Biotypic Diversity in Greenbug (Hemiptera: Aphididae): Microsatellite-Based Regional Divergence and Host-Adapted Differentiation

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ABSTRACT Nineteen isolates of the cereal aphid pest greenbug, *Schizaphis graminum* (Rondani) (Hemiptera: Aphididae), were collected from wheat, *Triticum aestivum* L.; barley, *Hordeum vulgare* L.; or noncultivated grass hosts in five locations from Colorado and Wyoming. Parthenogenetic colonies were established. Biotypic profiles of the 19 isolates were determined based on their abilities to damage a set of host plant differentials, and 13 new biotypes were identified. Genetic diversity among the 19 isolates and five previously designated greenbug biotypes (E, G, H, I, and K) was examined with 31 cross-species transferable microsatellite (simple sequence repeat) markers. Neighbor-joining clustering analysis of marker data revealed host-adapted genetic divergence as well as regional differentiation of greenbug populations. Host associated biotypic variation seems to be more obvious in "agricultural biotypes," whereas isolates collected from noncultivated grasses tend to show more geographic divergence. It seems that the biotype sharing the most similar biotypic profiles and the same geographic region with current prevailing one may have the greatest potential to become the new prevailing biotype. Close monitoring of greenbug population dynamics especially biotypic variation on both crop plants and noncultivated grasses in small grain production areas may be a useful strategy for detecting potentially new prevailing virulent biotypes of the greenbug.

KEY WORDS *Schizaphis graminum*, microsatellite, simple sequence repeat, biotype, genetic diversity

Biotypic differentiation is a common phenomenon in many insect pests, which is also of major concern in identification and deployment of host resistance genes in crop plants. The definition of biotype varies in different insects and is often confusing. For example, biotypes have been described on the basis of different host plant affiliations, degrees of phytotoxic symptom induction, insecticide resistance, morphology, and behavior of the insects (Diehl and Bush 1984, Hsiao and Stutz 1985, Saxena and Barrion 1987, Gill 1992, Brown et al. 1995, Dres and Mallet 2002, Berry et al. 2004). In cereal aphids such as the greenbug, *Schizaphis graminum* (Rondani), or Russian wheat aphid, *Diuraphis noxia* (Kurdjumov) (Hemiptera: Aphididae), biotypes have been defined by their abilities to damage different plant genotypes (Puterka et al. 1988, 1992; Shufran et al. 1997; Haley et al. 2004).

The greenbug is one of the most important cereal aphid pests of wheat, *Triticum aestivum* L.; barley, *Hordeum vulgare* L.; and sorghum, *Sorghum bicolor* (L.) in the southern Great Plains in the United States and many other parts of the world. Biotypic variation among greenbug populations has been well characterized (Porter et al. 1997, Burd and Porter 2006), which also has been a driving force behind several small grain breeding programs. Wood (1961) was the first to designate greenbug biotypes. The greenbug strain that was virulent to greenbug resistant 'DS 28A' wheat was designated biotype B, with the presumption that all other greenbugs were avirulent, thus constituting biotype A. Since then, eight additional biotypes (C, E, F, G, H, I, J, and K) have been recognized (Porter et al. 1997). More recently, 13 new biotypes were reported based on damage responses of these greenbug isolates on a set of host resistance differentials from wheat, barley, sorghum, and rye, *Secale cereale* L. (Burd and Porter 2006). Of the 22 greenbug biotypes so far identified, only C, E, and I caused or are causing significant economic losses in small grain crops, which may be called "agricultural biotypes" (Shufran et al. 2000). All others are laboratory strains. Over the years, there has been a shift of prevailing biotypes from C to E and E to I. Biotypes E and I are currently the prevailing biotypes in the fields of the

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southern High Plains in the United States (Berzonsky et al. 2003).

Many studies have been conducted to characterize the interactions between greenbug biotypes and their resistant or susceptible hosts. Why and how biotypes have developed is still not well known, which is important for practical purposes in crop breeding for insect resistance. Theoretically, it is widely believed that selection pressure exerted by resistant cultivars may result in proliferation of biotypes. However, Porter et al. (1997) analyzed the history of deployment of host resistance genes and the appearance of new greenbug biotypes and did not find apparent correspondence between the two. Because the greenbug has a wide range of noncultivated grass hosts (Michels 1986), it was postulated that these poaceous grasses may play an important role in generating and maintaining diversity of greenbug biotypes (Anstead et al. 2003). Indeed, large-scale field surveys revealed a high degree of biotypic diversity among greenbug populations collected from noncultivated grasses, and it was proposed that the greenbug species complex was composed of host-adapted races that diverged on grass species independent of, and well before, the advent of modern agriculture (Porter et al. 1997, Shufran et al. 2000, Burd and Porter 2006).

The assessment of damage (virulence) to a set of resistant plants (differentials) is the only criterion used to identify a greenbug biotype. The genetic basis for identification of greenbug biotypes is plant based rather than insect derived. Molecular analysis based on mitochondrial DNA sequences found that a greenbug biotype is comprised of genetically diverse individuals sharing similar virulence genes (Shufran et al. 2000, Anstead et al. 2002, Lopes-Da-Silva et al. 2004). In our previous study, microsatellite markers were used to investigate genetic diversity among greenbug populations (Weng et al. 2007). We found host-adapted genetic divergence as well as regional differentiation of greenbug biotypes. However, only three previously designated biotypes (C, E, and I) and three new greenbug isolates collected from one location were used in our early study (Weng et al. 2007). In the past decade, many greenbug isolates from different plant hosts and geographic regions in the Great Plains have been collected (J.D.B., unpublished data). In the current study, 31 microsatellite markers developed from different aphid species were used to conduct biotypic profiling among 19 such greenbug isolates, to evaluate genetic diversity among them and biotypes E, G, H, I, and K.

Materials and Methods

Greenbug Collections in the Field. Collection dates, locations, and plant hosts of the greenbug clones used in this study are listed in Table 1. The 19 greenbug isolates were collected from five plant hosts (wheat, barley, intermediate wheatgrass [*Agropyron intermedium* (Host) Beauv.], volunteer wheat, and volunteer oat [*Avena sativa* L.]) in five locations of Colorado and Wyoming. Greenbugs were collected using a Stihl model 85 leaf blower-vacuum (Stihl Incorporated, VA Beach, VA) customized to function as a D-vac system through attachment of a fine mesh collection bag onto the vacuum tube (10 cm in diameter). Samples were discretely collected from cultivated wheat, sorghum, volunteer oat, and noncultivated grass species within 1–5 m from the respective cultivated field margins. Greenbugs were transferred from the collection bag to 'Schuyler' barley seedlings that were caged to prevent cross-sample contamination. Subsequent clonal colonies for evaluation of biotypes were established from a single, apterous greenbug from each sample. Test colonies were reared on Schuyler barley grown in caged pots and maintained in environmental chambers with a photoperiod of 16:8 (L:D) h at 20 and 18°C.

