Journal of Economic Entomology, 114(3), 2021, 1336–1344
doi: 10.1093/jee/toab058
Advance Access Publication Date: 3 May 2021
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Plant Resistance

Variation in Soybean Aphid (Hemiptera: Aphididae) Biotypes Within Fields

S. J. Bhusal,[1] R. L. Koch,[2] and A. J. Lorenz[1,3]

[1]Department of Agronomy and Plant Genetics, University of Minnesota, 411 Borlaug Hall, 1991 Upper Buford Circle, Saint Paul, MN 55108, USA, [2]Department of Entomology, University of Minnesota, 219 Hodson Hall, 1980 Folwell Avenue, Saint Paul, MN 55108, USA, and [3]Corresponding author, e-mail: lore0149@umn.edu

Subject Editor: Kristopher Giles

Received 29 August 2020; Editorial decision 3 March 2021

Abstract

Soybean aphid (*Aphis glycines* Matsumura (Hemiptera: Aphididae)) has been a major pest of soybean in North America since its detection in this continent in 2000 and subsequent spread. Although several aphid resistance genes have been identified, at least four soybean aphid biotypes have been discovered, with three of them being virulent on soybean cultivars with certain soybean aphid resistance genes. These biotypes are known to vary across years and locations, but information on their variation within single fields is limited. An investigation was conducted to study the variation of soybean aphid biotypes within single townships and fields in Minnesota. Screening of 28 soybean aphid isolates collected from seven soybean fields (six soybean fields in Cairo and Wellington Townships of Renville County, MN and one field in Wilmar Township of Kandiyohi County, MN) revealed the existence of multiple known biotypes of soybean aphid within single fields of soybean. We found up to three biotypes of soybean aphid in a single field. Two biotypes were found in five fields while only one field had only a single biotype. Three isolates presented reactions on a panel of resistant and susceptible indicator lines that were different from known biotypes. These results highlight the importance of characterizing soybean aphid biotypes in small geographical areas and utilizing generated knowledge to develop soybean cultivars pyramided with multiple resistance genes. The outcome will be decreased use of insecticides, thereby improving economic and environmental sustainability of soybean production.

Keywords: soybean aphid biotype, resistance gene, virulence

Soybean aphid (*Aphis glycines* Matsumura), native to eastern Asia, was first detected in the United States in 2000. Soybean aphid has spread across at least 30 states of the United States and three provinces of Canada (Ragsdale et al. 2011). Soybean aphid is a major pest of soybean in Minnesota, causing significant loss of yield (up to 40%) and reduced seed quality (Ragsdale et al. 2007). Soybean aphids damage plants by sucking sap from plant tissues, resulting in stunted plant growth and decreased photosynthetic and transpiration rates (Wang et al. 1962, Macedo et al. 2003, Ragsdale et al. 2004). Growth of sooty mold on aphids' excretion (i.e., honeydew) further worsen plant photosynthesis and transpiration (Wu et al. 2004). Plant pathogenic viruses such as soybean mosaic virus and alfalfa mosaic virus can be transmitted by soybean aphids, contributing to yield reduction (Hill et al. 2001).

Management of soybean aphid has been heavily dependent on use of chemical insecticides, resulting in a dramatic increase of insecticide use in soybean fields (Ragsdale et al. 2011, Hodgson et al. 2012). Sole reliance on insecticides is not an ideal option as it can cause unintended environmental impacts, kill beneficial insects, trigger frequent pest outbreak, and lead to the development of insecticide resistance (Pedigo and Rice 2009). Hanson et al. (2017) and Menger et al. (2020) reported soybean aphids that had developed resistance to widely used insecticides.

Deployment of aphid-resistant soybean varieties, on the other hand, can be an effective, economical, and eco-friendly option by providing a preventive measure against aphid outbreaks. The development of biotypes capable of overcoming aphid-resistant varieties complicates the deployment of varietal resistance as an effective management tool. The evolution of biotypes is generally common within aphid species because of several characteristics related to their life cycle and feeding habits (Michel et al. 2011). For example, soybean aphid completes its sexual reproduction on buckthorn (*Rhamnus*

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spp., a primary host) during fall and overwinters there (Ragsdale et al. 2004), which may provide a source of inoculum of different biotypes within a geographical area. Since soybean aphids were first discovered in North America, four biotypes have already been identified and characterized (Fig. 1). Biotype 1 is avirulent to soybean genotypes that carry any genes of soybean aphid resistance known as *Rag* (Resistance to *Aphis glycines*) genes. Biotype 2 is virulent on soybean genotypes with the *Rag1* (Kim et al. 2008) and biotype 3 is virulent to soybean genotypes with *Rag2*, *Rag3*, *rag4*, or *rag1c* (Hill et al. 2010). Biotype 4 is virulent to *Rag1*, *Rag2*, and *Rag1* + *Rag2* genotypes (Alt and Ryan-Mahmutagic 2013). Widescale surveying of soybean aphid biotypes in North America reported that specific biotypes are not limited to specific geographical locations, but are widely distributed (Cooper et al. 2015, Crossley and Hogg 2015, Alt et al. 2019).

Knowledge on variation in biotypes within a single field or a township is limited. Using microsatelite molecular markers, Michel et al. (2009) found genetic variation among clones within single aphid populations collected at single sampling sites, but biotyping was not performed. Providing information on the frequency and distribution of different biotypes within single townships and even single fields would be informative for soybean breeders assessing the usefulness of new cultivars with *Rag* genes and farmers growing such cultivars as part of an integrated pest management system. Such information could also contribute to the development of models forecasting soybean aphid population shifts in soybean fields, and guide selection of aphid-resistant varieties for future cultivation. Therefore, we aimed to investigate the variation in known soybean aphid biotypes at the level of single fields spread across single townships in Minnesota.

