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CATEGORIES OF RESISTANCE IN WHEAT TO GREEN BUG *SCHIZAPHIS, GRAMINUM* (RONDANI) THROUGH A NOVEL TECHNIQUE DIRECT CURRENT ELECTRICAL PENETRATION GRAPH (DC-EPG)

SHAH ALAM KHAN[1*], J.C. REESE[2], C. PREDEESH[2], M. MURUGAN[3] AND Y. HAYAT[1]

[1]*The University of Agriculture, Peshawar 25000, Pakistan.*
[2]*Department of Entomology, Kansas State University, Manhattan, KS, 66506-4004.U.S.A.*
[3]*Department of Plant Molecular Biology and Biotechnology, Centre for Plant Molecular Biology, Tamil Nadu Agricultural University, Coimbatore, 641003 Tamil Nadu, India*
[*]*Corresponding author's e-mail: profkhansa69@aup.edu.pk*

Abstract

The green bug, *Schizaphis, graminum* (Rondani) is a cosmopolitan pest of bread wheat *Triticum, aestivum* L; and sorghum *Sorghum, bicolor* L. Seven wheat lines were evaluated for the three known insect resistance categories, including antixenosis, antibiosis and tolerance. No antixenosis was found during the screening tests. Wheat accessions, 'Molly' exhibited antibiotic effects toward green bug in the form of reduced rate of natural increase. The (r_m) of green bug on wheat lines 'Molly, "Magnum and Caldwell" was significantly lower than that of *S. graminum* on plants of susceptible cultivar Jagger. Measuring leaf chlorophyll content, SPAD chlorophyll meter, was used for tolerance determination. The tolerance resistance in Molly proved to be the result of a combination of antibiosis and tolerance categories. Furthermore, using direct current electrical penetration graphs (DC-EPG) indicated that Parameters related to sieve element phases the aphid reached quickly on susceptible cultivar compared to 'Molly, Magnum and Caldwell plants. This pattern was followed by the potential drops. The percentage of time left available after first sieve element phase, S*chizaphis graminum* stayed for a significant longer period on susceptible cultivar 'jagger' compared to the tested entries; "Molly, Caldwell and Magnum" (7.10, 3.21%, 3.93 and 15.81% respectively. The aphid penetrated sieve elements phase and phloem ingestion behavior was initiated with unequal success on resistant and susceptible genotypes. EPG indicates that phloem ingestion behavior is significantly reduced on tested wheat lines "Molly, Magnum and Caldwell" genotypes compared to the susceptible cultivar 'Jagger'. The duration of probing the pathway phase by *S. graminum*, was significantly different between control plants and the tested genotypes (Molly, Magnum and Cladwell). Similar response was observed during the last parameter 'mean proportion time' where the aphid have spent significantly longer time on susceptible cultivar as compared to the resistant genotype

Keywords: Wheat, Barley, Green bug, Biotypes, *Schizaphis graminum*

Introduction

Aphid infestation causes a significant loss to grain yield in wheat. The decline in grain yield in various genotypes, ranging from 7.9 to 34.2% has also been reported by (Lal *et al.,* 2010). The use of arthropods–resistant plant/cultivar is an environmentally safe and cost-effective tool to manage green bug. Several green bug resistant cultivars have been identified so far by different researchers (Harvey *et al.,* 1997; Porter *et al*., 1997; Smith, 1989).

Different biotypes (E, I and K) have been have also been reported by (Kinleder & Spomer, 1986; Porter *et al*., 1988; Harvey *et al*., 1991; Shufran *et al.,* 2000; Anstead *et al*., 2003). Green bug biotype 'K' is becoming the most predominant biotype on Midwestern sorghum and will eventually affect the wheat crop. The identification and evaluation of resistant genotype may help to minimize the possible use of insecticides and to improve future integrated pest management programs (Mansoor *et al*., 2013). Knowledge of the resistance categories in green bug-resistance wheat will help breeders to develop cultivars with durable resistance, based on the resistance gene (genes) involved, the genetic background of the resistance lines, and the existence of current potential green bug biotype 'K' will offer new insight in to how wheat green bug gene interactions are related to wheat resistance categories.

