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Discovery of English Grain Aphid (Hemiptera: Aphididae) Biotypes in China

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ABSTRACT The English grain aphid, *Sitobion avenae* (F.) (Hemiptera: Aphididae), is an important pest insect of wheat, *Triticum aestivum* (L.), in China. Grain aphid biotypes are necessary to breed aphid-resistant wheat varieties; however, none have currently been identified. Here, we describe a method to identify grain aphid biotypes and survey the aphid biotype variation in the wheat growth area of China. Clones of *S. avenae* were collected from 11 locations in China and used to establish culture populations. These populations were then used to assess the resistance of 12 wheat varieties. Based on resistance responses, seven differential hosts were selected to identify the biotype of *S. avenae*: Amigo, 'Fengchan No. 3', Zhong 4 wumang, JP1, LL, 885479-2, and 'Xiaobaidongmai'. *S. avenae* was ultimately classified into five biotypes: EGA I, EGA II, EGA III, EGA IV, and EGA V. These methods provide a mechanism to detect the variation and evolution of grain aphids in different wheat-growing locations and also allow for selection of appropriate aphid-resistant germplasm for wheat breeding of commercial wheat cultivars.

KEY WORDS wheat, aphid biotype, wheat resistance to aphid, *Sitobion avenae*

Aphids are major agricultural insect pests. They have a global distribution, damaging crops by removing photo assimilates and vectoring numerous devastating plant viruses (Smith and Boyko 2007). The development of successful long-term resistance is hampered by their unparalleled reproductive capacity (Dixon 1998) and the frequent emergence of new races and biotypes able to manipulate host plant physiology (Blackman et al. 1990; Puterka et al. 1993; Sunnucks et al. 1997a,b; Wilson et al. 2003).

The English grain aphid, *Sitobion avenae* (F.) (Hemiptera: Aphididae), is a major wheat, *Triticum aestivum* (L.), insect pest, responsible for frequent and extensive damage in wheat-growing areas worldwide (van Emden and Harrington 2007). In China, *S. avenae* is one of primary cereal aphid species (Chen et al. 1997), sucking nutrients from a sieve tube inserted into wheat phloem and transmitting barley yellow dwarf virus. *S. avenae* damage affects ≈ 13 million hm^2/yr and causes wheat yield loss up to 40% (Duan et al. 2006). Insecticides are the major methods used to control the grain aphid, but the excessive and im-

proper use of pesticides can seriously impact the local economy, environment and food safety.

Plant resistance is the most effective and environmentally friendly pest control measure (Nuessly et al. 2008). To effectively manage aphid pests by plant resistance, it is important to understand the genetic basis of aphid-resistance in wheat cultivars. One of the prerequisites for developing grain aphid resistant cultivars is the identification and characterization of sources of resistance. Antixenosis, antibiosis, and tolerance are three patterns of host plant resistance to aphids (Painer 1951, Kogan and Ortman 1978, Panda and Khush 1995). Antixenosis and antibiosis are measured in terms of aphid responses to host plants, whereas tolerance is measured by host plants responses to particular levels of aphid infestation. Antixenosis deters or reduces colonization by insects. Antibiosis causes adverse effects on insect life parameters. Tolerance is the ability for plant to grow normally while supporting aphid infestation, which would limit growth and reproduction of a susceptible host (Hesler 2005).

Despite multiple studies examining *S. avenae* resistance in wheat, there is little consensus regarding the underlying genetic mechanisms. Studies have implicated a single incompletely dominant gene (Zheng et al. 1999), a single dominant gene (Duan et al. 2006), as well as quantitative causes (Di Pietro et al. 1998b; Wu et al. 1999). Although environmental and economic benefits of planting resistant crops plant are well documented, the lack of agreement regarding the

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Fig. 1. *S. avenae* sampling sites (see Sampling Sites and Aphid Populations for expansions of abbreviations).

genetic mechanisms of wheat resistance to *S. avenae* severely hinders breeding aphid-resistant wheat.