Methods

Preliminary (Pilot) Test

A pilot study was performed in 2017 by collecting soybean aphid isolates from two different fields approximately 3 km apart in northwestern Minnesota (Roseau); two isolates from central Minnesota (one isolate each from Saint Paul and Rosemount, about 30 km apart); and two isolates from two different fields approximately 3 km apart in southwestern Minnesota (Lamberton). The six collected isolates were quarantined for 72 h in a growth chamber to

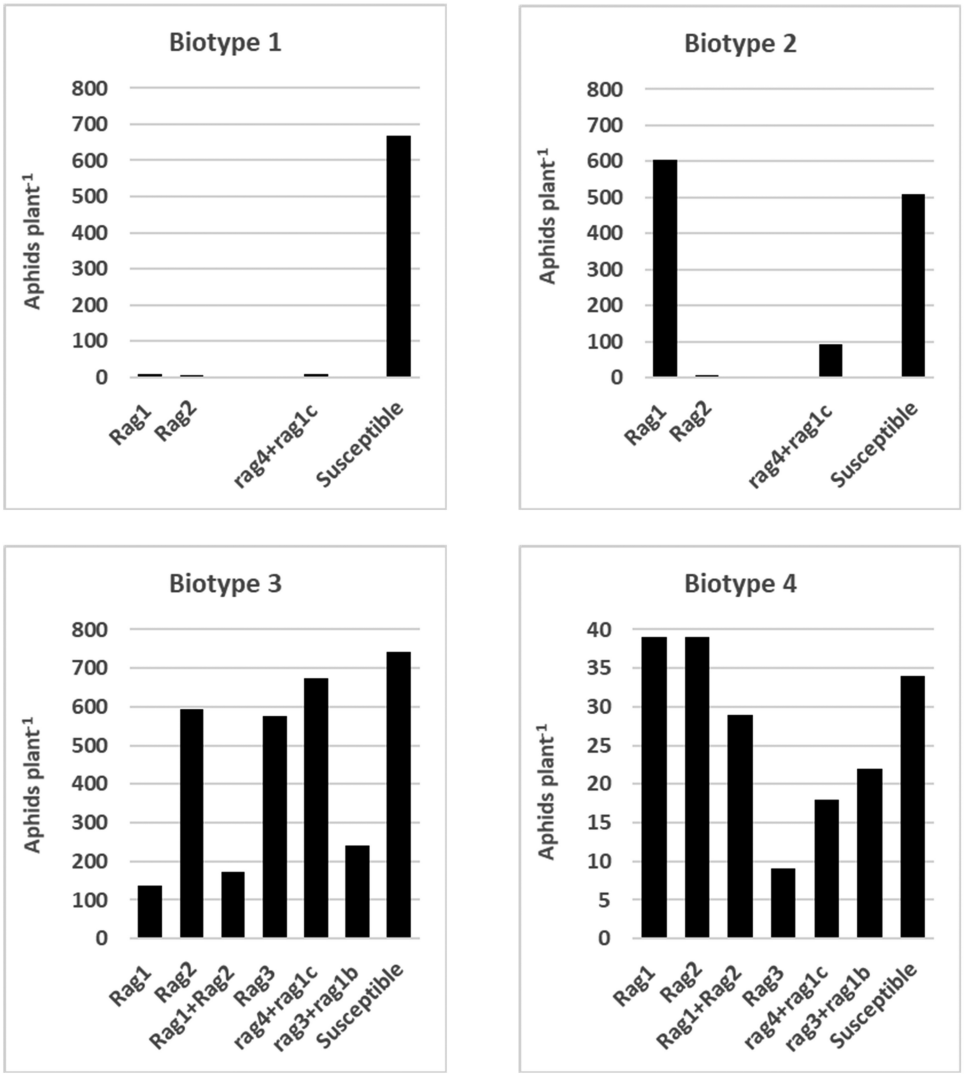

Fig. 1. Soybean aphids per plant on sets of soybean indicator lines carrying known resistance to *Aphis glycines* (*Rag/rag*) genes. Data were obtained from the original studies reporting the discovery of the biotypes. For biotypes 1 and 2 data were obtained from Kim et al. (2008). For biotype 3 data were obtained from Hill et al. (2010), and data were obtained from Alt and Ryan-Mahmutagic (2013) for biotype 4.

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develop parasitoid-free colonies. One percent agar media was prepared in advance and transferred to small clear plastic cups (1 oz capacity, Item #: 999P100C, Webstaurant Store, Lancaster, PA) with a fresh leaf disc (1.5 inches diameter) of aphid-susceptible soybean, Sheyenne, placed on top of the media. Approximately 20 adult soybean aphids were transferred from a single plant per site to a leaf disc in agar media and covered with a perforated lid. Aphids were allowed to reproduce over a period of 24 h in growth chamber at 25°C, a photoperiod of 16:8 (L:D) h, and approximately 70% humidity. After 24 h, adult aphids were removed and newly born nymphs were quarantined for another 48 h. Any aphids showing symptoms of parasitism were removed and the healthy nymphs were transferred to soybean plants of an aphid-susceptible cultivar, Sheyenne, for reproduction in collapsible cages (13.5 × 13.5 × 24 inches, BioQuip Products, Inc., Rancho Dominguez, CA).

The soybean aphid isolates were screened against a panel of indicator soybean lines including a susceptible check and six lines carrying different aphid resistance genes (Table 1). Many of these indicator lines were used to characterize aphid biotypes by previous studies (Hill et al. 2010, Alt and Ryan-Mahmutagic 2013, Cooper et al. 2015). The screening tests were conducted with no-choice experiments by using clear-cup cages (Bhusal et al. 2013) in the growth chamber (maintained at 25°C, 16:8 (L:D) h period, and approximately 70% humidity) with three replicates in a randomized complete block design. Such no-choice experiments specifically assess effects of antibiosis on the aphids. Other experimental details of plant maintenance, aphid inoculation and maintenance, aphid counts, and statistical analysis are described under, *Biotype Testing*, section below.