However, little is known about the categories of resistance involved in the response of these wheat lines to green bug biotype K. Therefore, the purpose of this study was to determine the categories of resistance in wheat lines "Caldwell, Ike, Iris, Molly, Magnum and Jagger" to green bug biotype K'.

Materials and Methods

Plant material: The wheat lines "Caldwell, Iris, Ike, Magnum, Molly and Jagger" were evaluated in this study. Jagger was used as susceptible check, all the other lines are resistant to Hessian fly. The wheat seeds of Hessian fly resistant were kindly provided by Dr. Ming-Shun Chen from USDA –ARS (Plant resistant to insect Laboratory, Department of Entomology at Kansas State University Manhattan U.S.A. Insect, green bug biotype K used in this study was provided from Dr. J C Reese Lab KSU Manhattan.

Antixenosis: Individual germinated seeds of different lines cultivars were planted equidistantly from one another in 10-cm diameter pot filled with PRO-MIX 'BX' potting mix (Hummert Inc, Earth City, MO). 7 plants per pot were used (10 Replications) of each genotypes and arranged in a complete randomized design (CRD). At the two leaf stages 40 adults biotype K green bug were released in the center of each pot respectively under (20-

25C with 70% R.H. and a photoperiod 14:10 [L: D] h). Each pot was caged in a plastic cylindrical cage with an open top covered with nylon cloth mesh. The number of adult green bugs on each plant was recorded at 12, 24 and 48h, after infestation to determine the expression of antixenosis (Flin *et al*., 2001).

Tolerance was quantified by simple and non-destructive method using the SPAD 502-chlorophyll meter (Minolta Camera Co., Ltd, Japan), Markwell *et al*., 1995). In our experiment 1.5-3.5 cm double –sided adhesive sticky foam leaf cages were placed on a single leaf of two plants of each lines for a total of eight cages for each lines. Single leaf from each plant was used to eliminate any variations occurring between different leaves of the same plant. Enough different instars of biotype 'K' were released into each cage to cover the exposed leaf area inside the cage.

Antibiosis: Antibiosis was assessed to determine the intrinsic rate of natural increase (r_m) of a single aphid on a plant of each wheat line. Briefly one germinated seed of each line and jagger was planted in a 10-cm pot filled with PRO-MIX 'BX' potting mix (Hummert Inc). The plants were allow to grow in the green house under a natural day length at the two leaf stage plants were arrange on a benches in growth room at 20-25C, 50-70 R.H and a photoperiod of 14:10 [L: D] h) in a RCB design with 10 replications. One late-instar biotype 'K' green bug nymph (P1) was removed and placed on the first leaf of each plant and caged in a translucent water drinking straw. After producing a first progeny (F1), the 'P1' was removed and placed on a second leaf of the same plant and caged with a separate drinking straw. Plants were checked twice a day. When 'F1' produced first offspring, the experiment was stopped, and the time period required for 'F1' to produce further offspring was noted d'. The total number of offspring produced by a 'P1' during'd' time was determined as 'Md'. The intrinsic rate of increase was calculated as r_m 0.738 (log e Md/d (Wyatt & White 1977)