The development of new biotypes (populations with the ability to damage normally resistant host plants) endangers the durability of plant resistance (Shufran et al. 2000, Burd and Porter 2006), and thus new biotypes clearly limit the usefulness of existing resistant cultivars (Weiland et al. 2008). Approximately 50% of the recognized insect biotypes on agricultural crops are aphids (Saxena and Barrion 1987), and it is reported that cereal aphids, such as greenbug, *Schizaphis graminum* (Rondani) (Burd and Porter 2006), and the Russian wheat aphid, *Diuraphis noxia* (Kurdjumov) (Weiland et al. 2008), have biotypic diversity. For example, 22 greenbug biotypes were reported previously (Burd and Porter 2006, Weng et al. 2010).

The *S. avenae* biotype is poorly documented despite the fact that *S. avenae* is a significant agricultural insect pest worldwide, and it is the primary species in most wheat-growing areas in China. The result of recent field screenings of aphid wheat resistance revealed germplasm with varying resistance levels. Specifically, some wheat cultivars demonstrated different fitness to *S. avenae* between two geographic populations: Hebei Langfang and Sichuan Jiangyou (J.-L.C., unpublished data). These differences among *S. avenae* populations validated the necessity to study aphid biotypes. In this study, we established a new method to

identify grain aphid biotypes and assess biotypic variation.

Materials and Methods

Sampling Sites and Aphid Populations. Populations of *S. avenae* were collected in 2009 from the winter wheat fields in 11 wheat-growing areas in China: HS, Hebei Shijiazhuang; ST, Shandong Taian; STA, Shanxi Taiyuan; HL, Henan Luoyang; AH, Anhui Hefei; JY, Jiangsu Yancheng; SB, Shaanxi Baoji; YH, Yunnan Honghe; SJ, Sichuan Jiangyou; HX, Hubei Wuhan; QX, Qinghai Xining (Fig. 1). All populations were maintained separately on seedlings of susceptible 'Beijing 837' wheat at $22 \pm 1^\circ\text{C}$, 40–60% RH, and a photoperiod of 16:8 (L:D) h. One viviparous adult aphid was selected from each population to establish a colony. To discriminate between colony and population from the same location, the aphid colony was designated as "individual," and the original aphid population as "population." All aphids were reared under conditions that minimized the risk of contamination between clones, e.g., the aphid isolates were transferred to potted wheat seedlings at two-leaf stage, and each pot was separated with a transparent plastic cylinder cage covered with gauze on the top (12 cm in height and 24 cm in diameter). Aphids and plants were maintained in a greenhouse at $22 \pm 1^\circ\text{C}$, with a photoperiod of 16:8 (L:D) h.

Table 1. Wheat variety reaction to *S. avenae* resistance category reference

Wheat variety	Previously known reaction to <i>S. avenae</i> and resistance gene if known	Main resistance mechanism	Reference
Amigo	Resistant/Gb2		Hollenhorst and Joppa (1983)
Fengchang No. 3	Resistant		Li and Jin (1998)
Zhong 4 wumang	Resistant	Antibiosis	Chen et al. (1997)
JPI	Resistant	Sntibiosis	Chen et al. (1997)
KOK	Resistant	Antixenosis	Chen et al. (1997)
Jinmai 31	Resistant		Zheng et al. (1999)
L1	Resistant	Antixenosis	Chen et al. (1997)
885479-2	Susceptible	Antibiosis	Chen et al. (1997)
Beijing 837	Susceptible		Chen et al. (1997)
Xiaobaidongmai	Resistant	Antixenosis	Chen et al. (1997)
Mingxian 169	Susceptible		Liu et al. (2001)
Hongmanghong	Susceptible		Chen et al. (1997)

Wheat Varieties. Twelve different wheat varieties were used (Table 1). Wheat varieties (Zhong 4 wumang, JPI, L1 and 885479-2) were selected based on two years of field data that indicated the presence of varying levels of aphid resistance between Langfang in Hebei Province and Jiangyou in Sichuan Province of China. Beijing 837, 'Hongmanghong', and 'Mingxian 169' were included as susceptible controls. The main resistance mechanism of the above-mentioned wheat varieties (Zhong 4 wumang, JPI, KOK, L1, 885479-2, and 'Xiaobaidongmai') is antixenosis or antibiosis (Chen et al. 1997).

