Virulence Analysis of Hessian Fly Populations From Texas, Oklahoma, and Kansas

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ABSTRACT In recent years, the number of wheat, *Triticum aestivum* L., fields heavily infested by Hessian fly, *Mayetiola destructor* (Say), has increased in the Great Plains of the United States. Historically, resistance genes in wheat have been the most efficient means of controlling this insect pest. To determine which resistance genes are still effective in this area, virulence of six Hessian fly populations from Texas, Oklahoma, and Kansas was determined, using the resistance genes *H3*, *H4*, *H5*, *H6*, *H7H8*, *H9*, *H10*, *H11*, *H12*, *H13*, *H16*, *H17*, *H18*, *H21*, *H22*, *H23*, *H24*, *H25*, *H26*, *H31*, and *Hdic*. Five of the tested genes, *H13*, *H21*, *H25*, *H26*, and *Hdic*, conferred high levels of resistance (>80% of plants scored resistant) to all tested populations. Resistance levels for other genes varied depending on which Hessian fly population they were tested against. Biotype composition analysis of insects collected directly from wheat fields in Grayson County, TX, revealed that the proportion of individuals within this population virulent to the major resistance genes was highly variable (89% for *H6*, 58% for *H9*, 28% for *H5*, 22% for *H26*, 15% for *H3*, 9% for *H18*, 4% for *H21*, and 0% for *H13*). Results also revealed that the percentages of biotypes virulent to specific resistance genes in a given population are highly correlated ($r^2 = 0.97$) with the percentages of susceptible plants in a virulence test. This suggests that virulence assays, which require less time and effort, can be used to approximate biotype composition.

KEY WORDS *Mayetiola destructor*, Hessian fly, biotype, wheat breeding, plant resistance

The Hessian fly, *Mayetiola destructor* (Say), introduced into the United States from the southern Caucasus region of Eurasia, is one of the most destructive pests of wheat, *Triticum aestivum* L. (Hatchett et al. 1987, Pauly 2002). Since it was first observed on Long Island, NY, around 1779, Hessian fly has gradually spread to nearly all wheat growing regions of the nation. Major Hessian fly control tactics have limited effectiveness and include the deployment of resistant cultivars, application of systemic insecticides at planting (seed treatment), and late planting to avoid the fall generation damage (fly-free date) (Zelarayan et al. 1991, Buntin et al. 1992). Applying systemic insecticide at planting is effective only during early growth and tillering (Morrill and Nelson 1975, Buntin and Hudson 1991, Buntin 1992). Late planting can be adopted only in certain regions of the northern United States and may result in a greater infestation of later Hessian fly generations (Buntin and Bruckner 1990). Fly-free dates also vary from year to year because of temperature fluctuation and may conflict with optimal planting and production practices. In addition, late planting may result in loss of forage, reducing wheat yield potential (Buntin et al. 1992). Much success for controlling this pest has been achieved by breeding wheat cultivars for Hessian fly resistance (Ratcliffe and Hatchett 1997). Because of its effectiveness, resistant breeding has been widely adopted in all major wheat-growing regions. The major challenge for the host resistance strategy is the development of new virulent biotypes that overcome resistance of specific genes after they are deployed (Ratcliffe et al. 1994, 2000).

Because of the occurrence of new biotypes, Hessian fly populations need to be analyzed periodically to provide wheat breeders and growers information on the effectiveness of wheat resistance genes and on biotype compositions of regional populations. The most recent studies of Hessian fly biotypes and the effectiveness of major resistance genes were conducted in the southeastern, midwestern, and northwestern (Ratcliffe et al. 2000, Clement et al. 2003) and eastern United States (Ratcliffe et al. 1996). In recent years, Hessian fly field populations have been steadily increasing, and the incidence of heavily infested wheat fields has been observed more frequently in Texas, Oklahoma, and Kansas (Peter-Blecha 2005,

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Royer 2005, Watson 2005, Comis 2007, Knutson and Swart 2007, Smith 2007, Whitworth 2007). However, before our study, no systematic analysis had been conducted in this region to analyze biotype compositions and the effectiveness of Hessian fly resistance genes in wheat in this region.