Tolerance was quantified by a simple, rapid nondestructive plant procedure using the SPAD 502-chlorophyll meter (Markwell *et al.,* 1995). In this experiments, 1.5- 3.5 cm double-sided adhesive/ sticky foam leaf cages were used, (Converters, Huntington Valley, PA), with a 0.5 cm diameter hole cut from the middle were placed on the upper side of uniformly green, lowest leaves of two leaf stage plants. Four cages were placed on a single leaf of two plants of each genotype, for a total of eight cages for each genotype. One leaf from each plant was used to eliminate any variations occurring between different leaves of the same plant. Enough mixed age biotype K green bugs were released into each cage to cover the exposed leaf area inside each cage with green bugs (Girma *et al.,* 1998). Cages were then closed with a piece of 1.5- 3.5 cm organdy cloth, attached to the adhesive top surface of each cage, to prevent the escape of green bugs, but allowing some exchange of air. After 5 days of feeding, aphids were collected and destroyed. The chlorophyll content of infested (caged area) and uninfested (green area adjacent to the caged area) leaf tissue was measured using a SPAD meter. On each leaf, five representative readings were taken in infested and uninfested areas and averaged SPAD Index (SI) values were calculated using the formula, SI ¼ (C _ T)/C, where, C ¼ mean SPAD meter value for the control (uninfested) wheat leaf and T¼ mean SPAD meter value for the treatment (infested) wheat leaf (Deol *et al.,* 2001). The SPAD index may be expressed as a percentage, by multiplying the decimal meter value by 100. We chose to express data as a percentage to more easily represent mean percent chlorophyll loss among the evaluated genotypes. Genotypes with (reduced chlorophyll loss) significantly lower than the susceptible control Jagger were considered tolerant (Girma *et al.,* 1998).

Data analysis: Data from each experiment was analyzed by using PROC GLM (SAS) Institute (Anon., 1987) Least Significant difference (LSD) was used to determine the significance of mean among the genotypes.

Electrical Penetration Graph Technique and experimental design: EPG experiments were carried out in the laboratory with continuous illumination by fluorescent ceiling-mounted lamps, 21-24 ºC, and 40-45% relative humidity. Adult apterous aphids were starved for 8 h in a Petri-dish padded with sterile Whatman® No.1 filter paper. A gold wire electrode (~12 µm in diameter by 1-2 cm in length) (Sigmund Cohn Corporation, Mount Vernon, NY) was attached to the dorsum of each aphid with a small drop of high purity silver conductive paint. A copper wire (2mm in diameter by 10 cm in length), which served as the plant electrode, was inserted into the plant soil. Both electrodes were connected to a Giga-8 DC-EPG amplifier with 10^9 Ω input resistance and an adjustable plant voltage (Wageningen, The Netherlands). After the 8 h starvation period, aphids were carefully moved onto a fully developed open second leaf of each test plant. The gain mode in the Giga-8 was selected at 50X, and the plant voltage source was adjusted (± 5V). These settings helped to get positive voltage signals when stylet tips are inserted intercellular, while negative voltage signals for the stylet insertion are intracellular (Tjallingii, 2006). Simultaneous recording of waveforms was done on eight plants (four susceptible check plants and four resistant genotype plants) placed at random in a Faraday cage. Fifteen to 20 replications, each containing 8 hrs of complete feeding data, were subjected to EPG analysis PROBE 3.0 (Windows) software (Wageningen Agricultural University, Wageningen, The Netherlands).

Aphid Feeding Waveform analysis: The main objective of this study was to identify and compare the resistant factors associated with wheat genotypes of the total time of *S.graminum* spent in the phloem phase or sieve element phase (SEP) in susceptible and resistant plants. Therefore, waveforms E1 (salivation) and E2 (ingestion phase including continuous salivation) were labeled as SEP. The parameters recorded (Table 2) in each of the four entries were the mean time from start of recording to first probe.

A total of 11 parameters were used: time from start of experiment to first probe of an aphid, mean time difference from first probe to first sieve element phase E, umber of potential drop (pds), Xylem phase (G), pathway phase (A, B, C and F), Non-feeding phase (np) mean duration of xylem phase (calculated by dividing xylum phase (G) by total number of xylem activities), and mean proportion (calculated by dividing mean sum of all SEP bouts by mean available SEP activities) were marked from each replicate of EPG recording that had 8 h complete feeding by the *S. graminum* individual aphids. Data were analyzed by a two-way analysis of variance (ANOVA) and PROCGLM (Anon., 1985). Means, when significant, were separated by the Tukey's studentized range (HSD) test procedure ($p \leq 0.05$).

