Characterization of Greenbug (Homoptera: Aphididae) Resistance in Synthetic Hexaploid Wheats

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ABSTRACT Twelve greenbug (*Schizaphis graminum* (Rondani)) biotype E-resistant synthetic hexaploid wheats synthesized by crossing *Triticum dicoccum* Schrank. and *Aegilops tauschii* (Coss.) Schmal. were evaluated for the three known insect resistance categories, including antibiosis, antixenosis, and tolerance. Different methods were evaluated for calculating antibiosis and tolerance. Calculating intrinsic rate of population increase and measuring leaf chlorophyll content with a SPAD chlorophyll meter proved to be time- and labor-efficient for antibiosis and tolerance determination, respectively. The resistance in all synthetic hexaploids proved to be the result of a combination of antibiosis, antixenosis, and tolerance, which makes them valuable sources of greenbug resistance. To assist plant breeders in selecting the best germplasm for greenbug resistance, a plant resistance index was created that revealed differences among the synthetic hexaploid wheats.

KEY WORDS *Schizaphis graminum*, synthetic hexaploid wheat, antibiosis, antixenosis, tolerance

ONE OF THE MAJOR insect pests on wheat worldwide is the greenbug (*Schizaphis graminum* (Rondani)), which is especially a serious threat to farmers in developing countries in which chemical control is often not an option. Considered one of the most important components of integrated pest management, genetic resistance of cereals is probably the best way to control greenbugs and would certainly benefit both farmers and the environment.

Damage and yield losses because of greenbugs occur as a result of the insect feeding on the plant phloem. While feeding, the greenbug injects toxic salivary enzymes, which induce chlorosis, thereby increasing the concentration of free amino acids around the feeding site (Dorschner et al. 1987). The feeding damage caused by greenbugs is recognizable as chlorotic and necrotic spots, which form the basis for determining the level of resistance in germplasm (Starks and Burton 1977).

New greenbug biotypes have been reported continuously since the middle of last century (Porter et al. 1997). From 1961 to 1992, 11 biotypes were reported in the United States alone, underlining the importance of identifying and describing new sources of greenbug resistance. Genes for resistance to greenbug have been located in wild and cultivated relatives of bread wheat, such as *Triticum turgidum* L. variety *durum* (Curtis et al. 1960), *Secale cereale* L. (Sebesta and Wood 1978, Porter et al. 1991), *Aegilops tauschii* (Coss.) Schmal. (Joppa et al. 1980, Martin et al. 1982, Flinn et al. 2001), *Aegilops spelledoides* Tausch (Tyler et al. 1985), and *Hordeum chilense* Roem et Schult (Castro et al. 1994).

Resynthesis of hexaploid wheat through interspecific hybridization between tetraploid *Triticum* sp. and *Ae. tauschii* is an effective method of transferring disease and insect resistance genes from *Ae. tauschii* to bread wheat (Joppa et al. 1980, Innes and Kerber 1994, Villareal et al. 1994, Kema et al. 1995). The purpose of this study was to investigate the level of resistance in *Triticum dicoccum* Schrank. x *Ae. tauschii*-derived synthetic hexaploid wheats, previously found to confer resistance to greenbug (Lage et al. 2003). Greenbug resistance can be partitioned into three categories, including antibiosis, antixenosis, and tolerance, as defined by Painter (1951), and different procedures for estimating each category are discussed. By using these measurements, plant resistance indices were produced (Inayatullah et al. 1990). These indices indicate the relative level of greenbug resistance and serves as a useful tool for selecting superior germplasm for resistance breeding programs.