The overall goal of this research was to identify resistance genes that are effective against current Hessian fly populations in Texas, Oklahoma, and Kansas through virulence analyses of field samples. Additionally, we also sought to determine whether variations in the virulence of Hessian fly populations are correlated with differences in biotype frequencies. Determining biotype composition with the currently available protocol is very labor-intensive, and it is impractical to analyze a large number of field samples (Ratcliffe et al. 1994, 1996, 1997, 2000). Correlations between population virulence and frequencies of specific biotypes may lead to the establishment of a new, simpler protocol for the estimation of biotype compositions of field collections. In this article, we report the effectiveness of major resistance genes against Hessian fly populations collected from heavily infested wheat fields in Texas, Oklahoma, and Kansas, the results of a preliminary analysis of biotype compositions, and the correlation between the percentages of virulent biotypes against specific resistance genes and the percentages of susceptible plants of the corresponding resistance genes in a virulence test.

Materials and Methods

Field Sample Collection. Hessian fly pupae (flaxseeds) were collected from heavily infested wheat fields in early April to late May 2005–2008. Wheat stubble (20–50 pounds, depending on infestation levels) containing ≈6,000 flaxseeds was packed into boxes and sent through overnight delivery to the USDA–ARS Hessian Fly Research Laboratory, Manhattan, KS, where the samples were analyzed. The samples were either immediately processed for virulence analysis (below) if the insects were not in diapause (adults emerged within 10 d at room temperature) or stored in a cold room for 3 mo to break diapause before virulence testing.

Establishment of Populations from Field Collections. To establish a population from a field collection, wheat stubble containing Hessian fly pupae was put into a screened cage ($8 \times 2 \times 3$ ft) and held at room temperature to facilitate the emergence of Hessian fly adults from pupae. Water was sprayed daily onto the stubble to keep moisture. When adults began to emerge, 50–500 wheat seedlings (depending on the number of flies emerging) of 'Karl-92' (a susceptible cultivar) at the 1.5 leaf stage were put into the same cage. Adult flies oviposited onto the wheat leaves. To achieve uniformity of the developmental stage of insects in the next generation, the wheat seedlings with Hessian fly eggs were removed from the cage the next day, and replaced with fresh wheat seedlings for egg deposition. This process was repeated for ≈2 wk. Wheat seedlings with Hessian fly eggs were further cultured in a greenhouse or growth chamber to allow eggs to hatch and larvae to develop to pupation. Pupae were collected daily and stored at 4°C in a cold room. At the end of the process, all new pupae obtained on different days were combined as a population representing that field collection. Approximately 90% of all pupae yielded adults.

Table 1. Populations developed and tested in this study

Pop[a]	County, state	Population nature
Grayson-TX-GH-07	Grayson, TX	Greenhouse-increased one generation
Grayson-TX-FD-08	Grayson, TX	Tested with field samples directly
Fannin-TX-GH-07	Fannin, TX	Greenhouse-increased one generation
Kay-OK-GH-06	Kay, OK	Greenhouse-increased one generation
Kay-OK-GH-07	Kay, OK	Greenhouse-increased one generation
Scott-KS-GH-05	Scott, KS	Greenhouse-increased one generation

[a] GH, greenhouse-increased; FD, field direct.

In 2007, a population was established from samples collected from Grayson and Fannin counties, TX, where large infestations were present. The samples were increased in a greenhouse to establish representative populations as described previously. The two populations were designated Grayson-TX-GH-07 and Fannin-TX-GH-07. To examine whether the greenhouse-increased population was representative of a field population, a collection of samples was acquired during spring 2008 from the same field in Grayson County, which was planted to the same cultivar as in 2007. This population (Grayson-TX-FD-08) was tested with flies collected directly from the field without increase in the greenhouse. Populations from Oklahoma were collected in Kay County in 2006 (designated Kay-OK-GH-06) and 2007 (Kay-OK-GH-07). The Kansas population was collected from Scott County, KS, in 2005 (Scott-KS-GH-05). The six populations from Texas, Oklahoma, and Kansas are listed in Table 1.

Virulence Analysis. To characterize the virulence of a field population to different resistance genes, seedlings of wheat lines carrying a different resistance gene (or a gene combination) were planted in a flat with dividers. These lines carried one of the following resistant genes or a gene combination: *H3*, *H4*, *H5*, *H6*, *H7H8*, *H9*, *H10*, *H11*, *H12*, *H13*, *H16*, *H17*, *H18*, *H21*, *H22*, *H23*, *H24*, *H25*, *H26*, *H31*, and *Hdic*. Each divider within a flat contained 15–25 plants of each wheat line for each test. At the 1.5 leaf stage (second leaf just emerged), the plants were infested with Hessian fly eggs (eight eggs per plant). Three weeks after infestation, resistant and susceptible plants were categorized and recorded. Susceptible plants were stunted and dark green in color, whereas resistant plants grew normally with light green color. Resistant plants contained dead Hessian fly larvae, and plants that escaped

infestation had no larvae. Each assay was repeated three times.