Determination of Biotypes. The biotype status of each test colony was determined using previously established plant differentials of barley, rye, sorghum, and wheat (Burd and Porter 2006). Fourteen host plant differentials were used in greenbug biotype profiling, including six (*Gb1* to *Gb6*) from wheat, two (*Rsg1* and *Rsg2*) from barley, two from rye, and four from sorghum (Table 2). Greenbug-resistant sources, resistance gene designations, and susceptible checkplants used in this study were the same as described in Burd and Porter (2006). Seeds of each plant genotype were planted in separate rows, at a rate of 10 seeds per 15-cm row, with four replications, in flats on greenhouse benches. Genotypes of plants were randomly assigned to rows. Barley, rye, and wheat plants were tested separately from sorghum. Test plants were caged and subsequently infested at the two-leaf stage by cutting and placing infested leaves next to each row of test plants. The tests of barley, rye, and wheat plants were done under supplemental artificial light, with a photoperiod of 16:8 (L:D) h and 22 ± 5°C, in a greenhouse. The conditions for the sorghum tests were the same except the temperature was maintained at 28 ± 5°C. Once the susceptible control plants were killed (usually within 7–14 d), the test was terminated and plants were scored as alive (resistant) or dead (susceptible). A greenbug isolate was considered a new biotype when its plant response profile was unique. New biotypes were denoted with regard to the state from which they were collected and numbered sequentially. After each test, vouchers of the aphids were collected and deposited at the Cereal Insect Genetic Resource Library, USDA–ARS, Plant Science Research Laboratory, Stillwater, OK.

Microsatellite Markers. Thirty-one cross-species transferable microsatellite markers were evaluated. Detailed information for each marker is listed in Table 3. Twelve simple sequence repeats (SSRs) were developed from microsatellite-enriched genomic DNA sequences of the greenbug (Sgg1 to Sgg13, excluding Sgg4) and 19 were from other aphid species, including *Sitobion miscanthi;* bird cherry-oat aphid, *Rhopalosiphum padi* (L.); and the pea aphid, *Acyrthosiphon pisum* (Harris).

Aphid Genomic DNA Isolation and Polymerase Chain Reaction (PCR). Each of the 19 greenbug isolates was established from a single parthenogenetic

Table 1. Date, location and host information of collected greenbug clones used in this study

Colony	Yr	Date	State	Location	Host	Notes
21 A	2003	9 July 2003	Wyoming	Worland	Barley	8 km north of Worland on barley
21 MC	2003	9 July 2003	Wyoming	Worland	Barley	8 km north of Worland on barley
38 A	2003	10 July 2003	Wyoming	Powell	Barley	Highway 295, south of Powell on barley
38 B	2003	10 July 2003	Wyoming	Powell	Barley	Highway 295, south of Powell on barley
38 MC	2003	10 July 2003	Wyoming	Powell	Barley	Highway 295, south of Powell on barley
42 A	2003	10 July 2003	Wyoming	Lovell	Barley	Highway Alt. 14, 1.6 km west of Lovell on barley
42 B	2003	10 July 2003	Wyoming	Lovell	Barley	Highway Alt. 14, 1.6 km west of Lovell on barley
E	2008	Continuous	Oklahoma	Stillwater	Wheat-barley-sorghum	Greenhouse culture
G	2008	Continuous	Oklahoma	Stillwater	Wheat-barley-sorghum	Greenhouse culture
H	2008	Continuous	Oklahoma	Stillwater	Wheat-barley-sorghum	Greenhouse culture
I	2008	Continuous	Oklahoma	Stillwater	Wheat-barley-sorghum	Greenhouse culture
K	2008	Continuous	Oklahoma	Stillwater	Wheat-barley-sorghum	Greenhouse culture
WB5A	2004	29 Oct. 2004	Colorado	Walsh	Wheat	Highway 160, 8 km west of Walsh, CO
WB5B	2004	29 Oct. 2004	Colorado	Walsh	Wheat	Highway 160, 8 km west of Walsh, CO
WB5MC	2004	29 Oct. 2004	Colorado	Walsh	Wheat	Highway 160, 8 km west of Walsh, CO
WB6A	2004	29 Oct. 2004	Colorado	Walsh	Wheat	Highway 160, 8 km west of Walsh, CO
WB6B	2004	29 Oct. 2004	Colorado	Walsh	Wheat	Highway 160, 8 km west of Walsh, CO
WB6MC	2004	29 Oct. 2004	Colorado	Walsh	Wheat	Highway 160, 8 km west of Walsh, CO
WY10A	2005	26 July 2005	Wyoming	Powell	Barley	4.8 km south of Powell on barley
WY10B	2005	26 July 2005	Wyoming	Powell	Barley	4.8 km south of Powell on barley
WY2A	2005	25 July 2005	Wyoming	Wheatland	Wheat-volunteer	8 km east of Wheatland on volunteer wheat from edge of wheat field
WY3A	2005	25 July 2005	Wyoming	Wheatland	Oat-volunteer	8 km east of Wheatland on volunteer oat from edge of wheat field
WY4A	2005	25 July 2005	Wyoming	Wheatland	Intermediate wheatgrass	8 km east of Wheatland on Intermediate wheatgrass from edge of wheat field
WY4B	2005	25 July 2005	Wyoming	Wheatland	Intermediate wheatgrass	8 km east of Wheatland on Intermediate wheatgrass from edge of wheat field

aphid. One isolate of bird cherry-oat aphid also was used, which was established from a single nymph from local greenhouse populations at Bushland, TX. Aphids from each colony were stored in a −80°C freezer until DNA extraction.

The CTAB method (Murray and Thompson 1980) was used to extract genomic DNAs from 20 greenbugs for each isolate or biotype. Each PCR contained 10 ng of template DNA, 0.5 μM each of two primers, and 1× PCR master mix (Promega, Madison, WI) in a total volume of 10.0 μl, which was performed in a PTC-200 thermocycler (Bio-Rad Laboratories, Hercules, CA). A single, touch-down PCR program of Weng et al. (2007) was used for all markers.