Aphid Infestation and Resistance Test. Ten seeds of each wheat variety were planted and germinated in a plastic pot. For each aphid population, a single pot of each variety, 12 pots total, were placed together by random arrangement and covered by a single, large nylon mesh cage (55 cm in length, 45 cm in width, and 60 cm in height). When wheat seedlings reached the two-leaf stage, each seedling was manually infected with two second-instar nymphs from the same aphid population. After being infected, the 12 pots were recovered with a single cage immediately. Three replicates were done for each aphid population. Ten days after infection, the number of aphids per pot was counted. The same treatment was conducted for each aphid population. Wheat resistance to *S. avenae* was evaluated according to the ratio of aphid quantity. The ratio of aphid quantity is the average aphid number on each wheat variety divided by the average aphid number per pot in one cage. A wheat plant was designated resistant (R; 0–0.9) or susceptible (S; >0.9) based on the ratio of aphid quantity that is consistent with the documented classifications (Li et al. 1998, Qu et al. 2004).

Statistical Analysis. Data analysis and computations were done with SAS (SAS Institute 2001) by using the analysis of variance (ANOVA) procedure, and when appropriate, means were separated by Tukey's studentized range (honestly significant difference [HSD]) test ($P < 0.05$).

Results

Aphid Numbers on Wheat Varieties. Twenty-two isolates (including populations and individuals) of *S.*

avenae were used to evaluate the fitness to 12 wheat varieties (Tables 2 and 3). There were high aphid numbers for all isolates on three wheat varieties: Beijing 837, Mingxian 169, and Hongmanghong. These varieties were identified by previous field screenings as *S. avenae* susceptible. However, for other wheat varieties significant differences in aphid numbers were observed. For example, Amigo demonstrated low aphid number for all but one of the *S. avenae* isolates (HX). The results indicated that some *S. avenae* isolates are capable of overcoming the existing aphid resistance of wheat cultivars.

Many *S. avenae* isolates showed significant differences with regard to aphid numbers among wheat varieties. The populations of HS, ST, STA, and HL, however, had no significant differences in the aphid numbers among many wheat varieties. HS, ST, and HL populations showed no significant differences on 'Fengchang No. 3', Zhong 4 wumang, JPI, L1, 885479-2, Beijing 837, Xiaobaidongmai, Mingxian 169, and Hongmanghong. STA population showed no significant differences on Fengchang No. 3, Zhong 4 wumang, 'Jinmai 31', L1, 885479-2, Beijing 837, Xiaobaidongmai, Mingxian 169, and Hongmanghong.

Evaluation for Wheat Resistance to Different *S. avenae* Isolates. For the ratio of aphid quantity, *S. avenae* populations of HS, ST, STA, and HL showed no clear differentiation among many wheat varieties (data not shown). Seven aphid populations were selected to demonstrate aphid host reactions: AH, JY, SB, YH, SJ, HX, and QX. The resistance of 12 wheat varieties to these seven populations is given in Table 4. AH and JY populations showed a similar pattern of aphid-host reactions among the 12 wheat varieties, as did the SB and YH populations. However, SJ, HX, and QX each showed patterns unique from that of other populations.

The resistance of the 12 wheat varieties to *S. avenae* individuals is provided in Table 5. Interestingly, it is not the same as that seen among populations. HS, ST, and AH individuals showed a similar pattern of reaction; STA, JY, YH, and SJ individuals showed a similar pattern of reaction; SB and QX individuals showed a similar pattern of reaction. However, the HX individual showed a reaction unique from all others tested.