Materials and Methods

Insect and Plants

The greenbugs used for all experiments were derived from aphids collected in the fields around the International Maize and Wheat Improvement Center (CIMMYT), El Batan, State of Mexico, Mexico. The greenbugs were determined to be biotype E by using a set of differentials, and not biotype G as was previously suspected (Lage et al. 2003). The greenbug population was maintained on 'Centinela' barley

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Table 1. Synthetic hexaploid wheats rated moderately resistant to resistant to greenbug biotype E in previous seedling screening

Synthetic hexaploid wheat pedigree	CWI[a] number
TK98-506 H83-1631-1/*Ae. tauschii* (409[b])	CWI 76315
T. dicoccum P194623/*Ae. tauschii* (895)	CWI 76352
T. dicoccum PI94623/*Ae. tauschii* (897)	CWI 76360
T. dicoccum PI94623/*Ae. tauschii* (1027)	CWI 76364
T. dicoccum PI225332/*Ae. tauschii* (372)	CWI 76436
T. dicoccum PI254157/*Ae. tauschii* (518)	CWI 76477
T. dicoccum PI254169/*Ae. tauschii* (219)	CWI 76500
T. dicoccum PI254169/*Ae. tauschii* (458)	CWI 76513
T. dicoccum PI347230/*Ae. tauschii* (1027)	CWI 76572
T. dicoccum CI7779/*Ae. tauschii* (385)	CWI 76633
T. dicoccum CI7779/*Ae. tauschii* (458)	CWI 76644
T. dicoccum CI9309/*Ae. tauschii* (409)	CWI 76658

[a] CWI, CIMMYT Wheat Introduction number (Lage et al. 2003).
[b] 'WX' (wide cross) identification number used at CIMMYT for *Ae. tauschii* accessions.

(*Hordeum vulgare* L.) in cages in a greenhouse at CIMMYT, at a temperature between 16 and 20°C and a relative humidity of 70% under conditions of natural daylight.

Plant materials consisted of 12 synthetic hexaploid wheat lines (Table 1) produced by CIMMYT's Wheat Wide Crosses Unit from interspecific crosses between tetraploid *T. dicoccum* and diploid *Ae. tauschii*, as described by Mujeeb-Kazi et al. (1996). All synthetics were maintained by the CIMMYT Wheat Germplasm Bank. The entries were selected based on results of previous screening for greenbug resistance (Lage et al. 2003), and were rated from moderately resistant to resistant. Resistance in all synthetic hexaploid wheats is derived from the *Ae. tauschii* parents, and the test entries represent hybrids derived from eight different *Ae. tauschii* accessions. The bread wheat cultivar 'Seri M82' was included in all the experiments as a susceptible control.

Experimental Design and Data Analysis

Antibiosis. Two seeds of each entry were planted in pots (9 × 9 × 8 cm) filled with a soil mixture (soil: peat:sand, 2:1:1) and grown in a greenhouse under natural light. Seedlings were thinned to one plant per pot 2 wk after planting and transferred to the laboratory for testing at 20°C and a photoperiod of 12:12 h (L:D) h. Before the study, small greenbug colonies were preconditioned for two generations to the respective test entry. Reproduction was studied by placing a single greenbug (instar 2–4) within a clip cage (2 × 1 cm) on the test plant and allowed to reproduce. When reproduction commenced, the adult and all progeny, with the exception of one, were removed. The remaining aphid was allowed to develop and, once reproduction started, offspring nymphs were counted and removed every 48 h, until death of the aphid. Data recorded or calculated included days to reproductive maturity (DTR), total nymph reproduction (Fec), average daily nymph production (DNP), nymphipositional period (number of days from production of first to last progeny) (NP), adult longevity (Long), and intrinsic rate of increase (r_m). Estimates of r_m were calculated using the formula developed by Wyatt and White (1977): $r_m = 0.738(\log_e M_d)/DTR$, where M_d is the number of progeny produced for a time equal to DTR. Because of space limitations, the experiment was divided into two incomplete blocks. Each block comprised six synthetic wheats and 'Seri M82', each represented by 10 plants.