Sampling Design

Based on preliminary results finding different biotypes in the same region, we designed our sampling strategy to investigate variation of soybean aphid populations within and between fields of single townships in Minnesota. Four separate isolates were collected from each field sampled. In 2018, three fields were sampled in Cairo Township of Renville County and one field in Wellington Township of Renville County (Table 2). In 2019, we sampled two fields in Cairo Township of Renville County and one field in Wilmar Township of Kandiyohi County (Table 2). The fields in Cairo and Wellington Townships of Renville County in 2018 and 2019 are displayed in Fig. 2. In total, 28 soybean aphid isolates were collected from seven fields. For each isolate, we collected approximately 20 adult aphids from a single plant (preferably from a single leaf). The collected isolates were quarantined to rear parasite-free aphid populations as described above.

Biotype Testing

Collected isolates were biotyped using the panel of indicator lines (Table 1). We performed a whole-plant bioassay in growth chambers, because bioassays with whole plants differentiate the biotypes more accurately than using detached-leaf assays (Lagos-Kutz et al. 2020). Three replicates of the biotyping were performed, with each replicate forming a complete block of the indicator lines listed in Table 1. In each replication, the indicator lines were randomized such that the susceptible check was always placed in the middle and the indicator lines carrying aphid resistance gene(s) were randomized around the susceptible check inside a rearing cage. This allowed uniform movement of aphids between the susceptible check and surrounding indicator lines. Such bioassays performed over the duration described below enable assessment of the combined effects of antixenosis and antibiosis on the aphids.

Three seeds of each line were planted in a 10.2 cm × 10.2 cm × 10.2 cm plastic pot filled with Berger BM2 germination mix (Berger Horticultural Products Ltd., Sulphur Springs, TX). After germination, plants were thinned so that each pot contained two soybean seedlings. At the unifoliate stage (Fehr and Caviness 1977), 10 mixed-aged apterous aphids were transferred to the first unfolding trifoliate leaf of each of the two plants in each pot. The experimental arrangements with aphid-infested plants were maintained in fine mesh insect rearing cages in growth chambers at 25°C, a photoperiod of 16:8 (L:D) h, and approximately 70% humidity. Plants were bottom-irrigated in the holding trays to avoid interference of irrigation water to soybean aphid infestations. Total number of aphids on each plant was counted at 2 wk after inoculation. Mean aphid counts per plant per replication were calculated and mean number of aphids across the replications of each treatment were plotted in the bar graphs. Analysis of variance was performed separately for each isolates using a model including soybean line and replication as fixed effects. Bonferroni correction was used to separate means at $P < 0.05$. Aphid isolates in resistant indicator lines were determined virulent when their infestation was similar to susceptible checks or different from other resistant indicator lines.

Results

Pilot Test

The results of the pilot test are presented in Fig. 3 and Supp Table 1 (online only). 'Roseau Field 1' isolate was similar to soybean aphid biotype 1 as it was virulent to the susceptible check (Sheyenne) but avirulent to all soybean indicator lines (Kim et al. 2008). However, 'Roseau Field 2' isolate was virulent to the soybean indicator lines that carried aphid resistance genes *Rag2* (PI 200538), *Rag3* (PI 567543C), or *rag4* + *rag1c* (PI 567541B) demonstrating responses similar to biotype 3 (Hill et al. 2010). 'Saint Paul' isolate was similar to biotype 1 except it was partially virulent to *Rag3*. 'Rosemount' isolate and 'Lamberton Field 2' isolate were virulent to *Rag1*, *Rag2*, and the *Rag1* + *Rag2* combination similar to biotype 4 (Alt and

Table 1. A panel of indicator soybean lines used for biotype screening

Line	Maturity group	Gene(s) of aphid resistance[a]	Reference
PI 548663 (Dowling)	VIII	*Rag1*	Hill et al. (2006)
PI 200538	VIII	*Rag2*	Hill et al. (2009)
Viking 2188	II	*Rag1* and *Rag2*	Albert Lea Seed House, Albert Lea, MN
PI 567543C	III	*Rag3*	Zhang et al. (2010)
PI 567541B	III	*rag4* and *rag1c*	Zhang et al. (2009)
PI 567598B	III	*rag3* and *rag1b*	Bales et al. (2013)
Sheyenne	0	None	

[a]The presence of upper case R in the gene name indicates the allele conferring resistance is dominant, whereas a lower case r indicates the allele conferring resistance is recessive.

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Table 2. Details of fields sampled for soybean aphid isolates (four isolates collected per field) from Minnesota in 2018 and 2019

Sampling location				GPS coordinates			
Field	Township	County	Sampling date	Isolate 1	Isolate 2	Isolate 3	Isolate 4
Field 1	Cairo	Renville	4 August 2018	44.470N, 94.720W	44.469N, 94.717W	44.473N, 94.717W	44.473N, 94.718W
Field 2	Cairo	Renville	4 August 2018	44.481N, 94.719W	44.481N, 94.716W	44.480N, 94.717W	44.479N, 94.720W
Field 3	Cairo	Renville	4 August 2018	44.500N, 94.701W	44.500N, 94.698W	44.502N, 94.700W	44.503N, 94.701W
Field 4	Wellington	Renville	4 August 2018	44.555N, 94.737W	44.556N, 94.732W	44.552N, 94.733W	44.550N, 94.735W
Field 5	Cairo	Renville	14 August 2019	44.509N, 94.693W	44.513N, 94.692W	44.514N, 94.686W	44.511N, 94.689W
Field 6	Cairo	Renville	14 August 2019	44.531N, 94.735W	44.530N, 94.732W	44.529N, 94.727W	44.532N, 94.726W
Field 7	Wilmar	Kandiyohi	5 August 2019	45.130N, 95.096W	45.129N, 95.098W	45.129N, 95.095W	45.130N, 95.094W