Results

Antixenosis test. In antixenosis experiment the green bug biotype K' showed no preference toward any of the lines and no statistical differences were found in the number of *Schizapohis graminum* on different genotypes compared to control plants Jagger, after 12h, (df = 6, 66 f = 0.02, p = 0.09), 24h (df = 6, 61, f = 0.46, p, = 0.83) and 48hr (df = 6 59, f =.00, p = 0.43) post infestation

Antibiosis test. In antibiosis experiment, the total progeny production (Md) of greenbugs biotype k' confined on the wheat lines: Newton, Ike and Iris showed similar response compared to the susceptible lines, Jagger, but the genotypes 'Magnum, 'Molly and 'Caldwell' produced significantly less number of aphids interims of progeny production compared to the control plant Jagger. (df = 6, 77; f = 4.22; p = 0.001). While the duration of pre-reproduction time indicated as (d) where *S. graminum* took significantly more time on genotype 'Molly' compared to all the tested genotypes including Jagger (df = 6, 77; f = 2.22; p = 0.050). This trend was also observed in column 2, table 1; rate of intrinsic increase (r_m) for *S. graminum,* where the genotype, 'Molly' (0.124), had the minimum rate of intrinsic increase (r_m) compared to the susceptible genotype 'Jagger' (0.224) (df = 6, 77; f = 5.61; p = 0.0001) (Table 1)

Table 1. Mean time required for green bug biotype K' F1 to produce first progeny(d) number of progeny produced by P1 during d (md), and intrinsic rate of increase (r_m) on different wheat lines

Genotype	Total Progeny production (md)	(Pre-reproduction Time (d)	Rate of intrinsic increase (r_m)
	Mean ± SE	Mean ± SE	Mean ± SE
Newton	40.70 ± 3.07 AB	6.90 ± 3.92 AB	0.183 ± 0.006 AB
Magnum	38.93 ± 2.19 BC	6.87 ± 4.71 AB	0.177 ± 0.006 AB
Jagger	46.45 ± 3.47 A	6.41 ± 3.41 B	0.224 ± 0.008 A
Ike	37.12 ± 4.96 ABC	6.93 ± 6.59 AB	0.162 ± 0.005 AB
Molly	26.25 ± 5.55 C	7.45 ± 5.21 A	0.1240 ± 0.004 B
Iris	36.15 ± 1.76 ABC	6.80 ± 4.20 AB	0.164 ± 0.005 AB
Caldwell	30.33 ± 1.97 BC	7.00 ± 6.00 AB	0.1204 ± 0.016 AB

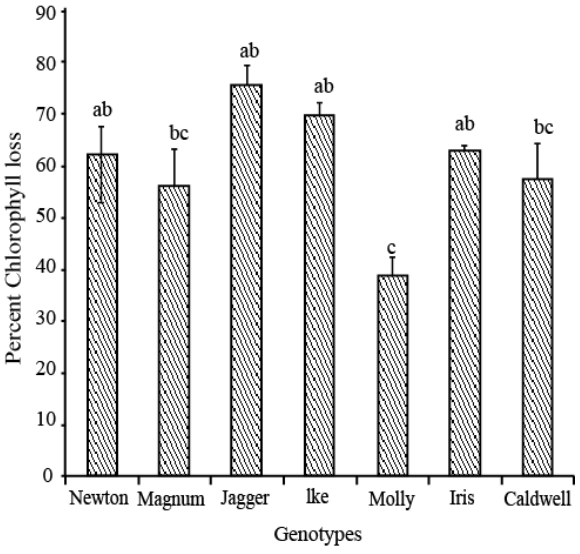

Fig. 1. Percent chlorophyll loss index of *S graminum* on different wheat lines.