Analysis of Biotype Composition. The biotype composition of the Grayson-TX-FD-08 population was determined using fly's virulence reactions to *H3*, *H5*, *H6*, *H9*, *H13*, *H18*, *H21*, and *H26*. These eight genes are representative of low, medium, and high level of resistance against Hessian fly populations from this region according to our virulence tests. The biotypes virulent to these genes were designated *vH3*, *vH5*, *vH6*, *vH9*, *vH13*, *vH18*, *vH21*, and *vH26* according to the corresponding resistance genes. Analysis of biotype composition was carried out as described by Ratcliffe et al. (1994) with modifications. Ten plants of each of four cultivars, Carol (*H3*), Erin (*H5*), Caldwell (*H6*), and Iris (*H9*), were planted in a pot with dividers. At the 1.5 leaf-stage, a single mated female (with ovipositor withdrawn) was caged in the test pot to infest the plants. Three weeks after infestation, the plants were phenotyped as susceptible or resistant. The same Hessian fly population was then subjected to a second assay using cultivars with four additional resistance genes, Molly (*H13*), Redland (*H18*), Hamlet (*H21*), and KSWGRC 26 (*H26*). This assay was conducted as described previously. One hundred females were tested against each set of resistance genes. However, not all females laid eggs; therefore, only 78–81% of the tests were successful.

Statistical Analyses. Chi-square tests were performed to determine whether different Hessian fly populations were dependent on or independent of wheat cultivars carrying different resistance genes in terms of the percentages of resistant plants in virulence tests. Analyses of variance were conducted to compare the percentages of resistant plants among different populations. Tukey's pairwise comparisons were used to identify significant differences for each cultivar. Bonferroni correction was used to control the family-wise error rate in significance tests (Bonferroni 1935). For example, in the comparisons of each cultivar with the Texas populations, a test was considered significant at the 0.05 level when the *P* value was equal to or $<0.05/21 = 0.0022$ (21 gene tests were conducted). A test was considered significant at the 0.05 level when the *P* value was equal to or $<0.05/20 = 0.0025$ for the Oklahoma populations (20 genes tested). Statistical software MINITAB and R (http://www.minitab.com/) were used for the analysis. Correlation analysis was carried out using software R (command cor.test) based on Spearman's rank measure of association and test (Hollander and Wolfe 1973). A Bonferroni correction is applied to judge the significance of each test.

Results

Virulence of Texas Populations. The virulence test of the Grayson-TX-GH-07 population revealed that 10 genes (*H3*, *H5*, *H12*, *H13*, *H21*, *H22*, *H23*, *H25*, *H26*, and *Hdic*) conferred resistance to 80% or more of plants containing one of these genes (Table 2). Five genes (*H4*, *H10*, *H11*, *H16*, and *H18*) conferred resistance in 60–80% of plants containing one of these genes. The other genes or a gene combination (*H6*, *H7H8*, *H9*, *H17*, *H24*, and *H31*) were less effective, with <60% of plants that were resistant.

Table 2. Response of wheat resistance genes to the Grayson-TX-GH-07 and Grayson-TX-FD-08 populations

Gene	Cultivar	Greenhouse[a]				Field[a]			
		R	S	%R	SD	R	S	%R	SD
H3	Carol	30	6	83	6.5	39	5	89	9.3
H4	Java	34	16	68	16	23	10	70	44
H5	Erin	39	4	91	7	32	4	89	8.7
H6	Caldwell	2	49	4	5.8	3	39	7	5.8
H7H8	Seneca	5	34	13	2.6	8	42	16	11
H9	Iris	22	27	45	3.8	18	24	43	19
H10	Joy	29	8	78	6.6	41	5	89	10
H11	Karen	38	12	76	11	35	9	80	10
H12	Lola	37	2	95	7	26	3	90	7
H13	Molly	57	1	98	2.3	49	1	98	2.9
H16	D6647-H16	37	15	71	16	43	7	86	14
H17	D6647-H17	28	22	56	11	35	16	69	37
H18	Reland	38	10	79	2.1	41	3	93	6.1
H21	Hamlet	43	7	86	13	44	2	96	5.2
H22	KSWRGC 01	36	4	90	7.4	42	10	81	12
H23	KSWRGC 06	37	1	97	3.5	52	1	98	2.3
H24	KSWGRC 03	24	40	38	5.1	37	16	69	18
H25	KSWGRC 20	39	0	100	0	41	1	98	2.9
H26	KSWGRC 26	38	0	100	0	45	1	98	4
H31	P921696A1-15-2-1	31	22	58	4.4	27	14	66	16
Hdic	KSWGRC 42	57	3	95	5.5	41	2	95	8.1