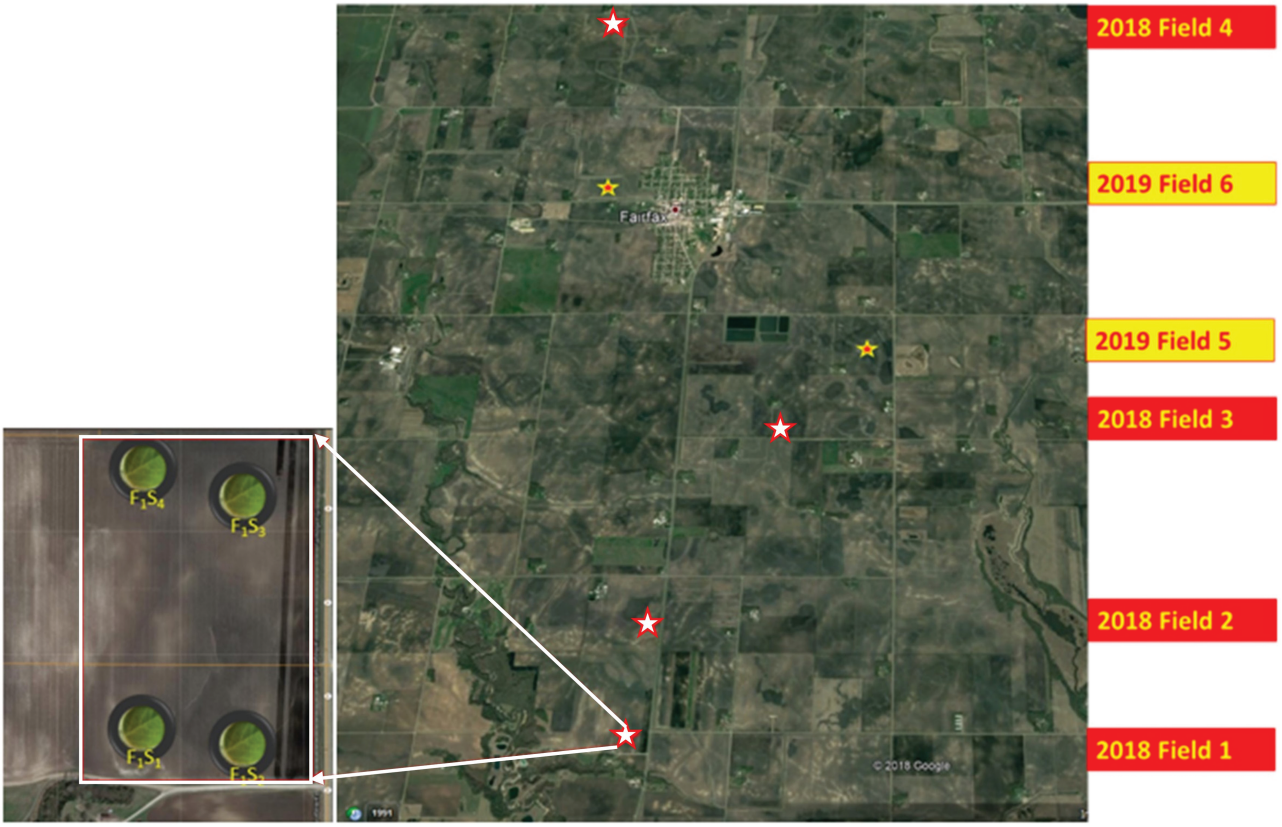

Fig. 2. Sampling sites (fields) of aphid isolates collected during 2018 and 2019 field season (Field 7 in Wilmar Township is not shown in the map because of its geographical distance from these field sites). Four isolates in all the fields were sampled to represent the corresponding field in different fashion such as from four corners in one field and three corners and deep in the field in other. The picture in the inset shows the location of four isolates sampled in field 1 in Cairo Township in 2018.

Ryan-Mahmutagic 2013). The 'Lamberton Field 2' isolate was collected from a *Rag1* + *Rag2* soybean cultivar growing at the University of Minnesota's Southwest Research and Outreach Center. Whereas 'Lamberton Field 1' isolate was found to be virulent to *Rag2*.

Biotype Screening Within Fields and Townships

Screening of aphid isolates against biotype indicator lines revealed different soybean aphid biotypes within fields and townships (Tables 3 and 4; Fig. 4). In 2018, three isolates collected from 'Field 1' in Cairo Township were avirulent to all of the resistant indicator lines and virulent to Sheyenne, the susceptible check. The remaining isolate from that field was virulent to susceptible check and *Rag2*, and partially virulent to all of the other indicator lines. Out of four isolates collected from 'Field 2', one isolate was avirulent to all of the resistant indicator lines, two isolates appeared to be virulent to *Rag1*, and the remaining isolate was virulent to *Rag2*, *Rag3*, or *rag4* + *rag1c*, indicating three different types of aphid populations in 'Field 2'. Two isolates from 'Field 3' and three isolates from 'Field 4' were avirulent to all of the resistant lines. The remaining isolates in these two fields were virulent to the indicator line carrying *Rag2*. Based on these results, out of 16 aphid isolates collected in 2018, nine isolates were similar to soybean aphid biotype 1 (Kim et al. 2008), two isolates were similar to biotype 2 (Kim et al. 2008), and one isolate was similar to biotype 3 (Hill et al. 2010). Three isolates were virulent to *Rag2*, but were unlike biotype 3 because they were avirulent to *Rag3* and the *rag4* + *rag1c* combination. One isolate ('Field 1–Isolate 2') was inconclusive (Table 4; Fig. 4).