Tolerance: During the Tolerance test plants of 'Molly' (38.36 %) exhibited tolerance to green bug biotype feeding damage based on, chlorophyll percent loss index (Fig. 1), compared to all other tested genotypes including the check plants 'Jagger' (75.01 %) (df = 6, 62; f = 7.41; p = 0.00010). Numerically the tested lines 'Caldwell' and 'Magnum' had reduced chlorophyll loss but statistically they were non-significant.

Feeding Behavior of *S. graminum* on Wheat plants**.** When allowed to feed on wheat entries, the *S. graminum* biotype K' recorded no significant difference in the parameters; mean time from start of experiment to first probe (df = 3, 47, F = 1.25, P = 0.303), mean time to reach xylem phase (df = 3, 47, F = 2.70, *P* = 0.67), were recorded (Table 1).

The parameters 3; mean time difference from first probe to first SEP (df = 3, 47, F = 125.98, *P* = 0.0001), *S. graminum* spent significantly shorter time to reach sieve element phase on susceptible plants compared to the tested wheat genotypes. While significance was also shown by the entry 'Caldwell' plants compared to the

'Molly and Magnum' plants. Parameter related to the short injuries, the mean number of potential drops (pds) *S. graminum* made significantly more attempts on susceptible plants compared to the other tested lines, (df =3, 47, F = 6.08, P = 0.0015),

The number of pathway phase (C) produced by *S. graminum* on 'Caldwell' plants recorded significantly more compared to all other genotypes including the susceptible 'Jagger' plants (df = 3, 47, F = 3.36, *P* = 0.0271). The number of SEP phase (df = 3, 47, F = 191.08, *P* = 0.0001) The green bug made significantly greater attempts on 'jagger' plants compared to 'Molly 'Magnum and 'Caldwell' wheat plants.

In total duration of pathway phase The aphids spent significantly short time on leaves of susceptible plants compared to all other genotypes, (df = 4, 47, F = 11.87, *P* = 0.0001) (Table 2 parameter 8). The total duration of non probing period was very short on susceptible plants than the other tested entries, however, significant differences were also recorded on plants of 'Caldwell' Molly' and Magnum' (df = 4,47, F = 30.52, *P* = 0.0001) .

The percentage of time (PT) left available after first sieve element phase, S*chizaphis graminum* stayed for a significant longer period on susceptible 'jagger' plants compared to the other entries; 'Molly, 'Caldwell and 'Magnum' (7.10, 3.21%, 3.93 and 15.81% respectively (df = 4, 47, F = 55.48, *P* = 0.0001).

Table 2. Feeding behavior (mean ± SE) of green bug biotype K during 8-h (480 min) period on the different wheat genotypes

Feeding behavior parameters	Jagger	Magnum	Caldwell	Molly	X^2	DF	P
1.Time from start of recording to first probe	16.67±2.33A	12.50±0.89A	10.92±2.87A	11.50±2.67A	8.05	3	0.0449
2. Time from start of recording to first xylem phase	119.83±8.15A	117.25±3.01A	136.67±4.00A	127.17±4.54A	9.68	3	0.0215
3. Time from start f recording to first sieve element phase	107.92±4.15C	183.17±3.96B	208.00±4.18A	194.58±3.67AB	33.60	3	<.0001
4. No of potential drops	69.83±3.70A	53.75±3.45B	49.25±2.38B	58.00±4.49AB	12.71	3	0.0053
5.No of pathway phase	6.08±1.73B	10.33±1.01AB	9.17±1.24A	12.00±1.70AB	13.51	3	0.0037
6.No of xylem phase	1.43±0.05A	1.18±0.13A	1.32±0.06A	1.26±0.07A	3.73	3	0.2924
7.No of sieve element phase	2.24±0.14A	0.19±0.01B	0.20±0.01B	0.23±0.01B	27.94	3	<.0001
8.Total duration pathway phase	168.67±4.86B	200.33±4.51A	201.92±5.63A	203.75±4.31A	19.22	3	0.0002
9.Total duration xylem phase	44.58±2.91A	42.17±2.16A	49.50±6.35A	39.83±2.27A	1.23	3	0.7456
10.Total duration non-probing	121.58±4.42C	154.50±3.05B	169.67±3.90A	160.75±3.69AB	28.75	3	<.0001
11.Percentage of time left available after first sieve element phase that was spent in the sieve element phase	15.81±1.41A	7.10±0.57B	3.93±0.07C	3.21±0.31C	39.08	3	<.0001