Table 2. Mean ± SE number of 11 *S. avenae* populations on 12 wheat varieties at 10 d after initial infestation of 20 aphids

Wheat variety	<i>S. avenae</i> isolate											
	HS	ST	STA	HL	AH	JY	SB	YH	SJ	HX	QX	
Amnigo	64.33 ± 2.37B	61.67 ± 1.45C	45.67 ± 2.03C	96.67 ± 1.76BC	41.67 ± 1.86F	24.33 ± 2.33E	33.33 ± 2.98F	40.67 ± 4.73F	39.00 ± 2.52GH	135.00 ± 2.65C	35.00 ± 2.08H	
Fengchan No. 3	101.33 ± 3.03A	105.67 ± 1.76A	104.67 ± 2.67A	104.67 ± 0.88AB	59.33 ± 2.33E	70.67 ± 3.48D	197.00 ± 6.24A	155.00 ± 7.00A	110.00 ± 4.16D	189.33 ± 2.33A	197.00 ± 2.52A	
Zhong 4 wumang	104.00 ± 1.25A	100.67 ± 2.03A	103.33 ± 2.19A	112.33 ± 2.06A	74.00 ± 2.08DE	62.00 ± 5.13D	58.67 ± 1.70DE	75.67 ± 4.73E	51.33 ± 1.86FG	70.67 ± 3.28EF	120.33 ± 6.12D	
JPI	100.00 ± 2.83A	96.67 ± 0.88A	69.33 ± 0.88B	109.67 ± 2.19AB	74.33 ± 2.96DE	59.00 ± 3.06D	180.33 ± 4.84A	141.00 ± 3.61AB	156.33 ± 3.28B	42.00 ± 3.79GH	103.67 ± 3.71E	
KOK	66.67 ± 1.19B	72.67 ± 1.76B	64.33 ± 3.18B	89.33 ± 2.85C	86.67 ± 2.40CD	68.00 ± 3.00D	71.67 ± 2.60D	73.67 ± 4.16E	60.00 ± 2.08F	30.33 ± 1.20H	68.00 ± 2.65FG	
Jinmai 31	71.33 ± 1.19B	66.33 ± 1.45BC	105.67 ± 1.20A	96.33 ± 1.45BC	84.33 ± 3.28CD	74.33 ± 3.53D	75.33 ± 3.38D	77.00 ± 6.24E	64.00 ± 3.79F	84.33 ± 1.86E	59.00 ± 3.21G	
LI	102.67 ± 1.52A	99.00 ± 2.08A	97.00 ± 5.51A	105.67 ± 1.76AB	76.00 ± 3.21DE	80.00 ± 5.51D	45.33 ± 4.06EF	56.00 ± 5.29F	89.33 ± 2.73E	116.00 ± 5.13D	147.00 ± 4.04C	
885479-2	108.00 ± 1.89A	100.00 ± 2.00A	99.67 ± 4.26A	106.33 ± 3.76AB	127.67 ± 4.48B	143.00 ± 5.57B	97.67 ± 5.70C	96.00 ± 5.57D	146.00 ± 2.65BC	57.00 ± 1.15FG	42.00 ± 1.53H	
Beijing 837	97.67 ± 2.23A	105.33 ± 4.18A	105.00 ± 2.06A	113.33 ± 2.19A	178.00 ± 5.51A	136.00 ± 3.21B	116.33 ± 4.63BC	116.67 ± 6.66C	97.00 ± 3.51DE	163.67 ± 3.84B	97.00 ± 2.00E	
Xiaobaidongmai	108.00 ± 3.30A	104.00 ± 1.53A	95.67 ± 1.86A	116.33 ± 1.86A	127.67 ± 3.71B	166.67 ± 3.84A	100.00 ± 4.36C	89.00 ± 5.00DE	27.33 ± 3.91IH	143.00 ± 6.00C	75.33 ± 3.18F	
Mingxian 169	108.33 ± 0.98A	106.00 ± 2.52A	103.67 ± 1.45A	111.33 ± 3.53A	130.00 ± 3.51B	114.00 ± 5.86C	121.33 ± 2.60B	135.00 ± 4.58B	174.33 ± 2.91A	107.33 ± 3.84D	168.00 ± 2.52B	
Hongnanghong	106.33 ± 1.19A	105.67 ± 2.19A	101.33 ± 1.86A	112.67 ± 3.84A	98.33 ± 3.18C	108.67 ± 3.28C	114.67 ± 2.96BC	104.67 ± 6.11CD	135.00 ± 3.06C	104.00 ± 3.06D	94.33 ± 1.20E	
ANOVA												
F	44.83	60.42	53.08	9.26	126.84	100.13	151.09	126.42	262.06	199.03	251.45	
df	11	11	11	11	11	11	11	11	11	11	11	
P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	

Means within columns followed by the same letter are not significantly different between varieties within isolates ($P < 0.05$; HSD test).

