Inheritance of Soybean Aphid Resistance in PI 567541B and PI 567598B

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ABSTRACT

In a previous study, two soybean [*Glycine max* (L.) Merr.] plant introductions (PIs), PI 567541B and PI 567598B, were found to possess anti-biosis-type resistance to the soybean aphid (*Aphis glycines* Matsumura). Plants with anti-biosis resistance negatively interfere with the reproduction of the aphid and thus control the insect effectively. Field studies were conducted to determine the inheritance of antibiosis resistance in PI 567541B and PI 567598B. The two resistant PIs were crossed with one or two susceptible soybean lines and the F_1 and F_2 plants and $F_{2:3}$ families were evaluated for aphid resistance. All F_1 plants were found to be susceptible to soybean aphids. The plants in seven F_2 populations segregated in a 15:1 susceptible/resistant ratio, which is the expected ratio for a trait controlled by two recessive genes. The $F_{2:3}$ families also segregated in a 15:1 susceptible/resistant ratio. Therefore, the segregation data suggest that two major recessive genes are involved in the resistance in PI 567541B and PI 567598B. This information will be useful to breeders for designing efficient breeding schemes for developing soybean cultivars with antibiosis resistance to aphids.

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Abbreviations: MG, maturity group; PI, plant introduction; SBA, soybean aphid.

THE SOYBEAN APHID (SBA; *Aphis glycines* Matsumura) was first discovered in eight midwestern U.S. states in 2000. Since then it has spread throughout the north–central United States and parts of Canada (North Central Soybean Research Program, 2004) and has become one of the major pests affecting soybean production in North America. Soybean aphid populations can double very quickly (McCornack et al., 2004), reaching thousands of aphids per plant. Aphid feeding reduces photosynthesis (Macedo et al., 2003) and reduces yield components including plant height, number of nodes and pods per plant, seed size, and bean quality (DiFonzo and Hines, 2002; Ostlie, 2001). In efficacy trials conducted in Michigan during SBA outbreak years, yield in untreated plots was 18 to 40% less than yield in treated plots (DiFonzo, 2006; DiFonzo and Hines, 2002).

Insecticides are still the primary means of controlling SBA, increasing production costs and human exposure. In 2005, an outbreak year for SBA across the Midwest, millions of acres were treated (USDA-National Agricultural Statistics Service, 2006). Insecticide applications also kill natural enemies of SBAs (Smith and Krischik, 1999) and may flare populations of other soybean pests such as spider mites. Host–plant resistance is the most effective means of control of insects. Soybeans resistant to SBA colonization

would eliminate or minimize the need for insecticides, reducing cost, environmental impacts, and exposure.

Since the discovery of SBA in the United States, significant effort has been put into the identification of sources of resistance. Hill et al. (2004) screened 1542 soybean accessions and identified seven, including Dowling and Jackson, with resistance to SBA. We evaluated 2147 soybean germplasm accessions in choice tests and identified four plant introductions (PIs), PI 567598B, PI 567541B, PI 567543C, and PI 567597C, with resistance to SBA (Mensah et al., 2005). In a no-choice test, PI 567598B and PI 567541B were found to possess antibiosis resistance (Mensah et al., 2005). Diaz–Montana et al. (2006) compared the reproduction of SBA on 240 soybean entries and found 11 entries with fewer nymphs than the susceptible checks. In a follow-up experiment they identified K1639 and Pioneer 95B97 as showing a strong antibiosis effect on SBA. Recently, Hesler et al. (2007) have also found two aphid resistance sources, PI 230977 with antibiosis resistance and G93-9223 (PI 595099) with antixenosis resistance. Currently only the resistance in Dowling and Jackson has been characterized; it was shown to be controlled by a single dominant gene (Hill et al., 2006a, 2006b). The inheritance of the other sources of aphid resistance has not yet been characterized.

Development of SBA-resistant cultivars is an objective in many public and private soybean breeding programs. For resistance sources to be useful in developing resistant plants, the genes conferring resistance must be characterized. The number of genes controlling resistance as well as the nature of the resistance determines the breeding method required to transfer this resistance into elite cultivars. The objective of this current study is to determine the inheritance of SBA resistance in the two antibiosis resistance sources: PI 567598B and PI 567541B.