Discussion

Our results demonstrate that no antixenosis was found during the screening tests. Antixenosis is much less useful in crop monoculture, and as such, was not considered a viable category of resistance for investigation. Antibiosis measurements based on the naturally intrinsic rate of population increase, have been found to be more reliable and less resource demanding compared to the other antibiosis measures (Webster & Porter, 2000; Flinn *et al.,* 2001; Lage *et al.,* 2003). The intrinsic rate of increase of green bug was significantly reduced when confined to the foliage of wheat plants 'Molly'. In this experiment the rate of increase of aphid on the susceptible cultivar 'Jagger' was significantly greater compared to the "Molly, 'Magnum and Caldwell". The antibiosis shown by accession 'Molly' is similar to that of Smith & Starkey (2003), Biona *et al.,* (2005), reported Gby gene in the Sando's '4040' wheat germplasm expressed as both antibiosis and tolerance characters.

The third category 'Tolerance' is an important trait for breeding resistance in wheat to aphids, as well as arthropod pests in general (Smith *et al.,* 1999). Measurements of tolerance based on the 'SPAD' meter, leaf chlorophyll loss index also proved an accurate measure of tolerance to green bug feeding damage. The reduced chlorosis caused by green bug on 'Molly' plants resulted as tolerance factor(s) to *S. graminum*. However the proportional chlorophyll loss index is in contrast with results of (Biona *et al*., 2005), who reported, Chlorophyll losses among Sando's 4040, KS89WGRC4 and 'TA1675' ranged from 27% to 35%. Furthermore, Flinn *et al*., (2001) have also reported proportional chlorophyll losses in 'TA1675' plants and its progeny '97-85-3', of 25% and 39%, respectively. But in our experiments, the proportional chlorophyll losses are ranged as 38.36 %, 57.23 % and 56.26 % on "Molly 'Caldwell' and 'Magnum' plants compared with the previous authors, possibly, they might have used less number of aphids or for shorter period of time as we, have used enough number of *S. graminum* on the leaves of infested plants. Thus the wheat line 'Molly' may represent potential new

sources for antibiosis resistance and tolerance to various biotypes of green bugs, such sources can be further used to develop multiple aphid resistant cereal germplasm for use in the Midwestern U.S. and other growing areas of wheat and sorghum.

Interestingly, the results of the second component regarding direct current electrical penetration graph technique (DC-EPG) study indicate that *S. graminum* feeding behavior is delimited by plant source, variety, and resistance category. Antibiosis resistance in wheat has been shown previously to have a strong affect on the feeding behavior of insect biotypes in case of wheat plants (Khan *et al*., 2009). EPG parameters related to sieve element phase (SEP) indicated that the performance of *S. graminum* on resistant genotypes was significantly different than that on susceptible wheat plants. As the number of sieve element phase (SEP) of *S. graminum* on susceptible 'Jagger' plants were prolonged compared to the other tested entries ('Magnum 'Molly and Caldwell'). Another important parameter, the mean duration of pathway phase (C) is of indicative that the *S. graminum* biotype K' spent significantly more time on probing 'Molly' 'Cladwell' and 'Mgmnum' plants when compared to the susceptible 'Jagger' plants. This denotes that part of resistance to *S. graminum* in all the three wheat genotypes is also contributed by intra- and inter-cellular factors that may delay the establishment of stylet path to reach the phloem.

The mean duration of SEP events (combined E1 & E2) indicated that both biotypes also spent significantly less time on ('Molly, 'Magnum and 'Caldwell) plants compared to susceptible 'Jagger' plants. In addition, most aphids reached SEP on 'Jagger' plants, where only less than 50% of those feeding on ('Magnum, 'Molly and 'Caldwell) reached SEP.