[a] R, resistant plants; S, susceptible plants; %R, percentage of resistant plants.

Results of the virulence trials of Grayson-TX-FD-08 (field-collected flies) revealed 14 genes that conferred resistance to 80% or more of plants with one of these genes (Table 2). These results were slightly different from the results obtained with the greenhouse-increased population (Grayson-TX-GH-07). However, statistical analyses revealed a high correlation ($r^2 = 0.95$) between the percentages of plants resistant to these two populations. Also, differences in virulence between these two populations were not statistically significant in either the overall hypothesis test ($P = 0.281$) or individual cultivar tests. *P* values for *H3*, *H4*, *H5*, *H6*, *H7H8*, *H9*, *H10*, *H11*, *H12*, *H13*, *H16*, *H17*, *H18*, *H21*, *H22*, *H23*, *H24*, *H25*, *H26*, *H31*, and *Hdic* cultivars are 0.44, 0.52, 0.74, 0.60, 0.52, 0.74, 0.11, 0.61, 0.52, 0.88, 0.43, 0.75, 0.021, 0.30, 0.27, 0.80, 0.041, 0.37, 0.37, 0.58, and 0.91, respectively. All values are >0.0024 (5%/21), the 0.05 significant level. These collective results suggest that the greenhouse-increased population was representative of the field population.

The virulence assessment of the Fannin-TX-GH-07 population identified 15 genes (*H3*, *H4*, *H5*, *H10*, *H11*, *H12*, *H13*, *H17*, *H18*, *H21*, *H22*, *H23*, *H25*, *H26*, and *Hdic*) that conferred resistance in 80% or more of plants with one of these genes (Table 3). Two genes (*H16*, *H24*) and a gene combination (*H7H8*) yielded 60–80% resistant plants. The remaining two genes, *H6* and *H9*, conferred resistance to <60% of plants with one of these genes. The correlation coefficient between the virulence values for the Fannin-TX-GH-07 and Grayson-TX-GH-07 populations was 0.83, and 0.80 between the Fannin-TX-GH-07 and Grayson-TX-

Table 3. Response of wheat resistance genes to the Fannin-TX-GH-07 population

Gene	Cultivar	R	S	%R	SD
H3	Carol	53	1	98	2.9
H4	Java	29	7	81	16
H5	Erin	50	3	94	5
H6	Caldwell	29	25	54	11
H7H8	Seneca	34	16	68	13
H9	Iris	22	18	55	4.2
H10	Joy	41	0	100	0
H11	Karen	44	1	98	3.5
H12	Lola	41	0	100	0
H13	Molly	44	0	100	0
H16	D6647-H16	35	10	78	3.2
H17	D6647-H17	38	6	86	9.5
H18	Reland	49	2	96	4.6
H21	Hamlet	55	0	100	0
H22	KSWRGC 01	31	0	100	0
H23	KSWRGC 06	36	0	100	0
H24	KSWGRC 03	34	17	67	5.7
H25	KSWGRC 20	43	0	100	0
H26	KSWGRC 26	34	3	92	7
H31	P921696A1-15-2-1	25	25	50	4.4
Hdic	KSWGRC 42	40	0	100	0

R, resistant plants; S, susceptible plants; %R, percentage of resistant plants.