Antixenosis. Ten plants of each entry were sown in square pots (7 × 7 × 6 cm) in the greenhouse. 'Seri M82' was sown in 40 pots. Ten plants of each entry and 20 of the control were transferred to the laboratory when the second leaf was fully expanded. The test was conducted according to Webster et al. (1994), except that the second fully expanded leaf from an intact plant was used instead of detached leaf sections. In each experiment, one leaf from each entry and two leaves from 'Seri M82' were compared in each Petri dish. The leaves from 'Seri M82' were placed opposite each other in the Petri dish, whereas the other leaves were placed randomly. Forty adult apterous greenbugs were released in the center of the Petri dish and allowed to settle on a leaf of their choice. The number of adult aphids on each leaf was recorded 24 h after release. The 12 synthetic hexaploids were divided into two incomplete blocks because of limitations of the Petri dishes.

Tolerance. Two seeds of each entry were planted in round pots (14 × 18 cm) in the greenhouse, with 18 pots of each entry. For the antibiosis tests, entries were divided into two incomplete blocks. Seven days after sowing, the plants were thinned to one plant per pot, and the height of the plants was measured. Plants were paired according to similar heights, for noninfested and infested treatments, resulting in nine replications for each entry, and then transferred to the laboratory. Each plant was covered with a clear cylindrical plastic tube (40–50 × 10 cm) with the top and seven to ten holes (4 cm) covered with fine mesh for ventilation. Aphids were added to the plants through a hole (3 cm), that could be tightly closed with a rubber cork. Twenty aphids of mixed stages were added daily to the infested plants to ensure high aphid pressure throughout the experiment. Aphids were added until the susceptible 'Seri M82' was near death. Height and dry weight of the infested and noninfested plants were measured.

According to Deol et al. (1997) and Flinn et al. (2001), measurement with a SPAD chlorophyll meter (SPAD-502 Chlorophyll Meter, Minolta Camera, Osaka, Japan) of the chlorophyll content of greenbug-infested and noninfested plant tissue can be used to estimate the level of tolerance. The SPAD meter readings correspond with the actual chlorophyll content, and SPAD meter readings can thus be used as an estimate of the chlorophyll content (Deol et al. 1997). All entries were tested in a leaf chlorophyll loss experiment. Seven plants of each entry were grown to the three-leaf stage in 9 × 9 × 8-cm pots in the greenhouse and subsequently moved to the laboratory. Entries were divided into two incomplete blocks, each with six synthetics and the susceptible 'Seri M82.'

Table 2. Antibiosis for greenbug biotype E in 12 synthetic hexaploid wheats and one susceptible control

Entry	Reps	DTR	Fec.	DNP	NP	Long.	r_m
Experiment 1							
CWI 76315[a]	10	7.1	59.4	2.3	25.5	37.7	0.137
CWI 76352	10	6.9	46.4	2.5	18.5	28.0	0.150
CWI 76360	10	6.8	48.4	3.2	15.8	24.6	0.152
CWI 76364	8	6.5	49.9	2.9	17.8	26.3	0.152
CWI 76436	10	6.7	51.2	2.8	18.4	28.5	0.150
CWI 76477	10	6.5	47.1	2.5	19.5	26.4	0.153
'Seri M82'	10	6.1	77.3	5.1	15.1	37.0	0.198
MSD[b]		0.73	18.8	0.88	6.0	8.47	0.019
Experiment 2							
CWI 76500	8	9.0	35.4	2.1	16.8	26.5	0.109
CWI 76513	7	8.3	42.4	2.5	17.0	27.3	0.122
CWI 76572	9	7.8	35.1	2.6	14.2	23.8	0.136
CWI 76633	10	8.0	42.5	2.3	18.9	28.4	0.124
CWI 76644	9	7.8	30.3	2.0	15.2	23.9	0.120
CWI 76658	9	7.4	43.2	2.5	17.3	24.8	0.137
'Seri M82'	10	7.4	73.0	4.2	17.7	34.3	0.161
MSD		0.86	21.7	1.00	8.79	10.0	0.021

DTR, days to reproductive maturity; Fec, fecundity (number of nymphs produced per female); DNP, daily nymph production per female; NP, number of days in nymphipositional period; Long, longevity of aphid in days; r_m, intrinsic rate of increase; MSD, minimum significant difference.