In our case *S. graminum* biotype K' spent less attempts on leaves of a comparatively resistant entries than on a susceptible line in-terms of 'mean sum of all bouts of SEP' and at the same time made more feeding attempts (mean duration of SEP events) against the resistant plants as compared to susceptible plants which indicated that ingestion by the aphid was less. As reported by (Spiller *et al.,* 1990) phloem consumption by aphids involves nutrient ingestion, while, our results are also in agreement with those of Oswald (1997) who determined that *D. noxia* spent longer periods in the sieve phase when probing susceptible barley plant leaves than probing leaves of the resistant barley line 'STARS-9301B'. But in our case the host/crop was changed and wheat plants were used in the experiment.

The duration of probing the pathway phase by *S. graminum*, was significantly different between control plants and the tested genotypes ('Molly, 'Magnum and 'Cladwell). Similar, response was observed during the last parameter 'mean proportion time' where the aphid have spent significantly longer time on susceptible cultivar as compared to the resistant genotype but the observations were not consistent with the finding by Brewer & Webster (2001) who found that the total duration of SEP was significantly shorter on RWA1-resistant barley plants than on susceptible barley plants. In the case of resistant plants, if the resistant factor were host volatilization mediated non-preference, the non-probing (NP) waveforms before first probing were prolonged, while mesophyll, mediated resistance factors changed the pathway activities (C wave). A prolonged E1 and shorter E2 were the outcome to a resistant factor in phloem (Tjallangii, 1988, 2006; and Hu *et al.,* 2006). High rate of potential drops (pd) upon reaching phloem tissue may be reflective of unsuccessful attempt to accept a sieve element for feeding due to a resistant factor lying in the phloem Gabrys *et al.,* (1997). In the present study we also found differences in potential drops (pd) of wheat lines compared to the susceptible 'Jagger' plants. Tjallingii (1991) indicated that wave form characteristics of xylem feeding may occur when aphids are on relatively unacceptable plants.

We conclude that aphid feeding differed by the plant host species and resistance mechanism shown by the plants. Furthermore, the tested genotypes 'Molly 'Magnum and 'Caldwell' are partly resistance to probing behavior lying in the cellular parts of leaves and partial resistance in the sieve element to the *S. graminum* biotype K'. Thus wheat line 'Molly' represents a potential new sources for both antibiosis and tolerance components of resistance for green bugs biotype 'K'. and such sources can be utilized to develop multiple aphid resistant cereal germplasm for use in the Midwestern U.S. and other growing areas of wheat and sorghum.

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References

Anonymous. 1985. SAS userÕs guide: basics. SAS Institute, Cary, NC.

Anonymous. 1987. SAS Institute. SAS/STAT user's guide for personal computers. SAS Institute, Cary, NC.

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

Boina, B., S. Prabhakar, C.M. Smith, S. Starkey, L. Zhu, E. Boyko and J.C. Reese. 2005. Categories of resistance to biotype I greenbug (Homoptera: Aphididae) in wheat lines containing the genes *Gbx* and *Gby. J. Kans. Entomol. Soc*., 78: 252-260.

Brewer, M.J. and J.A. Webster. 2001. Probing behavior of Diuraphis noxia and Rhopalosiphum maidis (Homoptera: Aphididae) affected by barley resistance to D. noxia and plant water stress. *Environ. Entomol*., 30: 1041-1046.

Deol, G.S., J.C. Reese, B.S. Gill, G.E. Wilde and L.R. Campbell. 2001. Comparative chlorophyll losses in susceptible wheat leaves fed upon by Russian wheat aphids or greenbugs (Homoptera: Aphididae). *J. Kansas Entomol. Soc*., 74: 192-198.

Flinn, M.B., C.M. Smith, J.C. Reese and B.S. Gill. 2001. Categories of resistance to green bug (Homoptera: Aphididae) biotype I in *Aegilops tauschii* germplasm. *J. Econ. Entomol.,* 94: 558-563.