Table 4. Response of wheat resistance genes to the Kay-OK-GH-06 and Kay-OK-GH-07 populations

Gene	Cultivar	2006 pop				2007 pop			
		R	S	%R	SD	R	S	%R	SD
H3	Carol	5	56	8	16	38	15	72	14
H4	Java	6	51	11	8.1	8	31	21	17
H5	Erin	10	52	16	9	10	42	19	14
H6	Caldwell	2	61	3	6.9	4	39	9	7
H7H8	Seneca	7	50	12	12	9	45	17	7.8
H9	Iris	12	58	17	4.9	5	32	14	12
H10	Joy	39	16	71	16	28	15	65	21
H11	Karen	4	61	6	2.6	18	38	32	3.1
H12	Lola	47	16	75	2.5	26	24	52	39
H13	Molly	43	11	80	13	48	0	100	3.5
H16	D6647-H16	29	35	45	11	26	27	49	8.5
H17	D6647-H17	14	42	25	23	26	25	21	8.3
H18	Redland	36	24	60	2.9	35	2	96	6.4
H21	Hamlet	59	0	100	0	45	0	100	0
H22	KSWRGC 01	59	2	97	2.6	50	3	94	8
H23	KSWGRC 03	44	14	76	2.6	38	3	93	5.7
H24	KSWRGC 06	19	44	30	24	26	22	54	6.2
H25	KSWGRC 20	50	8	86	1.5	51	2	96	10
H26	KSWGRC 26	55	6	90	14	39	5	89	14
Hdic	KSWGRC 42	54	10	84	2.1	40	8	83	7.2

R, resistant plants; S, susceptible plants; %R, percentage of resistant plants.

FD-08 populations. Despite the overall correlation between the Fannin and Grayson populations, individual cultivar tests revealed that the virulence of the Fannin-TX-GH-07 population was different from that of the Grayson-TX-GH-07 and Grayson-TX-FD-08 populations for the *H6* gene ($P = 0.0006$ and 0.0008, respectively) and *H7H8* gene ($P = 0.0012$ and 0.0019, respectively).

Virulence of Oklahoma Populations. Results of the virulence assessment of the population derived from a collection at Kay County, OK, in 2006 (Kay-OK-GH-06) determined that six resistance genes (*H13*, *H21*, *H22*, *H25*, *H26*, and *Hdic*) conferred resistance to 80% or more of plants with one of these genes (Table 4). Four genes (*H10*, *H12*, *H18*, and *H23*) conferred resistance to >60 to <80% of plants with one of the genes. The other 10 genes provided <60% resistant plants. Because this population was much more virulent than Texas and Kansas populations, a second sampling was carried out in the same county in 2007 (Kay-OK-GH-07). Assessment of the virulence of the Kay-OK-GH-07 population determined that two additional genes, *H18* and *H23*, conferred resistance to 80% or more of plants with one of these two genes in addition to the six resistance genes identified by the Kay-OK-GH-06 population (Table 4). Other resistance genes exhibited slightly different results. However, the overall virulence of these two populations was statistically similar ($r^2 = 0.80$). Only two genes, *H11* ($P = 0$) and *H18* ($P = 0.001$), showed significant differences in virulence between the two populations.

Virulence of a Kansas Population. An assessment of the virulence of the population collected from Scott County, KS, in 2005 (Scott-KS-GH-05) determined that seven genes (*H9*, *H13*, *H21*, *H23*, *H25*, *H26*, and *Hdic*) and a gene combination (*H7H8*) conferred resistance to 80% or more of plants with one of these genes (Table 5). Four genes (*H5*, *H6*, *H12*, and *H17*) conferred resistance to 60–80% of plants. The other eight genes (*H3*, *H4*, *H10*, *H11*, *H16*, *H18*, *H22*, and *H24*) were less effective, conferring resistance to <60% of plants

Biotype Analysis. The percentages of female Hessian flies from the Grayson-TX-FD-08 population virulent to genes *H3*, *H5*, *H6*, *H9*, *H13*, *H18*, *H21*, and *H26* were 15, 28, 89, 58, 0, 9, 4, and 22, respectively (Table 6). The percentages of virulence correlated well with the corresponding percentages of susceptible wheat seedlings obtained in a virulence test with insects from the same collection. For example, *vH6* was the most

Table 5. Response of wheat resistance genes to the Scott-KS-GH-05 population

Gene	Cultivar	R	S	%R	SD
H3	Carol	24	32	43	26
H4	Java	14	24	37	9.5
H5	Erin	38	15	72	3.5
H6	Caldwell	32	19	63	11
H7H8	Seneca	44	4	92	7
H9	Iris	49	3	94	6.6
H10	Joy	18	31	37	7.2
H11	Karen	14	30	32	3.5
H12	Lola	24	8	75	6.1
H13	Molly	57	0	100	0
H16	D6647-H16	27	37	42	3.8
H17	D6647-H17	37	22	63	9.8
H18	Redland	26	23	53	4
H21	Hamlet	60	0	100	0
H22	KSWRGC 01	30	37	45	10
H24	KSWRGC 06	26	32	45	11
H23	KSWGRC 03	42	8	84	17
H25	KSWGRC 20	65	0	100	0
H26	KSWGRC 26	65	1	98	2.9
Hdic	KSWGRC 42	56	0	100	0

R, resistant plants; S, susceptible plants; %R, percentage of resistant plants.