[a] CIMMYT Wheat Introduction number.

[b] Based on Tukey's studentized range test ($P = 0.05$).

Clip-cages used for the antibiosis experiment were placed in the middle of the second fully expanded leaf of each plant. Preliminary tests showed that the cage itself had a reducing effect on the SPAD measurement (data not shown), and empty cages were therefore placed next to the test cages to correct the SPAD measurements. Thirty aphids of mixed instars were confined to each test cage and allowed to feed for 4 d. The aphids were then removed, and measurements of chlorophyll content of infested and noninfested tissue were performed with the SPAD chlorophyll meter. Five SPAD meter readings were taken from each leaf site and averaged, yielding a mean SPAD value. SPAD index values were calculated using the formula: SPAD index = (C – T)/C (Deol et al. 1997), where C = SPAD unit value for noninfested leaf tissue, and T = SPAD unit value for infested leaf tissue.

Data from the antixenosis, antibiosis, and tolerance experiments were all analyzed using SAS PROC GLM (SAS Institute 1985) considering the replications as random effects. Differences between genotypes were partitioned into two parts: differences between the average of the synthetic hexaploids and 'Seri M82,' and differences among the synthetic hexaploids. Furthermore, Tukey's studentized range test for minimum significant differences ($P = 0.05$) were calculated for each measurement.

Plant Resistance Index. A plant resistance index (PRI) that combines measurements of the three resistance categories was calculated (Inayatullah et al. 1990) to facilitate comparison between plant genotypes. To calculate the PRI, average results from the individual resistance category are normalized by dividing each value (greenbugs per plant, etc.) from a plant entry by the highest mean value occurring in the same test. For tolerance measurements in which percent of noninfested control is calculated, the normalized indices are calculated using percent reduction instead. The normalized indices of antibiosis (X), antixenosis (Y), and tolerance (Z) are used to calculate the PRI based on the formula: PRI = 1/(XYZ).

Results

Antibiosis. Differences between all genotypes (df = 12, 116) were highly significant for DTR ($F = 5.82$; $P < 0.001$), Fec. ($F = 7.77$; $P < 0.001$), DNP ($F = 16.80$; $P < 0.001$), NP ($F = 2.57$; $P = 0.01$), Long. ($F = 4.89$; $P < 0.001$), and r_m ($F = 16.07$; $P < 0.001$). Partitioning the sums of squares (SS) for these genotypes revealed major average differences between the susceptible control 'Seri M82' and the 12 synthetic hexaploids. This comparison between the control and the synthetic hexaploids (df = 2, 116) was highly significant ($P < 0.001$) for DTR ($F = 11.12$), Fec. ($F = 40.70$), DNP ($F = 92.28$), Long. ($F = 14.41$), and r_m ($F = 84.18$) but not for NP.

Average differences between the control and the synthetic hexaploids were generally because of higher greenbug reproduction on the susceptible 'Seri M82.' Aphids on 'Seri M82' in both experiments reached maturity faster (DTR) and produced more nymphs (Fec) than aphids on synthetic hexaploids (Table 2). The average daily nymph production (DNP) for aphids on 'Seri M82' was approximately twice as high as for aphids on the synthetic hexaploids. Furthermore, aphids on 'Seri M82' had a longer life span (Long) than those on most synthetics (Table 2). The intrinsic rate of increase (r_m) (calculated based on the time required to reach maturity and nymphal production in the same amount of time after onset of production) were higher for aphids on 'Seri M82' than for aphids on any of the synthetics in both experiments.