MATERIALS AND METHODS

PI 567541B was crossed with E00075, and PI 567598B was crossed with 'Titan' and E00075. Both Titan and E00075 were susceptible to SBAs. Each F_1 plant was harvested separately to develop F_2 populations. The parental lines and F_1 plants of the cross Titan × PI 567598B were evaluated for SBA resistance in 2004 and the F_2 populations from the same cross were evaluated during 2005 in the field. Parental lines, F_1 plants, and F_2 populations from the crosses E00075 × PI 567541B and E00075 × PI 567598B were evaluated for aphid resistance in the field in 2005. The number of plants in each F_2 population is shown in Table 2. Evaluation of SBA resistance was performed in a 12.2 by 18.3 m aphid-proof cage in the field on the Michigan State University campus in East Lansing, MI. Two weeks after planting, when the plants were at the V2 stage (Fehr and Caviness, 1977), each plant was inoculated with two aphids according to the method described by Mensah et al. (2005). All aphids used in these tests were obtained from nearby naturally infested soybean fields. The F_1 plants were planted 30.5 cm apart with no replication and the parents were planted 5.1 cm apart with two replications. Each F_1, F_2, and parental plant was rated for aphid damage 2, 3, and 4 wk after inoculation using a rating scale of 0 to 4 described by Mensah et al. (2005).

Seeds from 376 individual F_2 plants in population 030104-8, which was developed from a single F_1 plant of the Titan × PI 567598B cross, were harvested individually during the fall of 2005. The 376 $F_{2:3}$ lines and the parents were evaluated for aphid resistance in the field during the summer of 2006. Depending on seed availability, up to 15 F_3 progenies from each F_2 plant were planted. Resistance evaluations were conducted in a field cage as described previously, but using a modified version of the rating scale described by Mensah et al. (2005). The rating scale used for F_1 and F_2 plants did not clearly distinguish between plants with low (one or two) versus moderate (tens of aphids) infestation. In 2006 half steps were added to the original 0 to 4 scale. The new scales were defined as: 0 = no aphids, plant is normal and healthy; 0.5 = less than 10 aphids per plant, no colony formation; 1 = 11 to 100 aphids per plant, plant appears normal and healthy; 1.5 = 101 to 150 aphids per plant, mostly on the young leaves of the plant; 2.0 = 151 to 300 aphids per plant, mostly on the young leaves and the tender stem at top of plant, plant appears normal and healthy; 2.5 = 301 to 500 aphids per plant, plant appears healthy; 3.0 = 501 to 800 aphids per plant, leaves slightly curly and shiny, young leaves and stems covered with aphids; 3.5 = more than 800 aphids per plant, plants stunted, leaves curled, slightly yellow, no sooty mold and few cast skins; and 4.0 = more than 800 aphids per plant, plants stunted, leaves severely curled, yellow, covered with sooty mold and cast skins. Each F_3 plant was rated weekly for three consecutive weeks starting 3 wk after inoculation.

When the susceptible parents first rated a score of 4.0, the data from that sample date were used to classify the F_2 or F_3 plants as resistant or susceptible. A plant with a rating of 1.5 or less was classified as resistant while a plant with a rating >1.5 was considered susceptible. The threshold of 1.5 was comparable to the threshold used to identify susceptible plants in our previous study (Mensah et al., 2005). Chi-square tests were performed to test the goodness of fit of observed segregation ratios among F_2 plants and $F_{2:3}$ families with different genetic ratios, with rejection at 0.05 level of probability.

RESULTS AND DISCUSSION

All F_1 plants from two of the three crosses were found to be susceptible with a rating greater than 1.5 (Table 1), indicating that resistance to SBA is controlled by recessive

Table 1. F_1 and parental soybean lines classified as resistant to soybean aphid.