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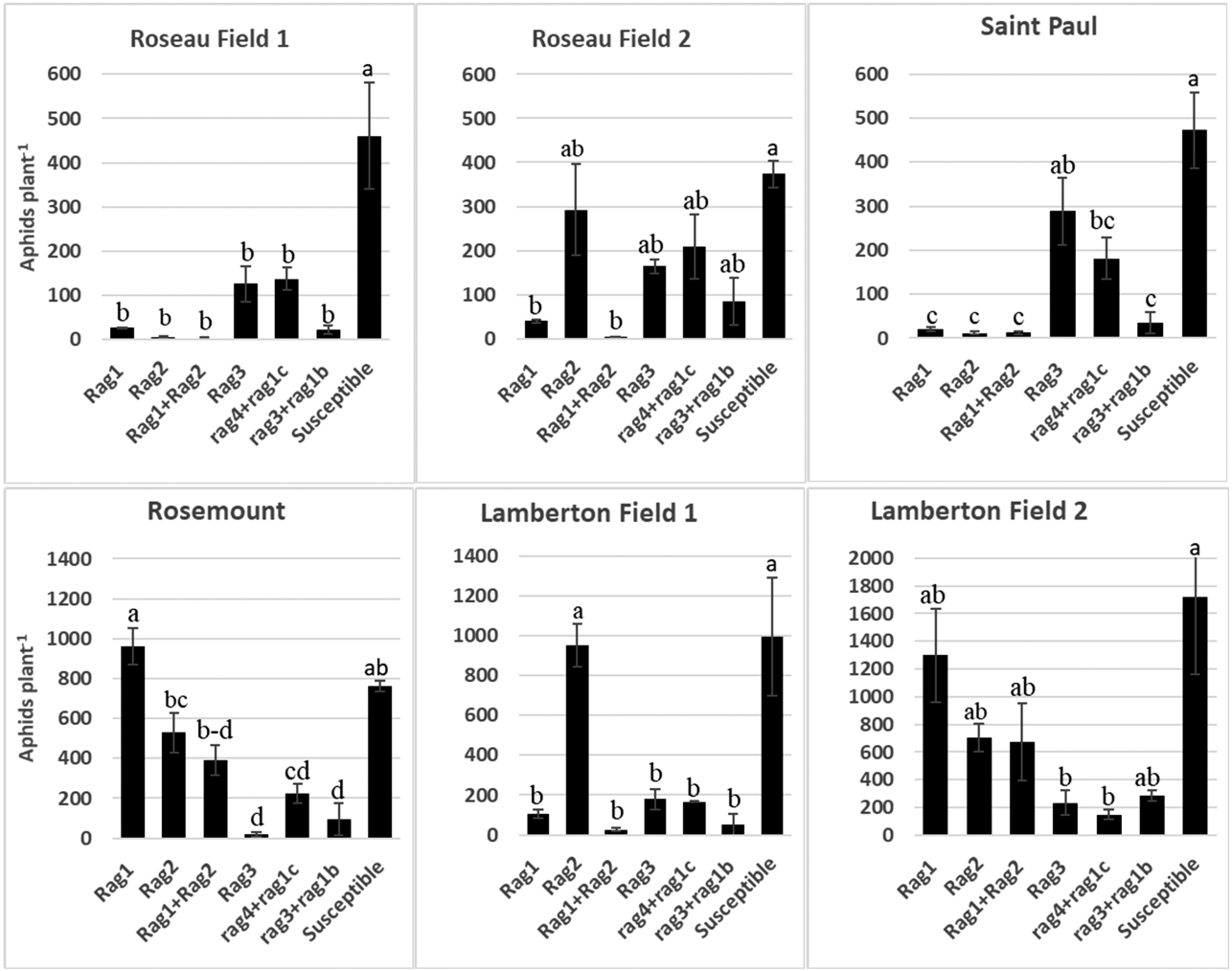

Fig. 3. Mean number of aphids per plant on different indicator lines observed in bioassay of the aphid isolates collected as part of the 2017 pilot test. Error bars represent the standard error of the means. Same letters above each bar within individual isolates are not different by LSD ($P > 0.05$).

In 2019, three isolates from 'Field 5', two isolates from 'Field 6', and two isolates from 'Field 7' were avirulent to all of the resistant indicator lines similar to biotype 1. One isolate from 'Field 5', two isolates from 'Field 6', and one isolate from 'Field 7' were virulent to *Rag1* demonstrating similar responses to biotype 2. Whereas 'Field 7–Isolate 1' was virulent to *Rag1* and *Rag2* individually, but not virulent to any resistant lines including the *Rag1* + *Rag2* combination, which suggests the presence of a mixture of biotype 1 and biotype 2 populations in that isolate. In summary, biotype 1 and biotype 2 were prevalent in all fields sampled in 2019 (Table 4; Fig. 4). The detailed results of screening of 2018 and 2019 collections are presented in Supp Table 2 (online only).

Discussion

A biotype is a population of an insect species that can survive on, reproduce on, and/or cause injury to a plant which is resistant to other populations of that insect species (Dogimont et al. 2010). The biotypic differentiation of soybean aphid is based on their reaction to soybean containing different genes of aphid resistance. Specific mechanisms of biotypic virulence are not well known (Alt et al. 2019) but the basis of virulence is hypothesized to involve the secreted effector proteins (Coates et al. 2020). Using a small number of single-nucleotide polymorphisms genotyped on different isolates, Wenger and Michel (2013) did not find significant genetic differentiation between isolates of soybean aphid biotype 1 and biotype 2 collected across northern Ohio. Based on this result, the authors speculated that variation in virulence does not stem from the development of a single, genetically distinct lineage of soybean aphid, but rather could be developed from ubiquitous genetic sources of virulence whereby virulence genes are broadly distributed in aphid populations at a low frequency. Other possible explanations include nongenetic causes of virulence and epigenetic variations. Similarly, Crossley and Hogg (2015) analyzed the clonal lineages of aphid isolates collected in Wisconsin during 2012 and 2013 and found that 41% and 8% of the isolates collected in 2012 and 2013, respectively, have matching multilocus genotypes. The dynamics of late-season dispersal and migration of soybean aphids to their primary winter host for sexual reproduction may cause admixture of a diverse sexual gene pool, and their clonal amplification in the following spring and summer may increase the heterozygosity in the aphid population (Orantes et al. 2012, Wenger and Michel 2013). A recent study that used whole-genome resequencing combined with population genomic analyses on different soybean aphid biotypes found that only a very small number of genomic regions were divergent between biotype 1 and biotypes 2, 3, and 4 (Coates et al. 2020). This finding suggests that a small number of loci control variation in virulence among soybean aphid biotypes.

