or halted. That may explain why aphid populations were higher when alatae were used compared with first instar nymphs. Population development on

**Table 5. Response of V<sub>1</sub>-stage plants of two resistant soybean genotypes (Dowling, Jackson), their ancestors, PI 71506, and susceptible genotypes to the soybean aphid in a choice test 21 d after transfer of aphid infested leaves (Table 1, Exp. 13).**

Genotype	Aphid index (0-9)†
'Dowling'	1.3a‡
'Jackson'	1.8a
'Palmetto'	2.0ab
'CNS'	2.0ab
PI 71506	2.2ab
'A.K. (Harrow)'	3.8bc
'Ogden'	4.2cd
'Illini'	4.6cde
'Clemson'	4.7cdef
'Tokyo'	4.9cdef
'Semmes'	5.3cdefg
'Komata'	5.4cdefg
'S-100'	5.4cdefg
'Volstate'	5.7cdefgh
'DKB 31-51'	6.1defgh
'KSC 3706 CRR'	6.2efgh
'Loda'	6.5fghi
'Pioneer 93B01'	7.2ghij
'Williams 82'	7.4hij
'Arksoy'	7.5hij
'Ralsoy'	8.3ij
PI 54610	8.8j
Mean	5.0

† Aphid index = aphid population (0, no live aphids, to 3, high aphid density) × aphid damage (0, no damage, to 3, severe damage).

‡ Mean of four replications of two plants. Means followed by the same letters are not significantly different by the least significant difference test ( $P = 0.05$ ).

CNS and PI 71506 was not significantly lower than susceptible cultivars in experiment.

### Variation among Aphid Clones

Different soybean aphid clones did not produce significantly ( $P = 0.92$ ) different aphid indices on resistant and susceptible cultivars in the test (Table 1, Exp. 16) of the three clones established from aphids collected from different geographic areas, indicating that the clones had similar virulence on the set of cultivars tested. There also was no significant ( $P = 0.12$ ) clone by cultivar interaction, indicating that there was no host specialization among the three aphid clones. Differences among cultivars were highly significant ( $P < 0.001$ ).

**Table 6. Populations of soybean aphids on V<sub>1</sub> plants of five resistant and four susceptible soybean genotypes in two nonchoice experiments. Population counts were taken 12 d after transfer of a single viviparous alate to each plant in Exp. 14 and 13 d after transfer of two first instar nymphs in Exp. 15.**

Genotype	Number of aphids per plant	
	Viviparous alate (Exp. 14)	Two first instar nymphs (Exp. 15)
'Jackson'	1a†	1a
'Palmetto'	1a	2a
'Dowling'	2a	1a
PI 71506	29bc	28bc
'CNS'	35c	15b
'Pana'	49c	not tested
'Williams 82'	57c	24bc
'Ina'	not tested	67c
'Loda'	104c	60c
Mean	13	11

† Mean of four plants. Means followed by the same letters are not significantly different by the least significant difference test ( $P = 0.05$ ).

### Aphid Control with Resistance Compared with Use of a Systemic Insecticide

There were significant ( $P = 0.006$ ) differences (Table 7) in aphid indices among the eight genotypes in the treatment without imidacloprid in the field cage experiment (Table 1, Exp. 17). Indices for Dowling, Jackson, and PI 71506 were significantly lower than Pioneer 93B01, Ina, Loda, and Pana; however, they were not significantly lower than Williams 82. Very few if any aphids were observed on Dowling plants throughout the season, and no plant damage occurred. There were up to about 20 aphids observed on Jackson plants and up to about 50 aphids on PI 71506 plants observed at any given time during the season. Discernable foliar damage did not occur on Jackson plants, while mild leaf yellowing and minor leaf distortion did occur on PI 71506, presumably caused by aphid feeding as it was not observed on plants in the imidacloprid treatment. Dense aphid populations built up on all five of the susceptible cultivars until they progressively declined as a result of decreased availability of susceptible tissue because of severe damage caused by aphid feeding. Peak populations reached several thousand aphids on susceptible plants. Moderate leaf yellowing and leaf distortion occurred on Williams 82, while severe foliar damage occurred on the other susceptible cultivars, including strong leaf yellowing, gross leaf distortion, and severe desiccation of plant tissues. All plants of Loda, Pana, and Pioneer 93B01 in the treatment without imidacloprid died before maturation (R<sub>7</sub>-stage).

Plants in the imidacloprid treatment were completely protected from aphid feeding until late in the season, when a few live aphids began to appear on susceptible plants, apparently because the protective effects of the insecticide began to wear off. No damage occurred from the late aphid feeding.