Differences in antibiosis among the synthetic hexaploids (df = 10, 116) were significant for DTR ($F = 4.77$; $P < 0.001$), Long. ($F = 2.99$; $P = 0.01$), NP

Table 3. Number of greenbug (Gb) biotype E per plant on leaves of 12 synthetic hexaploid wheats 24 h after infestation

Experiment 1		Experiment 2	
Entry	Gb/plant	Entry	Gb/plant
CWI 76352[a]	1.9	CWI 76315	5.1
CWI 76360	3.8	CWI 76477	2.2
CWI 76364	1.6	CWI 76513	5.1
CWI 76436	4.3	CWI 76572	1.4
CWI 76500	4.6	CWI 76633	3.6
CWI 76644	2.9	CWI 76658	2.5
'Seri M82'	7.1	'Seri M82'	8.4
MSD[b]	3.0	MSD	3.7

[a] CIMMYT Wheat Introduction number.
[b] MSD, Minimum significant difference based on Tukey's studentized range test ($P = 0.05$).

($F = 2.49$; $P = 0.05$), and r_m ($F = 2.42$; $P = 0.05$). Differences among synthetics for number of days to reproductive maturity were significant only in the second experiment ($F = 7.01$; df = 5, 55; $P < 0.001$) and were probably caused by slower aphid development on accession CWI 76500 when compared with the remaining synthetics in this experiment. Greenbugs on CWI 76500 took an average of 9.0 d to reach maturity, whereas aphids on the remaining five synthetics took only 7.4–8.3 d (Table 2, experiment 2).

Differences in the longevity of aphids among the synthetics were significant only for experiment 1 and were likely caused by extended longevity of greenbugs on CWI 76315 compared with that of aphids on the other five synthetics. Aphids on CWI 76315 lived on average 37.7 d, approximately the same as aphids on the susceptible control (37.0 d), whereas aphids on the remaining five synthetics in the experiment lived from only 24.6 to 28.5 d, on average (Table 2).

The slightly significant difference among synthetic hexaploids for number of days in nymphipositional period was found only in experiment 1 as a result of greenbugs on CWI 76315 having a considerably longer nymphipositional period than those on the remaining synthetics. The average nymphipositional period for aphids on CWI 76315 was 25.5 d, whereas those on the remaining synthetics had nymphipositional periods from 15.1 to 19.5 d. The significant difference among the synthetic hexaploids for r_m was found only in experiment 2 and was apparently caused by CWI 76500 having an r_m of 0.109, compared with 0.137 for CWI 76658 (Table 2).

Antixenosis. Significant differences ($F = 10.22$; df = 12, 128; $P < 0.001$) were found among all entries for the distribution of greenbugs on leaf tips 24 h after release of the aphids. On average, the susceptible control 'Seri M82' attracted more aphids (7.1 and 8.4) in both experiments than any of the synthetics, which attracted only 1.4–5.1 aphids per leaf tip (Table 3). Partitioning of the genotype SS also revealed a highly significant difference ($F = 46.28$; df = 2, 128; $P < 0.001$) between 'Seri M82' and all synthetic hexaploids. In addition, variation among the synthetics was significant in both experiments (experiment 1: $F = 2.96$; df = 5, 64; $P = 0.05$; experiment 2: $F = 3.04$; df = 5, 64; $P = 0.05$). Differences among the synthetics in experiment 1 may be caused by CWI 76364 attracting 1.6 aphids per leaf tip, whereas CWI 76500 attracted 4.6. In experiment 2, CWI 76572 attracted 1.4 aphids per leaf tip, whereas CWI 76315 and 76513 each attracted 5.1 aphids (Table 3).

Tolerance. In the tolerance experiment, differences among genotypes were highly significant for relative height ($F = 4.56$; df = 12, 112; $P < 0.001$) and relative SPAD chlorophyll measurements ($F = 22.75$; df = 12, 112; $P < 0.001$), whereas dry weight differences were nonsignificant. Partitioning of the genotype SS revealed that the major genetic effect for height was differences among the synthetic hexaploids ($F = 4.78$; df = 10, 112; $P < 0.001$) with a minor difference between the synthetics and the susceptible control 'Seri M82' ($F = 3.42$; df = 2, 112; $P = 0.05$). The highly significant variation among the synthetic hexaploids in height reduction upon infestation is most likely a result of CWI 76644 being considerably more tolerant to infestation than the remaining synthetics. Despite the large number of greenbugs, CWI76644 reached an average height of 55.2% of its noninfested counterpart, whereas the remaining synthetics developed to an average of 24.8% of the height of their noninfested counterparts (Table 4). The synthetic CWI 76658 had very little tolerance, because infested plants of this