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Table 3. Virulence of collected soybean aphid isolates against different indicator lines

	Indicator lines						
Aphid isolates	PI 548663 (*Rag1*)	PI 200538 (*Rag2*)	Viking 2188 (*Rag1* + *Rag2*)	PI 567543C (*Rag3*)	PI 567541B (*rag4* + *rag1c*)	PI 567598B (*rag3* + *rag1b*)	Sheyenne (NA)
2018							
Field 1–Isolate 1	A	A	A	A	A	A	V
Field 1–Isolate 2	PV	V	PV	A	A	PV	V
Field 1–Isolate 3	A	A	A	A	A	A	V
Field 1–Isolate 4	A	A	A	A	A	A	V
Field 2–Isolate 1	A	V	A	V	PV	A	V
Field 2–Isolate 2	A	A	A	A	A	A	V
Field 2–Isolate 3	V	A	A	A	A	A	V
Field 2–Isolate 4	V	A	A	A	A	A	V
Field 3–Isolate 1	A	A	A	A	A	A	V
Field 3–Isolate 2	A	V	A	A	A	A	V
Field 3–Isolate 3	A	V	A	A	A	A	V
Field 3–Isolate 4	A	PV	A	A	A	A	V
Field 4–Isolate 1	A	A	A	A	PV	A	V
Field 4–Isolate 2	A	PV	A	A	A	A	V
Field 4–Isolate 3	A	V	A	A	PV	A	V
Field 4–Isolate 4	A	A	A	A	A	A	V
2019							
Field 5–Isolate 1	PV	A	A	A	A	A	V
Field 5–Isolate 2	V	A	A	A	A	A	V
Field 5–Isolate 3	A	A	A	A	A	A	V
Field 5–Isolate 4	A	A	A	A	A	A	V
Field 6–Isolate 1	A	A	A	A	A	A	V
Field 6–Isolate 2	A	A	A	A	A	A	V
Field 6–Isolate 3	V	A	A	A	A	A	V
Field 6–Isolate 4	V	A	A	A	A	A	V
Field 7–Isolate 1	V	V	A	A	A	A	V
Field 7–Isolate 2	A	A	A	A	A	A	V
Field 7–Isolate 3	V	A	A	A	A	A	V
Field 7–Isolate 4	A	A	A	A	A	A	V

A = avirulent; V = virulent; PV = partially virulent.

Table 4. Number of aphid isolates from each field that displayed virulence reactions similar to known soybean aphid biotypes 1–4

	Biotypes				
Sampling fields	1	2	3	4	Other[a]
2018					
Field 1	3				1
Field 2	1	2	1		
Field 3	2				2
Field 4	3				1
2019					
Field 5	3	1			
Field 6	2	2			
Field 7	2	1			1

[a]Aphid isolates displayed different responses than already known biotypes of soybean aphid for such as 'Field 3–Isolate 3', 'Field 3–Isolate 3', and 'Field 4–Isolate 3' (Fig. 4). 'Field 1–Isolate 2' was inconclusive. 'Field 7–Isolate 1' potentially mixture of soybean aphid biotype 1 and 2. Detailed results are presented in Supp Table 2 (online only).

Current knowledge on the variation of soybean aphid biotypes at a small spatial scale is very limited. Several studies have found variation of soybean aphid biotypes between different geographical regions. Cooper et al. (2015) found variation in soybean aphid biotypes from year to year and across U.S. locations. The widest spectrum of variability in soybean aphid virulence was found in Wisconsin, which was the first state to report the soybean aphid in North America (Ragsdale et al. 2011, Cooper et al. 2015). Crossley and Hogg (2015) found all four soybean aphid biotypes (i.e., biotypes 1, 2, 3, and 4) in 42, 21, 17, and 4%, respectively, of 24 aphid collection sites in Wisconsin in 2013. Our study also found higher variation of aphid biotypes in 2018 which had overall higher levels of aphid infestation statewide compared to 2019.

This investigation of soybean aphid biotype variation within a small spatial scale was rooted from the results of a pilot test of screening aphid isolates collected from northwestern, central, and southeastern Minnesota in 2017. The pilot test revealed that multiple biotypes of soybean aphid were prevalent in Minnesota and even in individual regions (Fig. 3; Supp Table 1 [online only]). Two aphid isolates, which were collected only about 3 km apart, presented reactions similar to two different biotypes of soybean aphid both in northwestern and in southeastern Minnesota. There were also two different biotypes in central Minnesota. However, the aphid isolates in central Minnesota were collected from two different cities approximately 30 km apart. These results suggest we found three known biotypes as well as one isolate different than known biotypes of soybean aphid in a small collection of aphid isolates from a single field season. Alt et al. (2019) reported all four known biotypes of soybean aphid in Minnesota as a part of the large collection of aphid isolates in the United States and Canada during 2011–2013. Such variation at a small spatial scale observed in the pilot study triggered