As expected, development of Dowling (MG VIII) and Jackson (MG VII) plants was retarded compared with the entries adapted to the location (MGs II-IV)

and they did not fully mature before the end of the experiment; however, differences between the insecticide and noninsecticide treatments within a cultivar for some agronomic parameters were evident. There was a significant ( $P = 0.01$ ) treatment by cultivar interaction for 100-seed mass. In particular, single-degree-of-freedom contrasts or comparisons made between the treatments with and without imidacloprid indicated that Dowling resistance gave similar or equal protection against the soybean aphid as did imidacloprid (Table 7). No parameter means for Dowling in the treatment without imidacloprid were significantly different from the imidacloprid treatment means and the means were nearly identical for each parameter. Significant differences were found between the treatments in most parameters for Jackson and PI 71506, indicating that resistance in those cultivars did not protect the plants against the aphid as well as the resistance in Dowling did. In fact, Jackson and PI 71506 plants appeared to be stunted in the insecticide-free treatment, as indicated by significant differences in height between the imidacloprid and insecticide-free treatments. However, the agronomic performance of the susceptible cultivars, in particular Pioneer 93B01, Loda, Ina, and Pana, was much more severely affected by aphid feeding. Plants of those cultivars were not only severely stunted in the treatment without imidacloprid, but aphid feeding also killed many plants. Productivity of Ina and Loda was reduced to nearly zero by aphid feeding. Although aphid populations were also high on Williams 82, the effects of aphid feeding on agronomic performance were not as strong as on the other susceptible cultivars.

### DISCUSSION

This is the first report of the existence of resistance to the soybean aphid in North American soybean germplasm. A systematic approach and an efficient greenhouse screening method were used to find the resistance. The search began with a screen of current commercial

**Table 7. Comparisons of the agronomic performance of three resistant and five susceptible soybean genotypes under severe aphid infestation and when protected by the systemic insecticide imidacloprid in a field test conducted in Urbana, IL, in 2002 (Table 1, Exp. 17).**

Genotype	Aphid index (0-9) <sup>†</sup>	Height <sup>‡</sup>		Dry mass <sup>§</sup>		No. Pods <sup>§</sup>		No. Seeds <sup>§</sup>		Seed yield <sup>§¶</sup>		100-seed weight <sup>§¶</sup>							
		-	+	-	+	-	+	-	+	-	+	-	+						
		— cm —		— g —		— g —		— g —		— g —		— g —							
'Dowling'	1.1	93.8	90.6	ns <sup>††</sup>	64.3	66.8	ns	92	91	ns	129	124	ns	13.1	11.8	ns	2.1	1.7	ns
'Jackson'	2.0	65.6	93.1	*	37.1	64.0	**	49	78	**	80	131	*	10.3	17.9	0.02	2.3	3.0	ns
PI 71506	2.4	53.1	84.4	*	14.0	37.9	**	21	49	**	34	85	*	6.2	17.2	**	4.6	7.6	**
'Pioneer 93B01'	7.3	55.0	88.1	*	4.8	18.6	*	13	28	ns	27	92	**	2.4	11.7	**	1.5	2.5	*
'Ina'	7.9	64.4	90.6	*	2.3	20.9	**	6	43	**	9	90	**	1.0	10.3	**	2.7	2.4	ns
'Loda'	8.6	52.5	70.6	ns	1.6	15.4	*	5	26	*	8	58	*	0.7	8.8	**	1.6	2.5	ns
'Pana'	6.4	50.6	73.1	ns	9.3	20.3	ns	20	33	ns	40	76	ns	4.9	10.7	*	2.3	2.5	ns
'Williams 82'	3.9	72.5	94.4	ns	10.5	28.1	**	20	40	ns	35	100	**	4.1	15.7	**	2.0	2.6	ns

\* Significant at the 0.05 probability level.

\*\* Significant at the 0.01 probability level.

<sup>†</sup> Aphid indices on plots not treated with imidacloprid were calculated by multiplying a visual estimate of aphid population (0, no live aphids, to 3, high aphid density) × aphid damage (0, no damage, to 3, severe damage); the index values ranged from 0-9. LSD(0.05) = 4.2.

<sup>‡</sup> Mean plot height measured at the R<sub>6</sub> growth stage.

<sup>#</sup> - indicates no imidacloprid, + indicates imidacloprid applied.

<sup>§</sup> Mean per plant.

<sup>¶</sup> Weight at 10% moisture.

<sup>††</sup> ns, nonsignificant at the 0.05 probability level.

cultivars adapted to the North Central USA (Table 1, Exp. 1–4). Finding no resistance in those cultivars, it was hoped that resistance could be found in Asian cultivars (Table 1, Exp. 6), where the aphid originated. When none was found in those lines, a set of isolines possessing different pubescence traits was tested to determine if pubescence type or density might confer resistance (Table 1; Exp. 7, 8). It turned out that isolines having dense pubescence were more susceptible than glabrous or normal types (Table 2). Then, a set of ancestral germplasm was tested to determine if any resistance could be found in ancestors of North American soybean cultivars. Resistance in three ancestral genotypes, Dowling, Jackson, and PI71506, was identified (Table 3) in the test of ancestral germplasm (Table 1, Exp. 9). It was further characterized (Tables 4, 6) in subsequent choice and nonchoice tests (Table 1; Exp. 11, 12, 14, and 15), and established in a field test (Table 1, Exp. 17). Two ancestors of Dowling and Jackson, Palmetto and CNS (Table 1, Exp. 13), were found to be resistant (Table 5) and probable resistance donors. Resistance was identified in four additional germplasm accessions: Sugao Zairai, Sato, T260H, and PI 230977 in a choice test (Table 1, Exp. 10). Characterization of their resistance is in progress. Sugao Zairai and PI 230977 were sources of resistance to *Meloidogyne arenaria* race 2 (Luzzi et al., 1995).