Genotype	Total no. of plants tested	No. of resistant plants	Mean rating
PI 567541B	9	9	1.0
PI 567598B	12	12	1.0
E00075	8	0	4.0
'Titan'	13	0	4.0
(E00075 × PI567541B) F_1	6	0	3.3
(E00075 × PI567598B) F_1	12	0	3.0
(Titan × PI567598B) F_1	10	–	–

genes. Data for the F_1 plants in the third cross (Titan × PI 567598B) were not obtained due to poor infection in 2004 as a result of heavy rain and flood damage shortly after aphid inoculation. The overall frequency distribution of aphid colonization ratings in all F_2 populations was not normal and was skewed toward the susceptible parents (Fig. 1), suggesting that susceptibility was dominant over resistance. The distributions were continuous, indicating that more than one gene was involved in aphid resistance in the two PIs and the dominance of susceptibility over resistance was not complete. All the F_2 populations segregated in a 15:1 susceptible/resistant ratio (Table 2), which is the expected ratio for a trait controlled by two recessive genes with duplicate dominant epistasis. In both cases when E00075 was crossed with PI 567598B and PI 567541B the resulting F_2 populations also fit the 15:1 susceptible/resistant ratio confirming the recessive nature of the genes in a different population.

For the Titan × PI 567598B $F_{2:3}$ families, on average, eight seeds per family germinated. Out of the 376 $F_{2:3}$ families 25 were found to be resistant, fitting the 15:1 ratio with a P value of 0.258. Forty-five $F_{2:3}$ families derived from susceptible F_2 plants had segregated for resistance. The recessive nature of the resistance in PI 567598B and PI 567541B was confirmed in the $F_{2:3}$ families as all the resistant F_2 individuals produced resistant $F_{2:3}$ families. Due to the recessive nature of resistance in PI 567598B, it was expected that susceptible heterozygotes would segregate when the F_3 families were tested for aphid resistance. However, segregation was observed only in 45 $F_{2:3}$ families. This low number of $F_{2:3}$ segregating families might be due to low seed yield from susceptible F_2 plants and poor germination. Based on Fehr (1987), at least 11 plants are needed to have a 95% chance of identifying one resistant plant with a 0.25 expected frequency. On average, we had only eight plants per family; therefore many families did not have the minimum number of plants required to find a resistant plant in a segregating $F_{2:3}$ family.

The segregation data in the F_2 populations and $F_{2:3}$ families suggest that two major recessive genes are involved
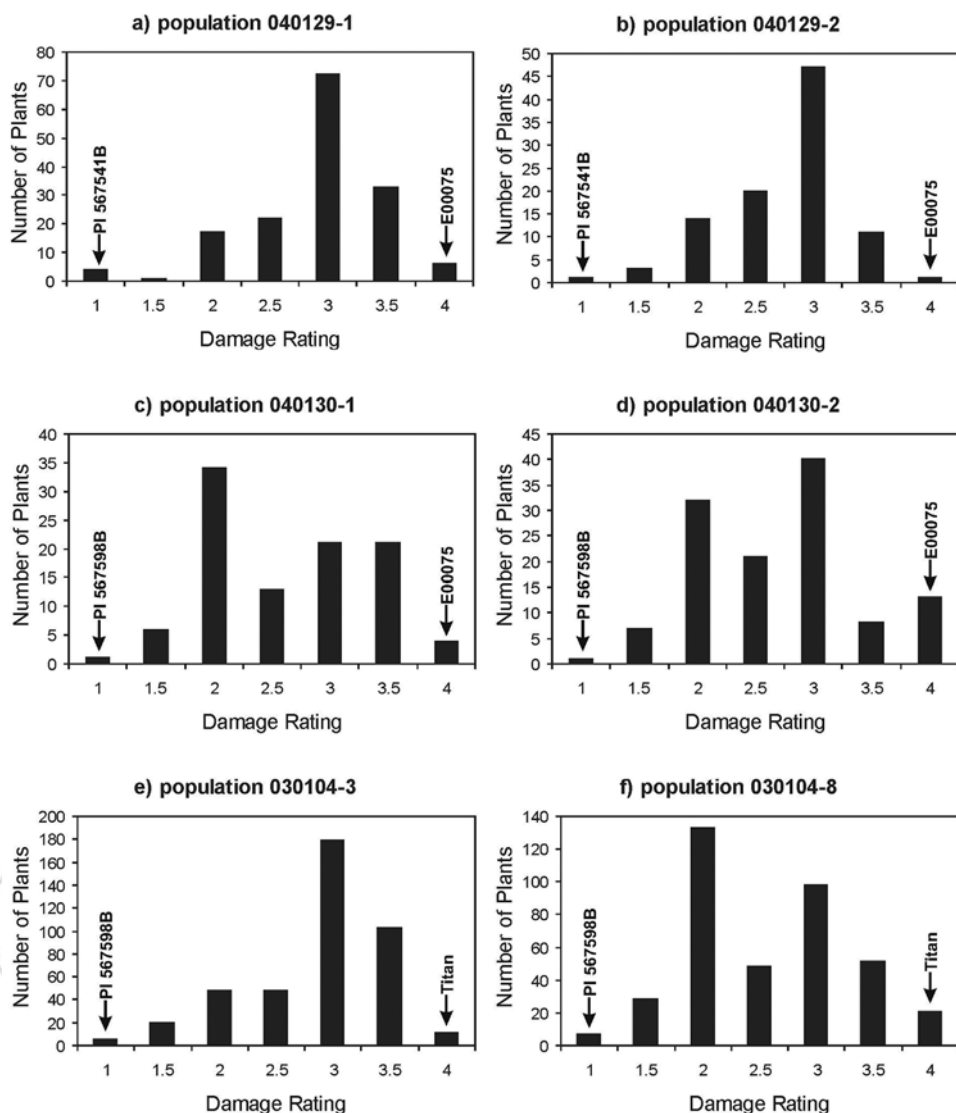