Table 3. Mean ± SE numbers of 11 *S. avenae* individuals on 12 wheat varieties at 10 d after initial infestation of 20 aphids

Wheat variety	<i>S. avenae</i> isolate											
	HS	ST	STA	HL	AH	JY	SB	YH	SJ	HX	QX	
Amnigo	24.67 ± 3.18E	39.67 ± 3.48G	36.33 ± 2.73G	45.33 ± 2.03HI	31.00 ± 2.65H	41.33 ± 3.18F	44.00 ± 4.16H	43.67 ± 3.18H	28.67 ± 3.18H	164.67 ± 3.48A	58.67 ± 3.41H	
Fengchan No. 3	46.33 ± 2.60D	60.67 ± 2.19EF	161.00 ± 1.53A	123.33 ± 3.48D	66.67 ± 3.58EF	157.67 ± 6.36A	139.33 ± 3.84AB	151.00 ± 4.04B	124.33 ± 3.18B	149.33 ± 1.20BC	140.00 ± 2.05A	
Zhong 4 wumang	49.00 ± 1.73CD	70.00 ± 3.61DE	49.00 ± 2.31G	77.67 ± 3.76GGF	54.33 ± 2.96GF	63.33 ± 3.38EF	115.67 ± 4.91CDE	69.67 ± 2.40G	52.67 ± 2.33E	41.33 ± 1.76E	125.00 ± 2.16ABC	
JPI	49.67 ± 1.45CD	49.33 ± 4.63FG	174.67 ± 3.38A	117.00 ± 4.93D	72.33 ± 1.86G	147.67 ± 5.78A	131.00 ± 4.04BC	189.00 ± 3.79A	139.67 ± 2.85AB	53.67 ± 3.28E	111.67 ± 2.68CD	
KOK	66.67 ± 3.28C	51.67 ± 3.91EFG	72.00 ± 3.21DE	91.33 ± 3.38EF	48.67 ± 0.88G	46.00 ± 4.04F	67.00 ± 5.03G	92.33 ± 3.48F	77.33 ± 2.75D	49.00 ± 3.61E	70.67 ± 1.19GH	
Jinmai 31	61.00 ± 4.04CD	59.00 ± 3.61EF	69.67 ± 4.70EF	61.67 ± 3.28CH	76.67 ± 4.41DE	82.33 ± 4.06DE	71.67 ± 0.88G	87.33 ± 2.40F	79.00 ± 4.04D	93.00 ± 3.46D	64.33 ± 3.41GH	
LI	66.00 ± 5.20C	84.67 ± 2.96CD	53.00 ± 3.79FG	187.33 ± 3.48B	64.67 ± 1.45EFG	61.33 ± 4.91EF	160.00 ± 3.79A	39.00 ± 1.73H	52.33 ± 2.96E	137.00 ± 2.65C	127.00 ± 2.62AB	
885479-2	184.00 ± 4.62A	211.33 ± 4.91A	102.33 ± 5.81C	109.33 ± 4.98DE	159.00 ± 3.21B	117.67 ± 5.49BC	31.33 ± 2.96H	133.67 ± 2.73C	143.00 ± 2.65A	53.67 ± 2.91E	41.67 ± 2.60I	
Beijing 837	165.67 ± 3.76A	194.00 ± 3.51A	96.67 ± 4.26C	143.67 ± 4.70C	184.00 ± 5.29A	103.67 ± 4.63CD	96.67 ± 4.06EF	127.00 ± 3.21CD	98.33 ± 3.18C	157.33 ± 2.85AB	88.67 ± 2.13EF	
Xiaobaidongmai	112.33 ± 6.64B	93.67 ± 2.33C	89.33 ± 3.76CD	32.33 ± 2.40I	109.00 ± 3.06C	90.67 ± 2.33D	83.67 ± 4.06FG	100.67 ± 2.60EF	100.67 ± 5.36C	101.33 ± 2.73D	79.33 ± 1.96FG	
Mingxian 169	107.33 ± 2.60B	118.00 ± 3.61B	140.00 ± 2.08B	211.00 ± 4.04A	114.00 ± 5.51C	99.00 ± 3.46CD	124.67 ± 3.53BCD	99.00 ± 3.61EF	91.00 ± 4.73CD	143.00 ± 3.51BC	113.00 ± 1.63BCD	
Hongnanghong	93.67 ± 2.60B	124.33 ± 3.84B	106.33 ± 2.60C	119.67 ± 3.28D	89.33 ± 0.88D	136.33 ± 3.84AB	104.33 ± 6.39DEF	114.33 ± 3.71DE	94.33 ± 1.76CD	99.00 ± 2.65D	99.00 ± 2.16DE	
ANOVA												
F	171.34	228.36	155.38	204.12	187.62	76.73	88.81	187.76	107.08	247.94	109.11	
df	11	11	11	11	11	11	11	11	11	11	11	
P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	