Table 6. Frequencies of biotypes virulent to eight major resistance genes in the Grayson-TX-FD-08 population

Gene	Virulent	Avirulent	% virulent	%S.P.[a]
H3	12	69	15	11
H5	23	58	28	11
H6	72	9	89	93
H9	47	34	58	57
H13	0	78	0	2
H18	7	71	9	7
H21	3	75	4	4
H26	17	61	22	2

[a] %S.P., percentage of susceptible plants carrying the corresponding resistance gene in a virulence test with the same fly population.

frequent (89%) virulent biotype. This result corresponded well with the percentage (93%) of susceptible wheat plants carrying the resistance gene *H6* (Table 6). Biotype *vH13* was not detected in our biotype analysis. In the corresponding virulence test, only 2% of plants with *H13* were susceptible. The overall correlation between the percentages of susceptible plants and the corresponding percentages of virulent biotypes was significant ($r^2 = 0.97$). Despite a high overall correlation, there were exceptions for specific genes. For example, the frequency of biotype *vH26* was relatively high (22%) in the biotype composition analysis, whereas the corresponding resistance gene *H26* resulted in a very low percentage (2%) of plants that were susceptible in the virulence test (Table 6).

Discussion

Variations in Population Virulence. There were variations in virulence to specific resistance genes among the six populations evaluated (Table 7). The Kay-OK-GH-06 population was the most virulent as only six genes conferred resistance to 80% or more of the plants. The most avirulent population was the Fannin-TX-GH-07 population, because 15 of the 20 genes tested conferred high (80%) levels of resistance.

We hypothesized that different biotype frequencies in different regions should be reflected in the differences in virulence among fly populations. However, unrepresentative sampling in the field, different larval densities during tests, and preferential increase in the greenhouse are factors that could potentially cause variations in the test results. To reduce variations due to artificial factors, careful measures were taken during each step in the testing procedure. First, a large number of Hessian fly pupae (≈6,000) were collected from fields for each sample to limit sampling errors. Second, approximately the same number of insects was targeted in each test to reduce variations due to different larval densities from test to test. As soon as the density reached around eight eggs per plant, plants were taken out of the infestation tent to stop further egg deposition. Third, a standard approach was followed to establish and increase Hessian fly populations from field collections (see Materials and Methods). We believe these measures limited variations in population virulence due to artificial factors to a tolerable level and that the variations in virulence of different populations primarily represent differences in biotype compositions. The fact that the two Oklahoma populations collected from the same county showed similar results ($r^2 = 0.8$) suggested that sampling errors during this study were small. Similarly, highly correlated results ($r^2 = 0.95$) between the Grayson-TX greenhouse-increased population and field-direct population (Grayson-TX-GH-07 and Grayson-TX-FD-08) indicated that preferential increases of field samples in the greenhouse were also within a tolerable level. Therefore, the variations in population virulence observed in our studies should

Table 7. Percentages of resistant plants and standard deviations in different Hessian fly populations