Figure 1. Distribution of damage rating scores in F_2 soybean populations: (a) 040129-1, (b) 040129-2, (c) 040130-1, (d) 040130-2, (e) 030104-3, and (f) 030104-8.

in aphid resistance in both PI 567598B and PI 567541B. However, the results cannot rule out the possibility that other minor genes are also involved in the resistance.

Soybean aphid resistance in the soybean cultivars Dowling and Jackson is controlled by a single dominant gene (Hill et al., 2006a, 2006b). Our study demonstrated that aphid resistance in the soybean PI 567598B and PI 567541B is controlled by two recessive genes, suggesting that different resistant genes from those in Dowling and Jackson underlie the resistance in these two PIs. Different genes and inheritance patterns for aphid resistance have also been reported in other crops. In wheat (*Triticum aestivum* L.), nine characterized genes (*Dn1, Dn2, dn3,* and *Dn4–Dn9*) are involved in resistance to the Russian wheat aphid [*Diuraphis noxia* (Kurdjumov)] (Du Toit, 1989; Harvey and Martin, 1990; Liu et al., 2001; Marais and Du Toit, 1993; Marais et al., 1998; Nkongolo et al., 1991a, 1991b; Schroeder-Teeter et al., 1994). Eight of the genes are independent dominant genes each conferring resistance in a

Table 2. Segregation of aphid resistance in F$_2$ soybean populations derived from susceptible (S) × resistant (R) crosses.

Population ID[†]	Susceptible parent	Resistant parent	Total no. of plants	Observed		Expected by a 15:1 (S/R) ratio		P value of χ^2 test[‡]
				S	R	S	R	
040129-1	E00075	PI 567541B	155	150	5	145.3	9.7	0.120
040129-2	E00075	PI 567541B	98	93	5	91.9	6.1	0.639
040130-1	E00075	PI 567598B	100	93	7	93.8	6.3	0.757
040130-2	E00075	PI 567598B	126	118	8	118.1	7.9	0.963
030104-3	'Titan'	PI 567598B	415	389	26	389.1	25.9	0.990
030104-8	'Titan'	PI 567598B	388	356	32	363.0	25.0	0.148
030104-10	'Titan'	PI 567598B	416	390	26	390.0	26.0	1.000

[†]Each F$_2$ population was developed from a single F$_1$ plant. F$_2$ populations developed from different F$_1$ plants of the same cross were considered different populations.

[‡]If the P value is larger than 0.05, the null hypothesis that the observed S/R ratio fits the expected 15:1 ratio is not rejected statistically.

different resistance source, while *dn3* is a recessive gene conferring the aphid resistance in *Triticum tauschii* (Coss.) Schmal. In barley (*Hordeum vulgare* L.), a single dominant gene controls the Russian wheat aphid resistance in the line S13 (Robinson et al., 1992), and two dominant genes control resistance in the line STARS-9577B (Mornhinweg et al., 2002).