Table 4. Tolerance of 12 synthetic hexaploid wheat lines and a susceptible control to greenbug biotype E feeding damage, expressed as percent of noninfested control for plant growth in height (cm), dry weight (g), and SPAD chlorophyll meter readings

Experiment 1				Experiment 2			
Entry	H	DW	SPAD[a]	Entry	H	DW	SPAD
CWI 76315[b]	26.8	60.9	91.5	CWI 76500	24.7	49.6	93.1
CWI 76352	27.4	69.8	88.7	CWI 76513	23.2	45.7	91.6
CWI 76360	29.2	71.5	92.3	CWI 76572	35.1	49.5	92.2
CWI 76364	27.4	65.8	91.5	CWI 76633	32.8	53.7	91.8
CWI 76436	38.6	64.2	87.9	CWI 76644	55.2	62.3	93.6
CWI 76477	34.5	67.0	92.3	CWI 76658	8.4	60.2	88.8
'Seri M82'	19.5	47.9	45.1	'Seri M82'	20.0	43.8	65.4
MSD[c]	21.5	24.3	13.4	MSD	24.0	25.9	15.3

H, height; DW, dry weight; MSD, minimum significant difference.
[a] SPAD index = (control SPAD units − treatment SPAD units)/control SPAD units.
[b] CIMMYT Wheat Introduction number.
[c] MSD based on Tukey's studentized range test ($P = 0.05$).

Table 5. Normalized indices for greenbug resistance categories and greenbug biotype E resistance indices of 12 synthetic hexaploid wheats and the susceptible control 'Seri M82'

Entry	Normalized indices			PRI[a]
	Ab (X)	Ax (Y)	Tol (Z)	
CWI 76315[b]	0.69	0.61	0.15	15.8
CWI 76352	0.75	0.27	0.21	23.5
CWI 76360	0.77	0.54	0.14	17.2
CWI 76364	0.77	0.23	0.15	37.6
CWI 76436	0.76	0.61	0.22	9.8
CWI 76477	0.77	0.26	0.14	37.1
CWI 76500	0.67	0.65	0.20	11.5
CWI 76513	0.77	0.61	0.25	8.5
CWI 76572	0.78	0.17	0.22	34.3
CWI 76633	0.77	0.43	0.24	12.6
CWI 76644	0.74	0.41	0.19	17.3
CWI 76658	0.83	0.30	0.33	12.2
'Seri M82'[c]	1.00	1.00	1.00	1.0

Ab, antibiosis; Ax, antixenosis; Tol, tolerance; PRI, plant resistance index.

[a] PRI = 1/(XYZ).

[b] CIMMYT Wheat Introduction number.

[c] Indices of the control are based on averages from two separate tests for each resistance category.

line developed to an average of only 8.4% of the height of their noninfested counterparts in the experiment (Table 4).

The highly significant genotypic differences found for the SPAD chlorophyll measurements were a result of the average difference between 'Seri M82' and the synthetic hexaploids ($F = 134.98$; df = 2, 112; $P < 0.001$), whereas the synthetics were homogenous and differences nonsignificant. In the presence of greenbugs, the SPAD values for 'Seri M82' reached from 45.1 to 65.4% of the untreated leaf areas in the two experiments, whereas SPAD values ranged from 87.9 to 93.6% for the synthetics (Table 4). Although there were no significant differences in dry weight measurements for all entries, partitioning nevertheless showed the comparison between 'Seri M82' and average of all synthetic hexaploids to be significant ($F = 5.60$; df = 2, 112; $P = 0.01$). This difference is most likely a result of the difference between 'Seri M82' and the synthetics in experiment 1, in which aphid-infested 'Seri M82' gained 47.9% of the weight of its noninfested counterparts, whereas the synthetics on average reached weights of 66.5% of the noninfested controls (Table 4).