Means within columns followed by the same letter are not significantly different between varieties within isolates ($P < 0.05$; HSD test).

Table 4. Response of 12 wheat varieties to different *S. avenae* populations

Wheat variety	<i>S. avenae</i> isolate						
	AH	JY	SB	YH	SJ	HX	QX
Amigo	R	R	R	R	R	S	R
Fengchan No.3	R	R	S	S	S	S	S
Zhong 4 wumang	R	R	R	R	R	R	S
JPI	R	R	S	S	S	R	S
KOK	R	R	R	R	R	R	R
Jinmai 31	R	R	R	R	R	R	R
L1	R	R	R	R	S	S	S
885479-2	S	S	S	S	S	R	R
Beijing 837	S	S	S	S	S	S	S
Xiaobaidongmai	S	S	S	S	R	S	R
Mingxian 169	S	S	S	S	S	S	S
Hongmanghong	S	S	S	S	S	S	S

In this study, KOK and Jinmai 31 were resistant to all *S. avenae* populations examined, in contrast to Beijing 837, Mingxian 169, and Hongmanghong, which were susceptible to all *S. avenae* populations. The remaining seven wheat varieties (Amigo, Fengchan No. 3, Zhong 4 wumang, JPI, L1, 885479-2, and Xiaobaidongmai) exhibited different resistant responses to the seven populations and 11 individuals tested. For example, Amigo was susceptible to the HX population and individual but resistant to all other populations and individuals. Thus, these seven wheat varieties were selected to establish the biotypes of *S. avenae*. Based on the resistant responses, five response patterns were established and are listed in Table 6: EGA I (AH population, JY population, HS individual, ST individual, and AH individual), EGA II (SB population, YH population, STA individual, JY individual, YH individual, and SJ individual), EGA III (SJ population and HL individual), EGA IV (HX population and HX individual, and EGA V (QX population, SB individual, and QX individual).

Discussion

Aphid biotype monitoring is essential to the development of aphid-resistant cultivars. Researchers need be able to identify the appearance of new aphid biotypes because they have the potential to defeat resistance genes that are being used or developed for

Table 5. Response of 12 wheat varieties to different aphid individuals

Wheat variety	<i>S. avenae</i> isolate											
	HS	ST	STA	HL	AH	JY	SB	YH	SJ	HX	QX	
Amigo	R	R	R	R	R	R	R	R	R	S	R	
Fengchan No.3	R	R	S	S	R	S	S	S	S	S	S	
Zhong 4 wumang	R	R	R	R	R	R	S	R	R	R	S	
JPI	R	R	S	S	R	S	S	S	S	R	S	
KOK	R	R	R	R	R	R	R	R	R	R	R	
Jinmai 31	R	R	R	R	R	R	R	R	R	R	R	
L1	R	R	R	S	R	R	S	R	R	S	S	
885479-2	S	S	S	S	S	S	R	S	S	R	R	
Beijing 837	S	S	S	S	S	S	S	S	S	S	S	
Xiaobaidongmai	S	S	S	R	S	S	R	S	S	R	R	
Mingxian169	S	S	S	S	S	S	S	S	S	S	S	
Hongmanghong	S	S	S	S	S	S	S	S	S	S	S	