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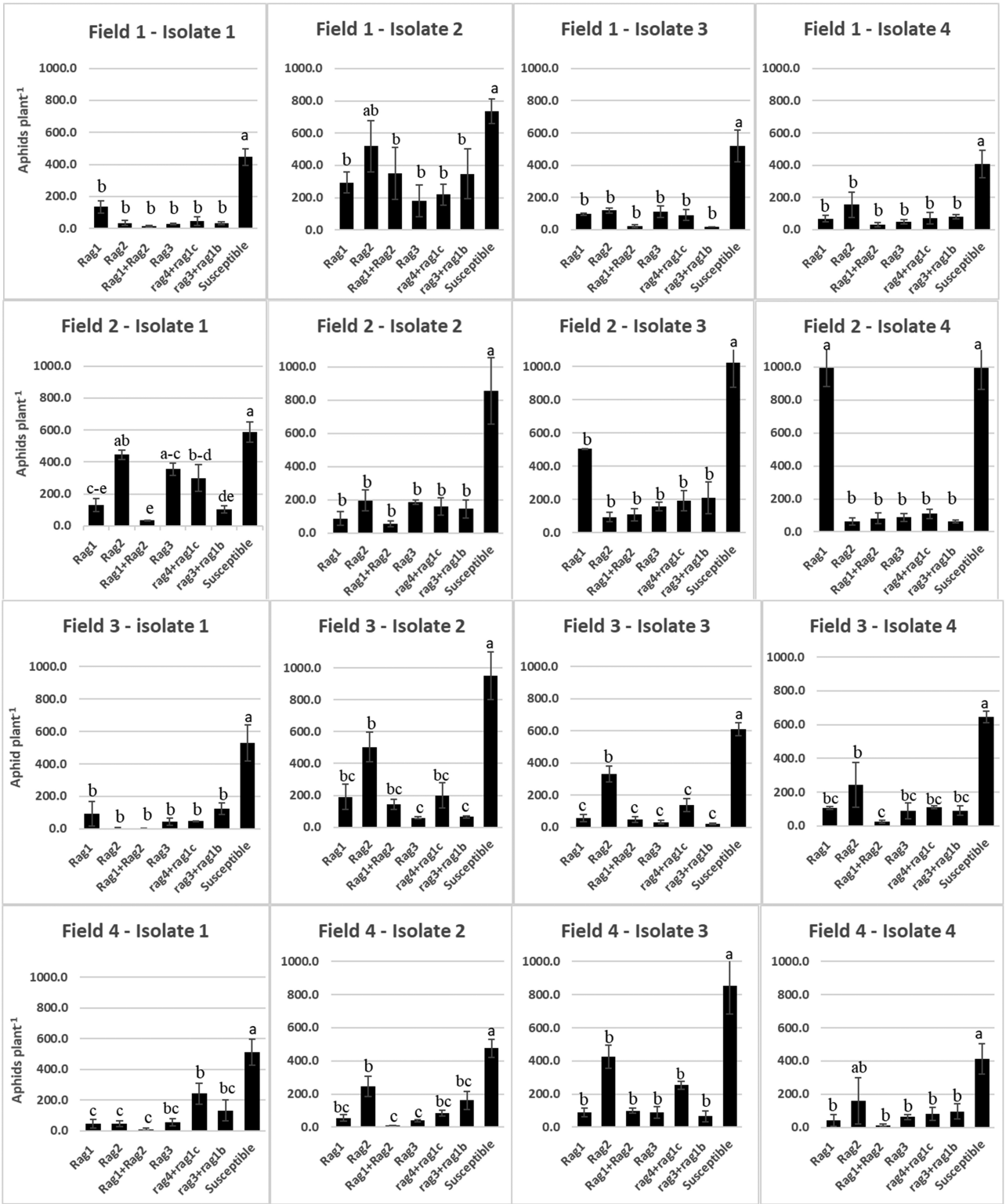

Fig. 4. Mean number of aphids per plant on different indicator lines observed in bioassay of the aphid isolates collected in 2018 and 2019 field season. Error bars represent the standard error of the means. Same letters above each bar within individual isolates are not different by LSD ($P > 0.05$).

an investigation into the variation of soybean aphid biotypes within township and within single fields.

Screening of aphid isolates collected in 2018 and 2019 revealed the presence of multiple biotypes of soybean aphid not only within a single township, but also within a single field. In 2018, four types of soybean aphid populations were prevalent including aphid populations similar to biotype 1, 2, and 3. The fourth type of aphid populations were different from previously known biotypes. The isolates different from previously known biotypes were virulent to *Rag2*, but unlike biotype 3 (Hill et al. 2010), they were avirulent to