Gene	Cultivar	Scott-KS-GH-05 %R/SD	Kay-OK-GH-06 %R/SD	Kay-OK-GH-07 %R/SD	Grayson-TX-GH-07 %R/SD	Grayson-TX-FD-08 %R/SD	Fannin-TX-GH-07 %R/SD
H3	Carol	43/26	8.1/16	72/14	**83**/6.5	**89**/9.3	**98**/2.9
H4	Java	37/9.5	11/8.1	21/17	68/16	70/44	**81**/16
H5	Erin	72/3.5	16/9	19/14	**91**/7	**89**/8.7	**94**/5
H6	Caldwell	63/11	3/6.9	9/7.1	4/5.8	7/5.8	54/11
H7H8	Seneca	**92**/7	12/12.1	17/7.8	13/2.6	16/11	68/13
H9	Iris	**94**/6.6	17/4.9	14/12	45/3.8	43/19	55/4.2
H10	Joy	37/7.2	71/16	65/21	78/6.6	**89**/10	**100**/0
H11	Karen	32/3.5	6/2.6	32/3.1	76/11	**80**/10	**98**/3.5
H12	Lola	75/6.1	75/2.5	52/39	**95**/7	**90**/7.2	**100**/0
H13	Molly	**100**/0	**80**/13	**100**/3.5	**98**/2.3	**98**/2.6	**100**/0
H16	D6647-H16	42/3.8	45/11	49/8.5	71/16	**86**/14	78/3.2
H17	D6647-H17	63/9.8	25/23	21/8.3	56/11	69/37	**86**/9.5
H18	REDLAND	53/4	60/2.9	**96**/6.4	79/2.1	**93**/6.1	**96**/4.6
H21	HAMLET	**100**/0	**100**/0	**100**/0	**86**/13	**96**/5.2	**100**/0
H22	KSWRGC 01	45/10	**97**/2.6	**94**/8	**90**/7.4	**81**/12	**100**/0
H23	KSWGRC 03	45/11	76/2.6	**93**/5.7	**97**/3.5	**98**/2.3	**100**/0
H24	KSWRGC 06	**84**/17	30/24	54/6.2	38/5.1	69/18	67/5.7
H25	KSWGRC 20	**100**/0	**86**/1.5	**96**/10	**100**/0	**98**/2.9	**100**/0
H26	KSWGRC 26	**98**/2.9	**90**/14	**89**/14	**100**/0	**98**/4.1	**92**/7
H31	P92169A1-15-2-1	ND	ND	ND	58/4.4	66/16	50/4.4
Hdic	KSWGRC 42	**100**/0	**84**/2.1	**83**/7.2	**95**/5.5	**95**/8.1	**100**/0

R, resistant plants; S, susceptible plants; %R, percentage of resistant plants.
Percentages of resistant plants >80% are bold. Percentages of resistant plants <20% are underlined.

Table 8. Correlation coefficients and *P* values from pairwise comparisons of the Hessian fly populations[a]

Corr *P* value	Scott-KS-GH-05	Kay-OK-GH-06	Kay-OK-GH-07	Grayson-TX-GH-07	Grayson-TX-FD-08	Fannin-TX-GH-07
Kay-OK-GH-06	1	**1.15E-05**	0.093191	0.011067	0.01341	0.11852
Kay-OK-GH-07	**0.803607**	1	0.460454	**0.000858**	**0.000356**	0.014087
Scott-KS-GH-05	0.375786	0.170313	1	0.636438	0.56243	0.16254
Grayson-TX-GH-07	0.542448	**0.671556**	−0.10954	1	**6.54E-11**	**2.76E-06**
Grayson-TX-FD-08	0.530258	**0.705234**	−0.13403	**0.948196**	1	**1.28E-05**
Fannin-TX-GH-07	0.351193	0.527057	−0.31623	**0.833089**	**0.80112**	1

[a] Bold values indicate r^2 significant at 0.05 level, i.e., $P = 0.0033$ (0.05/15).

reflect the differences in biotype composition in different regional populations.

Texas, Oklahoma, and Kansas are adjacent states. The four counties where Hessian fly populations were collected are located within 500 miles geographically. Grayson and Fannin counties are located on the northern border of Texas with Oklahoma, Kay County is near the northern border of Oklahoma with Kansas, and Scott County is near the southern border of Kansas with Oklahoma. Despite geographic proximity, however, the virulence patterns among the fly populations from different states were very different. The virulence of the Kansas (Scott-KS-GH-05) fly population was distinct and was not correlated with the virulence of either the Oklahoma or Texas populations (Table 8). The virulence data of the Kay-OK-GH-07 population exhibited moderate correlation with the Grayson-TX-GH-07 and Grayson-TX-FD-08 populations ($r^2 = 0.67$ and 0.70, respectively), but the Kay-OK-GH-06 population did not exhibit correlation with either of the Texas populations. Overall, the two Oklahoma populations were quite different from the Kansas and Texas populations, especially in virulence against *H4*, *H5*, and *H17*. The Texas populations from adjacent Grayson and Fannin counties, however, exhibited similar virulence, with correlation coefficients of $r^2 = 0.83$ and 0.80, respectively.