As with all host plant resistance to insects or pathogens, there is the fear that the resistance will be overcome. In wheat, the resistance gene *Dn4,* found in many varieties, was overcome by a new biotype of Russian wheat aphid found in Colorado in 2003 (Haley et al., 2004). In a follow-up experiment, Haley et al. (2004) found that only one of the nine resistance genes, *Dn7,* conferred resistance to the new biotype. In 2006, three new aphid biotypes were identified based on the foliar damage they caused; one biotype was virulent to eight of the nine sources of Russian wheat aphid resistance in wheat (Burd et al., 2006). Each of the eight sources carried different genes conferring resistance to Russian wheat aphid. The adaptive ability of aphids in general to overcome plant resistance through biotype differentiation highlights the need to explore the genetic diversity of SBA resistance. Variation of SBA biotypes has been observed in the United States (Ki-Seung et al., 2007; Mensah et al., 2007). Some biotypes have overcome the resistance from Dowling and Jackson but not the resistance from PI 567598B and PI 567541B (Ki-Seung et al., 2007; Mensah et al., 2007). Therefore, different sources of resistance must be used to develop SBA-resistant cultivars.

In general, resistance controlled by multiple genes is more durable than the resistance controlled by a single dominant gene (Duvick, 1999). Thus the resistance from PI 567541B and PI 567598B may be more durable than the single gene–controlled resistance from Dowling and Jackson. However, more effort will be required to incorporate the resistance from these two PIs into elite germplasm because larger progeny populations are required to recover at least one resistant progeny with the resistance.

The information on the recessive inheritance of the SBA resistance detected in this study is useful to breeders in developing special schemes in breeding programs to incorporate this resistance in elite breeding lines. In breeding for insect resistance, backcrossing is the major approach for introducing resistance into an otherwise superior cultivar. Selfing after each backcross can be used to select lines with the recessive resistance gene. If markers associated with the genes are identified, marker–assisted selection can be used to identify resistant lines faster, and therefore incorporation of the recessive genes into new cultivars will be easier and faster (Chen and Line, 1999). Genetic populations have been developed to test for allelism of genes controlling aphid resistance in these two PIs. Research is ongoing to identify molecular markers associated with the resistance genes in this study.

References
Burd, J.D., D.R. Porter, G.J. Puterka, S.D. Haley, and F.B. Peairs. 2006. Biotypic variation among North American Russian wheat aphid populations. J. Econ. Entomol. 99:1862–1866.

Chen, X.M., and R.F. Line. 1999. Recessive genes for resistance to races of *Puccinia striiformis* f. sp. *hordei* in barley. Phytopathology 89:226–232.

Diaz-Montano, J., J.C. Reese, W.T. Schapaugh, and L.R. Campbell. 2006. Characterization of antibiosis and antixenosis to the soybean aphid (Hemiptera: Aphididae) in several soybean genotypes. J. Econ. Entomol. 99:1884–1889.

DiFonzo, C.D. 2006. Soybean aphid chemical control: Foliar sprays. Available at www.ipm.msu.edu/cat06field/fc04-06-06.htm (verified 12 July 2008). Field Crop Advisory Team Alert Newsl. 21(2).

DiFonzo, C.D., and R. Hines. 2002. Soybean aphid in Michigan: Update from the 2001 season. Michigan State Univ. Ext. Bull. E-2748. Michigan State Univ., East Lansing.

Du Toit, F. 1989. Inheritance of resistance in two *Triticum aestivum* lines to Russian wheat aphid (Homoptera: Aphididae). J. Econ. Entomol. 82:1251–1253.

Duvick, D.N. 1999. Consequences of classical plant breeding for pest resistance. *In* Proc. of a Workshop on Ecological Effects

of Pest Resistance Genes in Managed Ecosystems, Bethesda, MD. 31 Jan.–3 Feb. 1999. Information Systems for Biotechnology, Blacksburg, VA.

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.

Harvey, T.L., and T.J. Martin. 1990. Resistance to Russian wheat aphid, *Diuraphis noxia*, in wheat (*Triticum aestivum*). Cereal Res. Commun. 18:127–129.

Hesler, S.L., K.E. Dashiell, and J.G. Lundgren. 2007. Characterization of resistance to *Aphis glycines* in soybean accessions. Euphytica 154:91–94.