All nine resistant genotypes belonged to MGs IV through VIII. It seemed possible that resistance could be found in current cultivars belonging to those MGs; however, no resistance was found in commercial cultivars belonging to those MGs that were tested in this study. This suggested that resistance present in ancestral germplasm did not persist through the development of current commercial soybean cultivars in the central and southern USA, probably because there was no selection pressure to aid breeders in identifying, selecting, and maintaining resistance without the presence of the soybean aphid in North America. The lack of resistance in the 106 Asian cultivars tested (Table 1, Exp. 6) was unexpected because they were developed where selection pressure was assumed to have existed, although there are no known reports of the existence of resistance in *G. max* in the Asian literature.

Nonchoice tests (Table 6) demonstrated that the resistance in Dowling, Jackson, and Palmetto had a strong antibiotic effect on the soybean aphid, as indicated by the lack of population development on those cultivars, apparently due to a negative impact on fecundity, and high mortality. In contrast, resistance in PI 71506 and CNS appeared to be primarily antixenosis or nonpreference type because high aphid populations were clearly discouraged on those cultivars in choice tests, while in the nonchoice tests, population development was not significantly lower than the development on susceptible cultivars. Although alates of uniform age were not used in Exp. 14 (Table 1), the magnitude of differences between resistant accessions and susceptible genotypes (Table 6) was great enough to limit the importance of variability in population development due to potential bias of the age of adult used to initiate colonies. The resistance in all five resistant genotypes appeared to be

expressed during all plant stages, suggesting that they would likely provide season-long protection.

Resistance in Jackson was probably derived from its parent Palmetto (USDA-ARS National Genetic Resources Program, 2003). The effect of both cultivars on aphid population development, survival, and fecundity were similar (Table 5). The origin of the resistance in Dowling was less clear because of the differences in the type of resistance expression in Dowling and its grandparent CNS (Table 5), as noted above. The genetic relationship between Dowling resistance and the resistance in Jackson is unknown; however, it seems likely that the resistance in PI 71506 is not genetically related to either the Jackson or Dowling resistance because of the differences in type of resistance expression. The inheritance and genetic relationships of these resistance sources is currently under study.

Dowling resistance, in particular, was demonstrated to provide protection to the soybean aphid equal to imidacloprid (Table 7). Imidacloprid was an effective aphicide in the field experiment, but it is not labeled for use in production of soybean. Other chemical substances that are labeled for use on soybean may be as effective in controlling the soybean aphid (Kim et al., 1987; Ostlie, 2002); however, genetic resistance is likely to be most economical (Luginbill, 1969), particularly if combining its use in an integrated control program with chemical and other control methods (Wang et al., 1998) maximizes its durability.

In contrast to reported effects of soybean pubescence on insect pests, such as reduced damage due to feeding by plant hoppers, *Empoasca fabae* (Harris) (Elden and Lambert, 1992), reduced feeding of the Mexican bean beetle, *Epilachna varivestis* Mulsant (Gannon and Bach, 1996), reduced leaf damage caused by the false melon beetle, *Atrachya menetriesi* Faldermann (Kanno, 1996), increased resistance to defoliation by lepidopterans (Lambert et al., 1992), and effects on the probing behavior of other aphid virus vectors (Gunasinghe et al., 1988), there was no protective benefit of dense pubescence against the soybean aphid (Table 2). In fact, the ability of the soybean aphid to colonize soybean irrespective of pubescence may have given it a selective advantage during its co-evolution with *G. max*. Soybean aphids feeding underneath trichomes may be protected from predation and parasitism.

Although a small sample of clones was tested (Table 1, Exp. 16), the lack of differences among the three clones indicated that the aphid population in the geographic region surveyed consisted of a single biotype or possibly the same clone. Nonsignificant clone by cultivar interaction indicated that variability in virulence toward the resistant cultivars, or host specialization, did not exist in the region. That suggested that the resistance would be effective throughout the region, initially at least, until genetic variability for virulence was introduced by mutation, migration, or another mechanism.

The screening methods used in this study proved to be efficient in screening large numbers of genotypes for resistance. Resistant phenotype expression, low aphid population densities with minimal plant damage, was



clearly distinguishable from susceptible responses. Resistance identified in the greenhouse tests held true in the field test.

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