PCR products were resolved in 4% high-resolution agarose gels stained with ethidium bromide. Primers amplified null alleles were repeated at least one more time to rule out the possibility of the failure of PCR amplification.

Marker Data Analysis. The discriminatory power of each marker used in this study was assessed with polymorphic information content (PIC), which was calculated as $PIC = 1 - \sum(p_i^2)$, where p_i is the frequency of the *i*th allele detected in all 24 greenbug isolates or biotypes (Anderson et al. 1992). The PCR products were scored in binary format with the presence of a band being scored as 1 and its absence scored as 0. In evaluation of biotypic diversity, the binary matrix of different clones and markers was used as input in data analysis with the software package PHYLIP 3.66 (available at http://evolution.genetics.washington.edu/phylip.html; Felsenstein 1989). Bootstrapping, construction of pairwise distance matrices, and neighbor-joining (NJ) cluster analysis (Saitou and Nei 1987) were all following Weng et al. (2007). Bird cherry-oat aphid was used as the outgroup in consensus tree construction. The dendrogram was drawn with TreeView, version 1.6.6 (http://taxonomy.zoology.gla.ac.uk/rod/treeview/).

For verification purposes, the same data set also was analyzed with different methods. The binary data matrix was used to calculate Jaccard's pairwise similarity coefficients (J) (Jaccard 1901) among the 25 greenbug biotypes/isolates. J varies from 1 to 0, where 1 indi-

Table 2. Response of 13 uncharacterized greenbug isolates and biotypes E, G, H, I, and K to 14 barley, sorghum, rye, and wheat genotypes

Biotype	Original colony	Wheat						Rye		Barley		Sorghum			
		DS 28A (*Gb1*)	Amigo (*Gb2*)	Largo (*Gb3*)	CI17959 (*Gb4*)	CI17882 (*Gb5*)	GRS1201 (*Gb6*)	Elbon	Insave (*Gb2+Gb6*)	Post 90 (*Rsg1*)	PI426756 (*Rsg2*)	TX 7000	TX 2737	TX 2783	PI 550607
WY1	WY2A	R	R	R	R	R	R	R	R	R	R	S	R	S	S
WY2	WY3A	R	R	R	R	R	R	R	R	R	S	S	S	R	S
WY3	21A	R	R	R	R	R	R	R	R	S	S	S	S	R	R
WY4	38MC	R	R	R	R	R	R	R	R	S	S	R	S	S	R
WY5	38B	R	R	R	R	R	R	R	R	S	S	S	S	S	S
WY6	38A	R	R	R	R	R	R	R	R	S	S	S	R	S	R
E	n/a	S	S	R	R	R	R	S	R	R	R	S	S	R	R
I	n/a	S	S	R	R	R	R	S	R	R	R	S	S	S	R
K	n/a	S	S	R	R	R	R	S	R	R	R	S	S	S	S
CO1	WB 6MC	S	S	R	R	R	R	S	R	S	R	S	S	S	S
CO2	WB5 A	S	S	S	S	S	R	S	R	S	S	S	S	S	R
CO3	WB5 B	S	S	R	R	S	R	R	R	S	S	S	S	S	R
CO4	WB 5MC	S	S	S	S	R	R	S	R	S	S	S	S	S	R
H	n/a	S	S	R	S	S	S	S	S	S	S	—	—	—	R
G	n/a	S	S	S	S	S	R	S	R	R	R	S	S	S	R
WY7	WY10A	S	S	S	S	S	S	S	R	R	S	S	S	S	R
WY8	WY10B	S	S	S	S	S	S	S	S	R	S	S	R	S	S
WY9	WY4A/B	S	S	S	S	S	S	S	S	R	S	S	S	S	R

R, resistant; S, susceptible; n/a, not applicable; and —, not tested.

cates that a pair has identical banding patterns and 0 indicates that a pair has uniformly contrasting (opposite) banding patterns. Genetic distance (GD) estimates were calculated as the complement of J (i.e., GD = 1 − J) (Spooner et al. 1996). Unweighted pair-group method with arithmetic average (unweighted pair group method using arithmetic average) cluster analysis of GDs as well as principal components analysis (PCA) was performed to visualize similarities among the test subjects. All the computations and analyses were performed using the computer software NTSYS-pc, version 2.02i (Applied Biosystematics, Setauket, NY).

Results

Biotypic Profiling of Greenbug Isolates. Among the 19 greenbug isolates tested (Table 1), WB6A and WB6B had the same host reaction profile as biotype K. Isolates 42A, 42B, and 21MC had the same profile as biotype TX10 (Burd and Porter 2006). WY4A and WY4B had the same host reactions but were different from any known biotypes. Thus, 13 isolates had unique biotypic profiles that were different from previously designated greenbug biotypes, and they were consequently designated as new biotypes (Table 2).

Genetic Diversity Among Greenbug Populations. One hundred and eighty-one putative alleles were detected with 31 SSR markers among 25 greenbug and bird cherry-oat aphid isolates or biotypes. Of the 181 putative alleles, 22 were specific to bird cherry-oat aphid and 159 belonged to the greenbug. Thus, each SSR primer pair was able to amplify on average 5.1 bands among 24 greenbug DNA templates. The number of bands amplified by each SSR primer pair varied from 1 to 15, and the PIC values varied from 0 to 0.9028 (average PIC = 0.6340; Table 3). Apg20, developed from pea aphid genomic sequence (Weng et al. 2007), and SmS16b, from genomic DNA sequence of *S. miscanthi* (Wilson et al. 2004), detected the highest number of alleles, 14 and 15, respectively. Meanwhile, ApEST02 and SmS49 each was able to detect only one allele. The average numbers of alleles detected by SSRs from the greenbug, bird cherry-oat aphid, *S. miscanthi*, and the pea aphid were 4.9, 5.4, 6.4, and 5.0, respectively. This result indicated that both genomic and expressed sequence tag (EST)-SSRs from other aphid species are very useful in genetic diversity studies for the greenbug.

A dendrogram constructed for the 24 greenbug isolates based on 1,000 bootstrapping repetitions was shown in Fig. 1. The majority of the nodes in the tree were supported by >50% probabilities (that is, the same particular branching point appeared in at least 500 times in 1,000 trees built from bootstrapping), suggesting that the consensus tree generated from the SSR data were highly reliable.