Hill, C.B., Y. Li, and G.L. Hartman. 2004. 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.

Fehr, W.R. 1987. Principles of cultivar development. McGraw-Hill, New York.

Fehr, W.R., and C.E. Caviness. 1977. Stages of soybean development. Special Rep., Agric. and Home Economics Exp. Stn. No. 80. Iowa State University, Ames.

Ki-Seung, K., C. Hill, G. Hartman, and B. Diers. 2007. Identification of a new soybean aphid biotype. *In* Annual meeting abstracts [CD-ROM]. ASA, CSSA, and SSSA, Madison, WI.

Liu, X., C.M. Smith, B.S. Gill, and V. Tolmay. 2001. Microsatellite markers linked to six Russian wheat aphid resistance genes in wheat. Theor. Appl. Genet. 102:504–510.

Macedo, B., C.S. Bastos, L.G. Higley, K.R. Ostlie, and S. Madhavan. 2003. Photosynthetic responses of soybean to soybean aphid (Homoptera: Aphididae) injury. J. Econ. Entomol. 96:188–193.

Marais, G.F., and F. Du Toit. 1993. A monosomic analysis of Russian wheat aphid resistance in the common wheat PI 294994. Plant Breed. 111:246–248.

Marais, G.F., W.G. Wessels, M. Horn, and F. Du Toit. 1998. Association of stem rust resistance gene (*Sr45*) and two Russian wheat aphid resistance genes (*Dn5* and *Dn7*) with mapped structural loci in common wheat. S. Afr. J. Plant Soil 15:67–71.

McCornack, B., D.W. Ragsdale, and R.C. Venette. 2004. Demography of soybean aphid (Homoptera: Aphididae) at summer temperatures. J. Econ. Entomol. 97:854–861.

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.

Mensah, C., C. DiFonzo, and D. Wang. 2007. A case for the presence of soybean aphid biotypes in Michigan. *In* 2007 annual meeting abstracts [CD-ROM]. ASA, CSSA, and SSSA, Madison, WI.

Mornhinweg, D.W., D.R. Porter, and J.A. Webster. 2002. Inheritance of Russian wheat aphid resistance in spring barley germplasm line STARS-9577B. Crop Sci. 42:1891–1893.

North Central Soybean Research Program. 2004. Soybean aphid research update. Available at www.planthealth.info/aphids_researchupdate.htm (verified 12 July 2008). Plant Health Initiative, NCSRP, Urbandale, IA.

Nkongolo, K.K., J.S. Quick, A.E. Limin, and D.B. Fowler. 1991a. Sources and inheritance of resistance to Russian wheat aphid in *Triticum* species *amphiploid* and *Triticum tauschii*. Can. J. Plant Sci. 71:703–708.

Nkongolo, K.K., J.S. Quick, F.B. Peairs, and W.L. Meyer. 1991b. Inheritance of resistance of PI 372129 wheat to the Russian wheat aphid. Crop Sci. 31:905–907.

Ostlie, K. (ed.). 2001. Soybean aphid reduces yields: Harvest results from insecticide strip trials. Available at www.soybeans.umn.edu/crop/insects/aphid/studyresults.htm (verified 12 July 2008). Univ. of Minnesota, St. Paul.

Robinson, J., P.A. Burnett, H.E. Vivar, and F. Delgado. 1992. Russian wheat aphid in barley: Inheritance of resistance and yield loss. p. 94–97. *In* W.P. Morrison (comp.) Proc. Russian Wheat Aphid Conf., 5th. Great Plains Agric. Counc. Publ. 142, Rapid City, SD.

Schroeder-Teeter, S., R.S. Zemetra, D.J. Schotzko, C.M. Smith, and M. Rafi. 1994. Monosomic analysis of Russian wheat aphid (*Diuraphis noxia*) resistance in *Triticum aestivum* line PI 137739. Euphytica 74:117–120.

Smith, S.F., and V.A. Krischik. 1999. Effects of systemic imidacloprid on *Coleomegilla maculata* (Coleoptera: Coccinellidae). Environ. Entomol. 28:1189–1195.

USDA-National Agricultural Statistics Service. 2006. Agricultural chemical usage 2005 field crops summary. USDA-NASS, Washington, DC.