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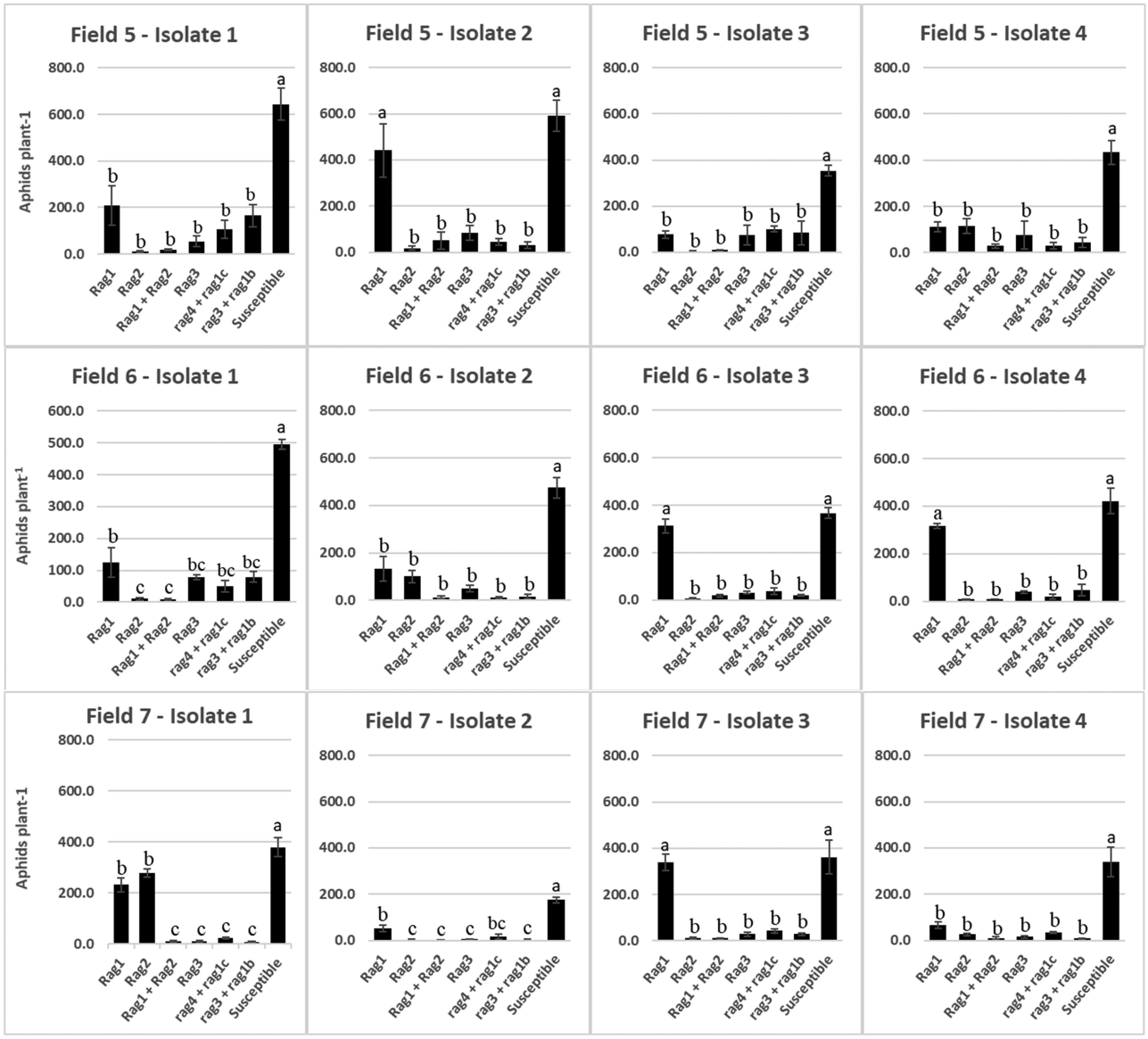

Fig. 4. Continued.

Rag3 and the *rag4* + *rag1c* combination (Table 4; Fig. 4). Lagos-Kutz (2020) also found a soybean aphid clone from Wooster, OH which readily colonized *Rag2* but did not colonize *Rag1* or *Rag3*. However, the authors did not screen this aphid clone against other resistant genes. The inconclusive results of an isolate from 'Field 1–Isolate 1' may have been attributed by the presence of intrapopulation or intrabiotypic variability as observed by Alt et al. (2019).

Soybean aphid infestation in Minnesota was not as widespread in 2019 compared to 2018, and therefore we were able to collect soybean aphid isolates from only two fields in Cairo Township of Renville County, MN, in 2019. We also collected aphid isolates from one field of its neighboring county, Kandiyohi. The aphid populations collected in 2019 were similar to either biotype 1 or biotype 2. Both types of aphid populations were present in all three fields sampled, but the populations similar to biotype 1 were more prevalent in each case (Table 4). The 'Field 7–Isolate 1', which was virulent to both *Rag1* and *Rag2* but avirulent to the *Rag1* + *Rag2*, makes it distinct from biotype 4. Because we collected several adult aphids from a single plant, the isolate may have been a mixture of two different biotypes. It is likely to have admixture of different populations/biotypes in natural aphid isolates due to their dispersal throughout the season (Wenger and Michel 2013). Alt et al. (2019) also reported intrabiotypic variability. It is important to further study genetic differences between potentially new soybean aphid biotypes to determine virulence attributes.

Detailed knowledge of how soybean aphid biotypes vary at different spatial scales will inform integrated pest management strategies for the control of this agriculturally damaging insect species. Previous studies of soybean aphid biotype variation were conducted at large geographical scales (e.g., state or regional [multistate] levels). Our current investigation examined variation of biotypes at the scale of townships and single fields. We found up to three different biotypes of soybean aphid in a single field. Six out of seven fields sampled were found to have more than one biotype. We found all four known biotypes of soybean aphid populations in Minnesota as well as populations that were different from known biotypes. These results highlight the importance of continually discovering new sources of aphid resistance

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and developing soybean cultivars pyramided with multiple resistance genes. Varieties with pyramided *Rag* genes are crucial in integrated pest management of soybean aphid because they confer stronger and more durable resistance, and significant yield protection (McCarville et al. 2014, Zhang et al. 2018). Such pyramided resistant varieties and new genetic sources conferring resistance to multiple biotypes will help in decreasing insecticide inputs thereby improving economic and environmental sustainability of soybean production.

Supplementary Data

Supplementary data are available at *Journal of Economic Entomology* online.

Acknowledgments

We would like to acknowledge James Menger for helping in preparing and arranging logistic of the investigation. We would also like to thank Donn Vellekson for sampling soybean aphids from Roseau, MN. Bruce Potter and Steve Commerford are also acknowledged who helped in finding locations of aphid infestation. We are much thankful to Tom Palmer, Tom Runcke, and Jason Fussy who allowed us to collect aphids in their field. Funding for this investigation was provided by the Minnesota Invasive Terrestrial Plants and Pests Center through the Minnesota Environment and Natural Resources Trust Fund.

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