Clustering analysis placed the 24 greenbug biotypes and isolates into three major groups I, II, and III (Fig. 1). Group I included six isolates and three agricultural biotypes, which were further divided into three subgroups. Noticeably, the three agricultural biotypes E, I, and K were in one subgroup. The isolates WB5A, WB5B, WB5MC, WB6A, WB6B, and WB6MC collected from wheat in Walsh, CO, in 2004 formed two other subgroups. Group II had two subgroups. One subgroup contained biotype G and isolates WY4A and WY4B (collected from wheatgrass in Wheatland, WY, in 2005), and the other subgroup included isolates WY10A and WY10B collected on barley from Powell, WY, in 2005 (Table 1). Group III included nine greenbug isolates, seven of which (42A, 42B, 21A, 21MC, 38A, 38B, and 38MC) were all collected from the barley in 2003 from Wyoming. The remaining two isolates (WY2A and WY3A collected in 2003 from volunteer wheat and oat, respectively) formed an-

Table 3. Information of molecular markers from different sources used for assessment of greenbug diversity in this study

No.	Marker	No. alleles	PIC	Species origin	Nature	Left primer (5′→3′)	Right primer (5′→3′)	GenBank no.	Reference
1	ApEST02	1	0.0000	*Acyrthosiphon pisum*	EST	atggcattcagtatttcgat	ttatgtgcaggacacaacag	DY230061	Weng et al. (2007)
2	ApEST05	6	0.7918	*A. pisum*	EST	tttcccatcgaacaagatac	tagattctgagtggagcgat	CV845551	Weng et al. (2007)
3	ApEST18	5	0.7258	*A. pisum*	EST	cgcagtgatatgcttccta	aaacaatggatggattatgc	CN757895	Weng et al. (2007)
4	ApEST22	2	0.3550	*A. pisum*	EST	atacagtcggtggatctcag	tgatggtgatgtccactatg	DY224487	Weng et al. (2007)
5	ApEST27	2	0.5000	*A. pisum*	EST	ccatgcaacaagtacaacag	ggaccgggtatttctacagt	CN583958	Weng et al. (2007)
6	ApEST38	2	0.4444	*A. pisum*	EST	gacggaataagacaaactgc	cgagacccgttcatttatta	CN754849	Weng et al. (2007)
7	ApEST41	5	0.8714	*A. pisum*	EST	tcttgcttaactgcacacac	tcttgcttaactgcacacac	EC389329	Weng et al. (2007)
8	ApEST42	5	0.6319	*A. pisum*	EST	ctcgctcactccgcactc	ctcgctcactccgcactc	DW012560	Weng et al. (2007)
9	Apg20	14	0.9079	*A. pisum*	Genomic	ccgatgcagtagttctcatt	acacacacacacacacacaa	N/A	Weng et al. (2007)
10	R2.73	5	0.6893	*Rhopalosiphum padi*	Genomic	cgtagaccgccgcggg	gtcgtttctggtcagcggcc	AF277466	Simon et al. (2001)
11	R5.10	7	0.7519	*R. padi*	Genomic	ccgactaagcttaatattgtttg	cggttcggagaacataagag	AF277462	Simon et al. (2001)
12	R5.29.b	2	0.3299	*R. padi*	Genomic	catgagtgtgtccctttttaac	gatggacgaggggacac	AF277464	Simon et al. (2001
13	R5.50	6	0.8159	*R. padi*	Genomic	tgttacgcggagtgtgtagg	ccacagagcgttgtcatc	AF349550	Simon et al. (2001)
14	R6.3	7	0.7324	*R. padi*	Genomic	cgaaatgtacccactataaac	caaatttaaatgtataatcaatg	AF277465	Simon et al. (2001)
15	Sgg01	7	0.8230	*S. graminum*	Genomic	gccctgttaatttgtcgacg	agaagccccccagtcgacgc	DQ887280	Weng et al. (2007)
16	Sgg02	8	0.7734	*S. graminum*	Genomic	gcccgtatatagttaatgtatgacatgtcc	ggtattattccccgtaactgc	DQ887281a	Weng et al. (2007)
17	Sgg03	7	0.7967	*S. graminum*	Genomic	gaataatacccgtttattatggtatcgg	aagccccgaaacctcaaccg	DQ887281b	Weng et al. (2007)
18	Sgg05	4	0.4356	*S. graminum*	Genomic	cgaggacaattcagtctagg	aagtggaacgatgatattgg	AF321574	This study
19	Sgg06	2	0.4970	*S. graminum*	Genomic	taataataagtgcctgccgt	cctccttcacactggtagag	DQ845397	This study
20	Sgg07	4	0.5740	*Schizaphis graminum*	Genomic	atcaatccattggctacaac	agctgagtatcgaaccaaga	DQ845398	This study
21	Sgg08	6	0.8183	*S.graminum*	Genomic	ctttaacattcctcgctgac	cattatacgtgcacaaatcg	DQ845399a	This study
22	Sgg09	2	0.4753	*S. graminum*	Genomic	ttacgcactgcatatacgac	acgaagaccctgatacacac	DQ845399b	This study
23	Sgg10	4	0.6973	*S. graminum*	Genomic	cggtacgctaaggctaataa	gtcaccgataggcatgtact	DQ845400	This study
24	Sgg11	3	0.5893	*S. graminum*	Genomic	agatcaagctgaacaagagc	cgtctgacgtctaatcgaac	DQ845403	This study
25	Sgg12	6	0.7776	*S. graminum*	Genomic	caacgttctgaaggtgtttc	cgagctagtgctacacattg	DQ845404	This study
26	Sgg13	5	0.7040	*S. graminum*	Genomic	aaatcgagtgcgagagttta	gttgttgttgttgttgttgc	DQ887281	This study
27	SmS16b	15	0.9028	*Sitobion miscanthi*	Genomic	ataaaacaaagagcaattcc	gtaaaagtaaaggttccacg	AY349960	Wilson et al. (2004)
28	SmS17b	5	0.7324	*S. miscanthi*	Genomic	ttctggcttcattccggtcg	cgtcgcgttagtgaaccgtg	AY349961	Wilson et al. (2004)
29	SmS23	6	0.7991	*S. miscanthi*	Genomic	ggtccgagagcattcattagg	cgtcgttgtcattgtcgtcg	AY349963	Wilson et al. (2004)
30	SmS24	5	0.7089	*S. miscanthi*	Genomic	cccgaccccgtccattcaaa	cctccaccactactttcactcc	AY349964	Wilson et al. (2004)
31	SmS49	1	0.0000	*S. miscanthi*	Genomic	cgcatttaggaggtttcgac	catgtgcagtggagcaggaa	AY349970	Wilson et al. (2004)