Table 6. Response of seven wheat varieties to different biotypes of *S. avenae*

Wheat variety	Biotype				
	EGA I	EGA II	EGA III	EGA IV	EGA V
Amigo	R ^a	R	R	S ^a	R
Fengchan No.3	R	S	S	S	S
Zhong 4 wumang	R	R	R	R	S
JPI	R	S	S	R	S
L1	R	R	S	S	S
885479-2	S	S	S	R	R
Xiaobaidongmai	S	S	R	R	R

^a R, resistant; S, susceptible.

future use (Kim et al. 2008). Field screening data demonstrated variability in the response to wheat varieties to *S. avenae*. Based on unique virulence patterns, we established that there are five different biotypes. This is the first report of the existence of distinct biotypes of *S. avenae* from China.

There is currently no uniform standard for the identification of wheat resistance to *S. avenae* at seedling stage. Unlike for *S. graminum* and *D. noxia*, there are no obvious symptoms of *S. avenae* infestation on wheat plant. Therefore, an infected wheat plant cannot be rated based on the commonly used metrics of chlorosis and plant vigor (Porter et al. 1982). The procedure used to identify wheat resistance to *S. avenae* in the field is based upon aphid number on the wheat leaf and ear, and this method also is robust for the laboratory.

Multiple biotypes occur in several aphid species, such as *D. noxia* and *S. graminum* (Haley et al. 2004, Burd and Porter 2006, Zaayman et al. 2009). Extensive cultivation of resistant varieties as well as aphid mass migration seems to drive the evolution of new biotypes (Pasalu et al. 2004). However, cultivars with resistance genes of diverse sequence and function can be deployed to sustain resistance and help delay the development of virulent, resistance-breaking aphid biotype (Smith and Boyko 2007). Di Pietro et al. (1998a) reared *S. avenae* on resistant wheat for 2 yr and could find no selection for breaking the host plant resistance. Although we found biotype variation in *S. avenae* from China, there is further need to identify the resistance gene(s) or genetic mechanisms in the six different wheat varieties (Fengchang No. 3, Zhong 4 wumang, JPI, KOK, Jinmai 31, L1, 885479-2, Xiaobaidongmai) to improve our understanding of biotype variation in *S. avenae*.

There were no significant differences of the ratios of aphid quantity for the HS, ST, STA, and HL populations among many wheat varieties. These populations were all from locations in the north of China where *S. avenae* is unable to survive through the winter. The aphid has the capacity to overwinter in the south of China and may then migrate into the north in spring. *S. avenae* populations collected from these four northern locations may have immigrated from other regions. Thus, there may be several biotypes in one population. Differential host plant responses have

been observed for the AH, JY, SB, YH, SJ, HX, and QX populations. These observations may be explained by the following: 1) several isolations were from southern wheat regions where the aphids can overwinter and be collected in early spring, the collected aphids were local overwintering aphids; or 2) some localities contained unique environment. Although populations collected in the above-mentioned localities contained several biotypes, each of location contained a predominant biotype. Our results suggest that there is biotypic variation among *S. avenae* populations of China. Further research is needed to determine the distribution and diversity of English grain aphid biotypes within populations and the predominant biotype of each geographical population.

Development of resistant varieties seems to be the most sustainable approach in the management of the English grain aphid. However, a successful insect resistance breeding program requires an understanding of insect biotypes occurring in each relevant locality. Biotypes may overcome resistant plants and failure to recognize their existence may lead to severe infestations of formerly resistant cultivars. The current study shows that biotype identification of *S. avenae* population is the first step for any cereal breeding program to obtain resistant cultivars to this pest.

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