Effectiveness of Resistance Genes. Despite overall variations in virulence of different populations, some of the resistance genes were highly effective against all Hessian fly populations. *H13*, *H21*, *H25*, *H26*, and *Hdic* conferred resistance in 80% or more of plants to all fly populations (Table 7). *H12* and *H18* provided resistance in >50% of tested plants. The remaining genes, however, exhibited significant variation in resistance among different fly populations. Gene *H5*, for example, conferred high levels of resistance to Texas populations and moderate resistance to the Kansas population but was essentially susceptible to the Oklahoma populations. The *H7H8* gene combination and *H9* were highly resistant to the Kansas population but not to the Oklahoma and Texas populations. *H22* and *H23* provided high levels of resistance to the Oklahoma and Texas flies but were much less effective to the Kansas population. These results suggest that genes *H13*, *H21*, *H25*, *H26*, and *Hdic* would be good choices for wheat breeding programs for Hessian fly resistance in the south central Great Plains.

Biotype Composition and Population Virulence. Insect biotypes have been long used by entomologists and breeders for monitoring population changes and designing breeding strategies (Diehl and Bush 1984, Ratcliffe et al. 2000). Current Hessian fly biotypes were identified using three resistance genes (*H3*, *H5*, and *H6*) and a gene combination (*H7H8*) (Ratcliffe et al. 1994, 1996, 1997, 2000). Based on the differential reactions of the insect to these four genes or gene combinations, 16 biotypes were identified and named the Great Plain biotype (biotype GP) and biotypes A to O. Biotype GP, the most avirulent biotype, is avirulent to all four genes (gene combination); biotype L, the most virulent biotype, is virulent to all four genes (gene combination), whereas the other 14 biotypes are in the middle, between biotype GP and biotype L. Although these biotypes are useful for the studies of biotype genetics and wheat-Hessian fly interactions, they no longer provide much useful information for the management of this pest for two reasons. First, the four genes (gene combination) used for biotype identification are no longer effective against field populations in most regions (Ratcliffe et al. 2000). Second, the definition of these 16 biotypes provides no information on the virulence/avirulence of this insect to resistance genes other than the four genes (gene combination). The method is also very labor-intensive and impractical for analyzing large numbers of field populations. Currently, there are 32 resistance genes (and new genes are being identified). By following the same method, the 32 genes will have to be divided into eight groups, and each group will have to be tested separately with individual females. Usually, 200 data points are needed for each group of genes to reliably estimate the biotype composition (Ratcliffe et al. 2000). For eight gene groups, a total of 1,600 females will have to be analyzed for one HF population, assuming every single assay is successful. If there are 10 populations to be analyzed, the amount of work become formidable. Another problem with this approach is that the number of biotypes increases geometrically as the number of tested resistance genes increases. Clearly, a simpler method is needed for biotype monitoring of field populations.

Theoretically, the frequency of a biotype virulent to a specific resistance gene in a Hessian fly population should be proportional to the percentage of susceptible plants (obtained in a virulent test) that carry the corresponding resistance gene. A method for the estimation of biotype frequencies may be established on the basis of the correlation between the frequencies of virulent biotypes and the percentages of the corresponding susceptible plants. In an initial effort to establish such a correlation, we analyzed both the biotype frequencies and the percentages of susceptible plants with the Grayson-TX-FD-08 population using a set of resistance genes (Table 6). Our results demonstrated that the percentages of

biotypes virulent to specific resistance genes are highly correlated ($r^2 = 0.97$) with the percentages of susceptible plants in a virulence test with the same population. These results indicate that it is feasible to use data from a virulence test to estimate biotype compositions of field populations. Our data also indicated that the correlation may vary among different resistance genes. For example, the frequencies of biotypes *vH13* and *vH26* in the Grayson-TX-FD-08 population were 0 and 22%, respectively. However, the same population produced 2% of susceptible plants to both the *H13* and *H26* resistance genes. The observations of low (0%) *vH13* frequency versus low (2%) percentage of *H13* susceptible plants, but relatively high (22%) *vH26* frequency versus low (2%) percentage of *H26* susceptible plants, indicate that different correlation relationships exist between the frequencies of different biotypes within a Hessian fly population and the corresponding percentages of susceptible plants resulted from the same population. Further research to establish individual correlation equations with each resistance gene should allow the use of a simple virulence test to estimate biotype frequencies in Hessian fly populations. A virulence test is much less labor intensive because all resistance genes can be tested at the same time under a cheese tent, and therefore is feasible to screen a large number of field samples. A virulence test also can be done with direct field samples without greenhouse increase, eliminating the uncertainty of unproportional amplification during the increase process.

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