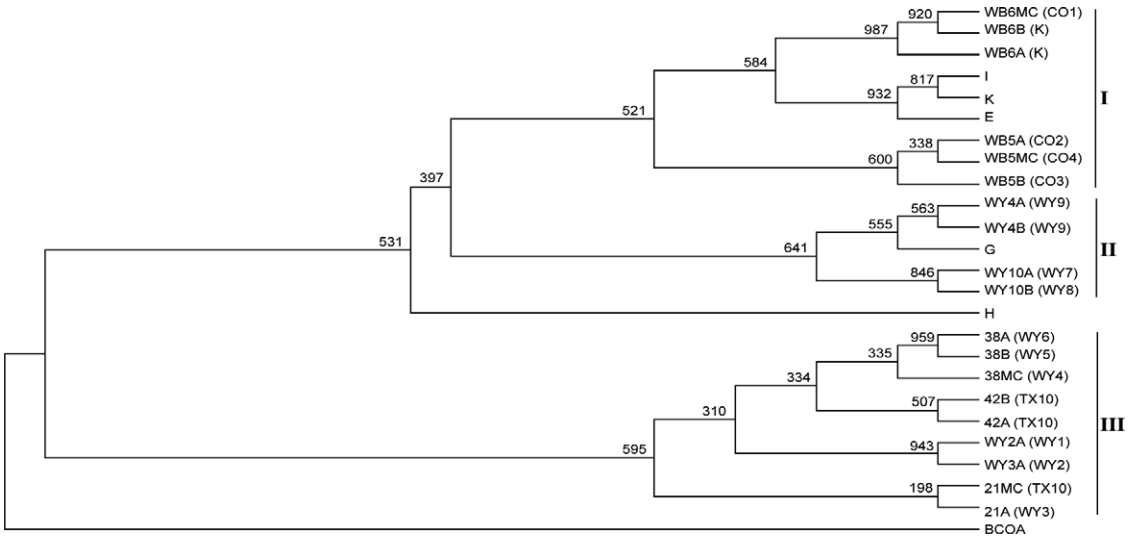

Fig. 1. NJ consensus tree for 19 greenbug isolates and five previously designated biotypes based on 31 microsatellite markers. Bird cherry-oat aphid (BCOA)) was the outgroup in clustering analysis. Bootstrap sampling of alleles was carried out for 1,000 repetitions, and the bootstrap value per 1,000 repetitions was shown at each node. Major groups (I, II, and III) were delimited by vertical bars to the right of isolate names. The new biotype symbol was shown in parenthesis after each isolate name.

other subgroup. Interestingly, biotypes H was genetically distant from other greenbug biotypes, and as such was not grouped with any of them.

Genetic distances among the 24 isolates calculated using Jaccard's similarity coefficient agreed with the consensus tree (data not shown). In addition, when SSR data were analyzed using principal component analysis (Fig. 2), the first and second components explained 19.0 and 10.3% of the total variance, respectively. This grouping of isolates was highly concordant with the consensus tree (Fig. 1), suggesting that molecular data obtained from the current study were robust and reliable.

Discussion

Greenbug biotypes have been defined by their virulence relationship to a selected group of plant genotypes (differentials). Twenty-two greenbug biotypes were previously designated with resistance differentials from wheat, barley, sorghum, and rye (Porter et al. 1997, Burd and Porter 2006). In the current study, 13 new biotypes were recognized. Obviously, the ability to identify new biotypes depends on the number of available differentials in host plants. For example, in Table 2, all six greenbug host differentials in wheat (*Gb1* to *Gb6*) had the same reactions to the first six biotypes, WY1 to WY6 (all resistant), which could have been classified as the same biotype if no other differentials were used. This is also true for biotypes WY7, WY8, and WY9, which were virulent to all six host resistance genes of wheat (Table 2). Because distinguishing between greenbug biotypes is based on the response of a host plant genotype, a greenbug biotype is a phenotypic expression of an indefinite number of genetically diverse individuals sharing similar virulence genes (Puterka and Peters 1990, Anstead et al. 2002). This early notion is well supported by the data herein. For example, the three isolates 42A, 42B, and 21MC had the same biotypic profile as the greenbug biotype TX10 (Burd and Porter 2006), but they were obviously heterogeneous at multiple SSR loci as evidenced from SSR analysis in this study (Fig. 1).

Previous field surveys (Burd and Porter 2006) and molecular marker analysis in a limited number of greenbug biotypes (Shufran et al. 2000, Anstead et al. 2002, Zhu-Salzman et al. 2003, Weng et al. 2007) have suggested that greenbug biotypes are host adapted races. Host-based divergence of insect populations also was observed in the pea aphid (Via et al. 2000, Simon et al. 2003, Frantz et al. 2006), Russian wheat aphid (Dolatti et al. 2005); lettuce root aphid, *Pemphigus bursarius* (L.) (Miller et al. 2005); and English grain aphid, *Sitobion avenae* (F.) (De Barro et al. 1995, Sunnucks et al. 1997). The molecular data herein based on 31 SSRs with 24 greenbug biotypes or isolates clearly supported the host-adapted nature of greenbug biotypes, but with a higher resolution. Although grouped into the same clade, biotypes E, I, and K could be further separated into two subgroups—I and K in one subgroup and E in the other subgroup (Fig. 1). This is consistent with the initial host association of the three greenbug biotypes. Both I and K were initially identified in sorghum (Harvey et al. 1991, 1997), and E was first identified as overcoming wheat resistance gene *Gb2* (Porter et al. 1982). Clearly, the higher resolving power was due to more SSRs used in the current study.