Plant Resistance Index. All synthetic hexaploids had greater PRI values than 'Seri M82,' based on r_m, distribution of greenbugs and SPAD chlorophyll measurements (Table 5). The synthetic hexaploids CWI 76364, CWI 76477, and CWI 76572 had the highest PRI mainly because of their high levels of antixenosis compared with the other synthetics.

Discussion

The current study found major differences between 12 new synthetic hexaploid wheats and the susceptible control 'Seri M82' for antibiosis, antixenosis, and tolerance. The total production of nymphs and the rate of nymph production were higher on the susceptible control than on any of the synthetics. Likewise, 'Seri M82' attracted more greenbugs in the antixenosis experiment than any of the synthetic wheats. Despite the presence of greenbugs, leaves of the synthetics stayed green longer than the leaves of 'Seri M82,' which were highly chlorotic, and in most synthetics, plant growth under heavy aphid pressure was higher than in 'Seri M82.' Such a mixture of resistance categories in greenbug-resistant plants seems to be common for other reported resistant sources. The bread wheat lines Largo, CI17882, GRS 1201, and PI240675 (Tyler et al. 1985, Curvetto and Webster 1998, Webster and Porter 2000) representing greenbug resistance genes *Gb3*, *Gb5*, *Gb6*, and another undesignated gene, each owe their resistance to all three described resistance categories, whereas line 97-85-3 (Flinn et al. 2001) is resistant because of tolerance alone.

There are several advantages of breeding for resistance expressed as more than one resistance category. For wheat viruses spread by aphids (such as barley yellow dwarf virus; Blackman and Eastop 1984), antixenosis is desirable because it often prevents the aphid from feeding long enough to successfully transmit the virus. Durability is often a goal in resistance breeding. Tolerance and antixenosis are believed to place less selection pressure on aphids, thus preventing the development of new virulent biotypes. However, a review by Porter et al. (1997) indicates that virulent greenbug biotypes do not develop as a result of the use of resistant cultivars, but are present before release of cultivars carrying new greenbug-resistance genes. Nonetheless, tolerance is a desirable trait because it allows aphids to be present in the crop without causing significant yield losses, and support a population of natural aphid enemies. Combined with reduced aphid fitness because of antibiosis, expression of all three resistance categories does appear ideal in a greenbug-resistant wheat cultivar.

The intrinsic rate of increase (r_m) is an estimate of insect population increase in an unlimited environment (Birch 1948) and has been used as a measurement of antibiosis in other studies of greenbug resistance (Kerns et al. 1989, Webster and Porter 2000, Flinn et al. 2001). The r_m incorporates fecundity and time to reproductive maturity into one measurement, and was proposed by Flinn et al. (2001) as a better method of expressing the antibiotic effects on greenbug biology than fecundity alone. Our results show that r_m produces results comparable to both daily nymphal production and the total number of nymphs produced per female. Data generation for r_m is much less resource-demanding than estimating daily nymphal production and total number of nymphs per female, thus supporting the use of r_m as a general estimate for antibiosis.

Loss in chlorophyll measured with a SPAD chlorophyll meter has been used by Deol et al. (1997) and Flinn et al. (2001) as a measurement of tolerance. The rationale for considering that chlorophyll measurements indicate tolerance is that plants need chlorophyll to produce biomass. Thus, if the plant does not

suffer extensive chlorophyll loss as a result of aphid feeding, then it must be tolerant.

We tested both reduction in height and biomass under heavy greenbug infestation of the entire plant and reduction in chlorophyll content estimated from SPAD readings. There were significant differences between the susceptible control and synthetic wheats for all three measurements, with the differences being most apparent for SPAD measurements. However, each measurement gave very diverse results for differences among synthetics, with only plant height reductions showing significant differences. This was probably a result of the low growth of CWI 76658 and the high growth of CWI 76644 (Table 4).