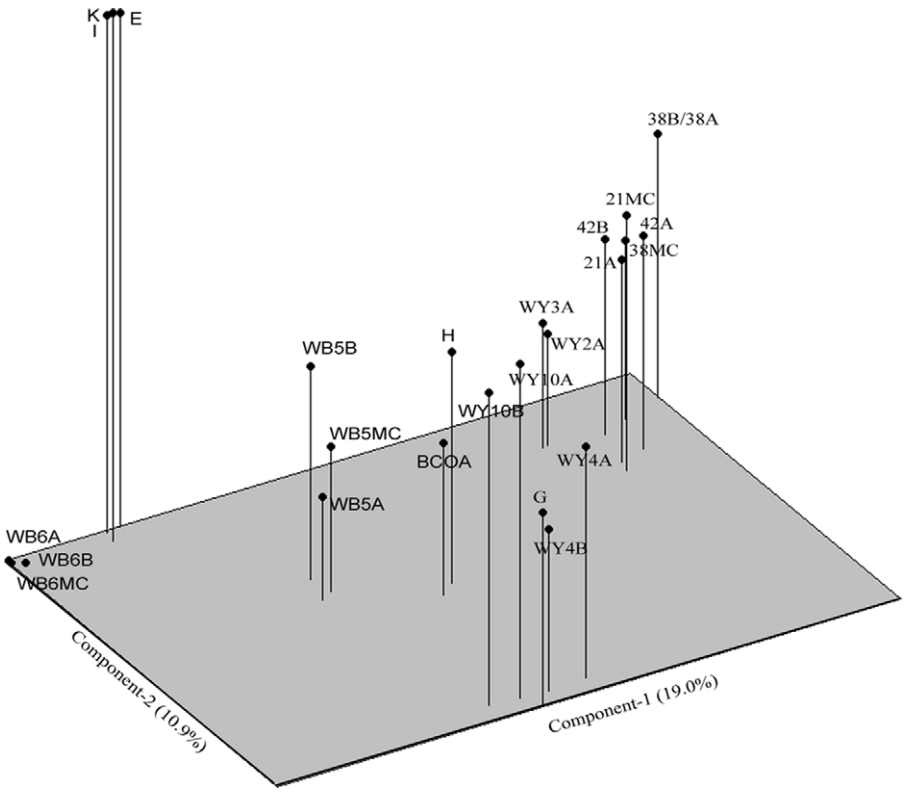

Fig. 2. Three-dimensional plot from PCA of 24 greenbug isolates/biotypes with 31 microsatellite markers. The percentage of total variations explained by the first two principal components (dimension-1 and -2) is given in parentheses. The third dimension (z-axis) was not shown in the plot.

Another example to support the host-associated nature of greenbug biotypes is the clad comprising of biotypes G and isolates WY4A and WY4B (Fig. 1). Both WY4A and WY4B were collected from the intermediate wheatgrass in Wheatland, WY (Table 1), and had the same biotype profile (designated as WY9; Table 2). Kerns et al. (1987) collected a greenbug (SCO) from wheat in Oklahoma that was later designated as biotype G (Puterka et al. 1988). Anstead et al. (2003) found that biotype G was almost exclusively found on *Agropyron* species rather than volunteer and cultivated wheat. It seems that biotype G is adapted to a limited set of noncultivated grass hosts especially *Agropyron* spp. That may explain why biotype G was grouped with WY4A/WY4B although they were collected in two locations that were geographically far away from each other.

Apart from host-associated biotypic genetic variation, regional differentiation among greenbug biotypes also seems evident. All greenbug isolates collected from Colorado (WB isolates) together with the three agricultural biotypes (E, I, and K) were placed in a large group (Group I). Isolates from Wyoming were clustered in two large groups (II and III), and those collected in the same location tended to be grouped in the same subgroup (Fig. 1). Geographical differentiation is well known in aphid species (Martinez-Torres et al. 1997; Simon et al. 1999, 2002; Dedryver et al. 2001; Dolatti et al. 2005; Guo et al. 2005). This is particularly obvious for isolates collected from the state of Wyoming, which seem to be more diverse genetically than those from Colorado at both the phenotypic level (Table 2) and DNA level (Figs. 1 and 2). It is not known if the higher degree of divergence among greenbug populations from Wyoming is due to sexual reproduction, thus genetic recombination among the populations in these areas. It is believed that sexual cycles exist among greenbug populations in regions 35° N parallel (Wadley 1931). The Wyoming isolates were all collected from locations ≈44° N parallel, which may have more chances for sexual reproduction among the greenbug populations.

Although the data here supported geographic divergence among greenbug populations, host association seems to be the determining factor of biotypic variation in agricultural biotypes. In recent field surveys, biotypes E and I exhibited the greatest host range including major small grain crops and a number of grasses, and they were the only biotypes collected in Nebraska, Kansas, Oklahoma, and Texas (Burd and Porter 2006). Nevertheless, these agricultural biotypes were consistently grouped into the same clade in several studies (Shufran et al. 2000, Zhu-Salzman et al. 2003, Weng et al. 2007; current study). This seems reasonable because monoculture of a single crop in

large areas may promote the movement and easy spread of agriculture biotypes. However, those biotypes that are adapted to particular noncultivated grasses but do not usually infest crop plants will have less chance of long-distance movement, thus exhibiting more geographical variations.

Biotype H did not belong to any group in the consensus tree (Fig. 1). Bush et al. (1987) collected a greenbug (WCT) from wheat in Texas, which was later designated as biotype H (Puterka et al. 1988). In a greenbug survey (Burd and Porter 2006), biotype H was found on the jointed goatgrass, *Aegilops cylindrica* Host, and intermediate wheatgrass, *Agropyron intermedium* Beauv. Biotype H has been shown to be the most divergent from other greenbug biotypes based on mitochondrial DNA sequence analysis (Black 1993, Shufran et al. 2000, Anstead et al. 2002). The result herein confirmed the more divergent nature of biotype H compared with other biotypes (Figs. 1 and 2).

The current study and previous studies have shown high degree of genetic variation among greenbug populations based on both phenotypic (Burd and Porter 2006) and genotypic assessments (Shufran et al. 2000; Anstead et al. 2002; Weng et al. 2007; this study). Greenbug biotypic variation is likely to be the interplay of host adaptation and geographic isolation that occurred long before the advent of modern agriculture (Porter et al. 1997). An interesting and important question is, if a biotype is already present in nature, what is the driving force to make it become an economically important prevailing biotype? Although selection pressure from host resistance may not direct emergence of a new biotype (Porter et al. 1997), it is possible that deployment of new host resistance gene(s) in small grain crops may change the population dynamics, and thus the frequencies of different virulence gene(s) that define a particular biotype of greenbug. Biotypes that can infest both noncultivated grasses and crop plants could then become prevalent.

Close examination of Table 2 and Figs. 1 and 2 indicated that biotypes in geographic proximity share more common host response profiles. For example, the biotypic profiles of the four new Colorado biotypes (CO1 to CO4) were more similar to those of the three agricultural biotypes (E, I, and K), whereas those of biotypes WY7, WY8, and WY9 from Wyoming are more similar with each other and that of biotypes G or H that are rarely found on small grain crops. Historically, there has been a shift of prevailing biotypes from C to E and E to I in the fields of the southern Plains. Biotype K also may be a potential threat for small grain crop production (Harvey et al. 1997). If we compare the biotypic profiles of C, E, I, and K against the host differentials, the only difference between C and E is their feeding responses on *Gb2* ('Amigo') in wheat (Burd and Porter 2006). For E and I, and I and K, the differences are their responses to resistance in sorghum differentials 'TX2783' and PI 560607, respectively (Table 2). This may suggest that the biotype sharing the most similar biotypic profiles and the same geographic region with current prevailing one may have the greatest probability to become the new prevailing biotype. Therefore, although the most virulent biotypes were collected from noncultivated hosts (Burd and Porter 2006), because they were found in geographically far away regions (Wyoming), these isolates do not necessarily pose immediate threat to small grain production in the southern Plains. However, close monitoring of greenbug population dynamics especially biotypic variation on both crop plants and noncultivated grasses in small grain production areas may be a useful strategy for detecting potentially new prevailing virulent biotypes of the greenbug.

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References Cited

Anderson, J. A., G. A. Churchill, J. E. Autrique, S. D. Tanksley, and M. E. Sorrells. 1992. Optimizing parental selection for genetic linkage maps. Genome 36: 181–186.

Anstead, J. A., J. D. Burd, and K. A. Shufran. 2002. Mitochondrial DNA sequence divergence among *Schizaphis graminum* (Hemiptera: Aphididae) clones from cultivated and non-cultivated hosts: haplotype and host associations. Bull. Entomol. Res. 92: 17–24.

Anstead, J. A., J. D. Burd, and K. A. Shufran. 2003. Oversummering and biotypic diversity of *Schizaphis graminum* (Homoptera: Aphididae) populations on noncultivated grass hosts. Environ. Entomol. 32: 662–667.

Berry, S. D., V. N. Fondong, C. Rey, B. D. Rogan, C. M. Fauquet, and J. K. Brown. 2004. Molecular evidence for five distinct *Bemisia tabaci* (Homoptera: Aleyrodidae) geographic haplotypes associated with cassava plants in sub-Saharan Africa. Ann. Entomol. Soc. Am. 97: 852–859.

Berzonsky, W. A., H. Ding, S. D. Haley, M. O. Harris, R. J. Lamb, R.I.H. McKenzie, H. W. Ohm, F. L. Patterson, F. B. Peairs, and D. R. Porter. 2003. Breeding wheat for resistance to insects. Plant Breed. Rev. 22: 221–296.

Black, W. C. IV. 1993. Variation in the ribosomal RNA citron among host-adapted races of an aphid (*Schizaphis graminum*). Insect Mol. Biol. 2: 59–69.

Brown, J. K., D. R. Frohlich, and R. C. Rosell. 1995. The sweet potato or silverleaf whiteflies: biotypes of *Bemisia tabaci* or a species complex? Annu. Rev. Entomol. 40: 511–534.

Burd, J. D., and D. R. Porter. 2006. Biotypic diversity in greenbug (Hemiptera: Aphididae): characterizing new virulence and host associations. J. Econ. Entomol. 99: 959–965.

Bush, L., J. E. Slooser, W. D. Worrall, and G. J. Puterka. 1987. Status of greenbug biotypes in Texas. Southwest. Entomol. 12: 229–235.

De Barro, P. J., T. N. Sherratt, C. P. Brookes, O. David, and N. Maclean. 1995. Spatial and temporal genetic variation in British field populations of the grain aphid *Sitobion avenae* (F.) Hemiptera: Aphididae) studied using RAPD-PCR. Proc. R. Soc. Lond. Ser. B 262: 321–327.

Dedryver, C. A., M. Hulle, J. F. Le Gallic, M. Caillaud, and J. C. Simon. 2001. Coexistence in space and time of sexual and asexual populations of the cereal aphid *Sitobion avenae.* Oecologia 128: 379–388.

Diehl, S. R. and G. L. Bush. 1984. An evolutionary and applied perspective of insect biotypes. Annu. Rev. Entomol. 29: 471–504.

Dolatti, L., B. Ghareyazie, S. Moharramipour, and M. R. Noori-Daloii. 2005. Evidence for regional diversity and host adaptation in Iranian populations of the Russian wheat aphid. Entomol. Exp. Appl. 114: 171–180.

Dres, M., and J. Mallet. 2002. Host races in plant-feeding insects and their importance in sympatric speciation. Philos. Trans. R. Soc. Lond. B 357: 471–492.

Felsenstein, J. 1989. PHYLIP: phylogeny inference package (version 3.2). Cladistics 5: 164–166.

Frantz, A., M. Plantegenest, L. Mieuzet, and J. C. Simon. 2006. Ecological specialization correlates with genotypic differentiation in sympatric host-populations of the pea aphid. J. Evol. Biol. 19: 392–401.

Gill, R. J. 1992. A review of the sweet potato whitefly in southern California. Pan-Pac. Entomol. 68: 144–152.

Guo, W., Z. R. Shen, Z. H. Li, and L. W. Gao. 2005. Migration and population genetics of the grain aphid *Macrosiphum miscanti* (Takahashi) in relation to the geographic distance and gene flow. Prog. Natl. Sci. 15: 1000–1004.

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., K. D. Kofoid, T. J. Martin, and P. E. Sloderbeck. 1991. A new greenbug virulent to E-biotype resistant sorghum. Crop Sci. 31: 1689–1691.

Harvey, T. L., G. E. Wilde, and K. D. Kofoid. 1997. Designation of a new greenbug, Biotype K, injurious to resistant sorghum. Crop Sci. 37: 989–991.

Hsiao, T. H., and J. M. Stutz. 1985. Discrimination of alfalfa weevil strains by allozyme analysis. Entomol Exp. Appl. 37: 13–21.

Jaccard, P. 1901. Étude comparative de la distribution florale dans une portion des Alpes et des Jura. Bull. Soc. Vaudoise Sci. Nat. 37: 547–579.