The 'stay-green' ability of the synthetic wheats after exposure to high levels of aphid infestation indicates they are tolerant to the effects of salivary enzymes injected by the greenbug during feeding. Injected enzymes cause extensive chlorosis in susceptible plants (Dorschner et al. 1987). However, our results demonstrated that the presence of greenbugs also reduced plant growth in the synthetic wheats, evidently because of their feeding. Although dry weight losses are considered as the optimal measurement for tolerance under the presence of greenbugs, chlorophyll measurements provide an estimate of a plant's ability to survive aphid attack and resume normal growth when greenbug pressure diminishes, a situation commonly observed in the field. The similar trend found for dry weight loss and SPAD measurements, and the reduced time spent on SPAD readings, made us recommend the SPAD technique for estimating tolerance.

The PRI developed by Inayatullah et al. (1990) was used by Webster and Porter (2000) to distinguish between two greenbug-resistant lines, both expressing various levels of antibiosis, antixenosis, and tolerance. In our study, the three resistance categories were weighted equally for the PRI. Based on this approach, it appears there are differences among synthetic hexaploids with regard to their levels of greenbug resistance. In a previous study including these synthetic hexaploid wheats (Lage et al. 2003), we postulated that resistance derived from *Ae. tauschii* accessions 409 (CWI 76315 and CWI 76658), 458 (CWI 76513 and CWI 76644), and 518 (CWI 76477) were different from each other. Although the synthetics cannot be differentiated based on resistance categories alone, differences in the PRI of each suggest there are differences between synthetics derived from *Ae. tauschii* accession 518 (PRI = 37.1) and those derived from accessions 409 (PRI = 15.8) and 458 (PRI = 8.5 and 17.3).

Furthermore, statistical analysis of data in the current study showed significant differences for antibiosis, antixenosis, and tolerance among the synthetic hexaploid wheats, indicating that this group of 12 synthetic wheats may contain different genes for greenbug resistance. Through crossing and selection, these resistance genes may be combined to produce a single wheat line expressing antibiosis, antixenosis, and tolerance. This should ensure a high level of resistance, which theoretically would be more durable than resistance based on a single gene.

Genetic analyses involving crosses between the synthetic hexaploids and other known greenbug resistance sources are needed to characterize resistance genes. *Ae. tauschii*-derived greenbug resistance has been reported in Largo (Joppa and Williams 1982), CI 17959 (Martin et al. 1982), and '97-85-3' (Flinn et al. 2001). The resistance in '97-85-3' is presumably at least partly different from that in these synthetics, because '97-85-3' is resistant as a result of tolerance only and not as a result of antibiosis or antixenosis (Flinn et al. 2001). Likewise, Largo was found not to express antixenotic properties but is likely resistant because of tolerance (Joppa et al. 1980). Both antibiosis and antixenosis contributed significantly to greenbug resistance in the synthetic hexaploids tested in the current study. This supports the theory that *Ae. tauschii* is a valuable source of new types of greenbug resistance in bread wheat breeding.

In summary, this study revealed differences for greenbug biotype E resistance in a group of 12 synthetic hexaploid wheats, based on different resistance categories. For overall performance, three synthetics (CWI 76364, CWI 76477, and CWI 76572) reached higher PRI than all other lines, and should therefore be candidates for greenbug-resistance breeding. For individual categories, CWI 76315 and CWI 76500 showed promising results for antibiosis; CWI 76352, CWI 76364, and CWI 76572 showed promising results for antixenosis; and CWI 76644 appeared superior for tolerance. Furthermore, our results show that by using the antixenosis method described by Webster et al. (1994), the intrinsic rate of increase measurement for antibiosis (Flinn et al. 2001), and the SPAD readings for tolerance (Deol et al. 1997), estimates of resistance categories present in wheat selected for greenbug resistance can be obtained in a resource-efficient way.

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