Kerns, D. L., D. C. Peters, and G. J. Puterka. 1987. Greenbug biotypes and greenbug resistant sorghum seed sales surveys in Oklahoma. Southwest. Entomol. 12: 237–243.

Lopes-Da-Silva, M., G.E.L. Tonet, and L.G.E. Vieira. 2004. Characterization and genetic relationships among Brazilian biotypes of *Schizaphis graminum* (Rondani) (Hemiptera: Aphididae) using RAPD markers. Neotrop. Entomol. 33: 43–49.

Martinez, T., D., A. Moya, P.D.N. Hebert, and J. C. Simon. 1997. Geographic distribution and seasonal variation of mitochondrial DNA haplotypes in the aphid *Rhopalosiphum padi* (Hemiptera: Aphididae). Bull. Entomol. Res. 87: 161–167.

Michels, G. J., Jr. 1986. Graminaceous north American host plants of the greenbug with notes on biotypes. Southwest. Entomol. 11: 55–66.

Miller, N. J., N. B. Kift, and G. M. Tatchell. 2005. Host-associated populations in the lettuce root aphid, *Pemphigus bursarius* (L.). Heredity 94: 556–564.

Murray, M. G., and W. F. Thompson. 1980. Rapid isolation of high molecular weight DNA. Nucleic Acids Res. 8: 4321–4325.

Porter, K. B., G. L. Peterson, and O. Vise. 1982. A new greenbug biotype. Crop Sci. 22: 847–850.

Porter, D. R., J. D. Burd, and G. Teetes. 1997. Greenbug (Homoptera, Aphididae) biotypes, selected by resistant cultivars or preadapted opportunists? J Econ. Entomol. 90: 1055–1065.

Puterka, G. J., and D. C. Peters. 1990. Sexual reproduction and inheritance of virulence in the greenbug, *Schizaphis graminum* (Rondani), pp. 289–318. *In* R. K. Campbell and R. D. Eikenbary [eds.], Aphid-plant genotype interactions. Elsevier, Amsterdam, The Netherlands.

Puterka, G. J., D. C. Peters, D. L. Kerns, J. E. Slosser, L. Bush, D. W. Worrall, and R. W. McNew. 1988. Designation of two new greenbug (Homoptera: Aphididae) biotypes G and H. J. Econ. Entomol. 81: 1754–1759.

Puterka, G. J., J. D. Burd, and R. L. Burton. 1992. Biotypic variation in a worldwide collection of Russian wheat aphid (Homoptera, Aphididae). J. Econ. Entomol. 85: 1497–1506.

Saitou, N., and M. Nei. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4: 406–425.

Saxena, R. C., and A. A. Barrion. 1987. Biotypes of insect pests of agricultural crops. Insect Sci. Appl. 8: 453–458.

Shufran, K. A., J. D. Burd, and J. A. Webster. 1997. Biotypic status of Russian wheat aphid (Homoptera: Aphididae) populations in the United States. J. Econ. Entomol. 90: 1684–1689.

Shufran, K. A., J. D. Burd, J. A. Anstead, and G. Lushai. 2000. Mitochondrial DNA sequence divergence among greenbug (Homoptera: Aphididae) biotypes: evidence for host-adapted races. Insect Mol. Biol. 9: 179–184.

Simon, J.-C., S. Baumann, P. Sunnucks, P.D.N. Hebert, J.-S. Pierre, J.-F. Le Gallic, and C. A. Dedryver. 1999. Reproductive mode and population genetic structure of the cereal aphid *Sitobion avenae* studied using phenotypic and microsatellite markers. Mol. Ecol. 8: 531–545.

Simon, J.-C., N. Leterme, F. Delmotte, O. Martin, and A. Estoup. 2001. Isolation and characterization of microsatellite loci in the aphid species, *Rhopalosiphum padi.* Mol. Ecol. Notes 1: A33.

Simon, J.-C., C. Rispe, and P. Sunnucks. 2002. Ecology and evolution of sex in aphids. Trend Ecol. Evol. 17: 34–39.

Simon, J.-C., S. Carre, M. Boutin, N. Prunier-Leterme, B. Sabater-Munoz, A. Latorre, and R. Bournoville. 2003. Host-based divergence in populations of the pea aphid: insights from nuclear markers and the prevalence of facultative symbionts. Proc. R. Soc. Lond. B 270: 1703–1712.

Spooner, D. M., J. Tivang, J. Nienhuis, J. T. Miller, D. S. Douches, and M. A. Contreras. 1996. Comparison of four molecular markers in measuring relationships among the wild potato relatives *Solanum* section *Etuberosum* (subgenus *Potato*). Theor. Appl. Genet. 92: 532–540.

Sunnucks, P., P. J. De Barro, G. Lushai, N. Maclean, and D. F. Hales. 1997. Genetic structure of an aphid studied using microsatellites: cyclic parthenogenesis, differentiated lineages and host specialization. Mol. Ecol. 6: 1059–1073.

Via, S., A. C. Bouck, and S. Skillman. 2000. Reproductive isolation between divergent races of pea aphids on two hosts. II. Selection against migrants and hybrids in the parental environments. Evolution 54: 1626–1637.

Wadley, F. 1931. Ecology of *Toxoptera graminum,* especially as to factors affecting importance in the Northern United States. Ann. Entomol. Soc. Am. 24: 325–395.

Weng, Y., P. Azhaguvel, G. J. Michels, Jr., and J. C. Rudd. 2007. Cross-species transferability of microsatellite markers from six aphid (Hemiptera: Aphididae) species and their use for evaluating biotypic diversity in two cereal aphids. Insect Mol. Biol. 16: 613–622.

Wilson, A.C.C., B. Massonnet, J. C. Simon, N. Prunier-Leterme, L. Dolatti, K. S. Llewellyn, C. C. Figueroa, C. C. Ramirez, R. L. Blackman, A. Estoup, and P. Sunnucks. 2004. Cross-species amplification of microsatellite loci in aphids: assessment and application. Mol. Ecol. Notes 4: 104–109.

Wood, E. A. 1961. Biological studies of a new greenbug biotype. J. Econ. Entomol. 54: 1171–1173.

Zhu-Salzman, K., H. Li, P. E. Klein, R. L. Gorena, and R. A. Salzman. 2003. Using high-throughput amplified fragment length polymorphism to distinguish sorghum greenbug (Homoptera: Aphididae) biotypes. Agric. For. Entomol. 5: 311–315.

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