Gabrys, B., W.F. Tjallingii and T.A. Van-Beek. 1997. Analysis of EPG recorded probing by abbage aphid on host plant parts with different glucosinolate contents. *J. Chem. Ecol.*, 23: 1661-1663.

Girma, M., K.D. Kofoid and J.C. Reese. 1998. Sorghum germplasm tolerant to greenbug (Homoptera: Aphididae) feeding damage as measured by reduced chlorophyll loss. *J. Kans. Entomol. Soc*., 71: 108-115.

Harvey, T.L., G.E. Wilde and K.D. Kofoid. 1997. Designation of a new green bug biotype, biotype K, injurious to resistant sorghum. *Crop Sci.,* 37: 989.

Harvey, T.L., K.D. Kofoid, T.J. Martin and P.E. Sloderbeck. 1991. A new green bug biotype virulent to E-biotype resistant sorghum. *Crop Sci.,* 13: 1689-1691.

Hu, X.S., X.F. Liu and H.Y. Zhao. 2006. Development and application of electrical penetration graph (EPG) technique. *Plant Protec.,* (China) 3: 1-4991.

Khan, S.A., M. Murugan, S. Starkey and C.M. Smith. 2009. Inheritance and categories of resistance in wheat to Russian wheat aphid (Hemiptera: Aphididae) biotype 1 and biotype 2. *J. Econ. Entomol*., 102: 1654-1662.

Kindler, S.D. and S.M. Spomer. 1986. Biotypic status of six green bug (Homoptera: Aphididae) isolates. *Environ. Entomol.,* 15: 567-572.

Lage, J., B. Skovmand and S.B. Andersen. 2003. Characterization of green bug (Homoptera: Aphididae) resistance in synthetic hexaploid wheats. *J. Econ. Entomol*., 96: 1922-1928.

Lal. H.A., M. Hussain, R.M. Iqbal, M. Amer and A.H. Tariq. 2010. Losses in grain yield caused by Russian wheat aphid *diuraphis noxia* (Mordvilko). *Sarhad J. Agric.*, 26(4): 625.

Markwell, J., J.C. Osterman and J.L. Mitchell. 1995. Calibration of the Minolta SPAD-502 leaf chlorophyll meter. *Photosyn. Res*., 46: 467-472.

Oswald, C.J. 1995**.** Russian wheat aphid and corn leaf aphid (Homoptera: Aphididae) competition and 412n bp0mplant response as mediated by plant water stress and aphid resistant and -susceptible barley, M.S. thesis, University of Wyoming, Laramie.

Porter, D.R., J.D. Burd, K.A. Shufran and J.A. Webster. 1997. Green bug (Homoptera: Aphididae) biotypes: selected by resistant cultivar or pre-adapted opportunists*? J. Econ. Entomol.,* 90: 1055-1065.

Porter, K.G., H. Paerl, R. Hodson, M. Pace, J. Priscu, B. Riemann, D. Scavia and J. Stockner. 1988. Microbial teractions in lake food webs. In: *Complex interactions in lake communities.* (Ed.): S.R. Carpenter. Springer- Verlag, New York, pp. 209-227.

Shufran, K.A., L.R. Kirkman and G.J. Puterka. 2007. Absence of mitochondrial DNA sequence variation in Russian wheat aphid (Hemiptera: Aphididae) populations consistent with a single introduction into the United States. *J. Kansas Entomol. Soc.,* 80: 319-323.

Smith, C.M. 1989. Plant resistance to insects - a fundamental approach. Wiley, New York.

Smith, C.M. and S. Starkey. 2003. Resistance to green bug (Homoptera: Aphididae) biotype I in *Aegilops tauschii* synthetic wheat. *J. Econ. Entomol*., 96: 1571-1576.

Smith, R.C., D. Ainley, K. Baker, E. Domack, S. Emslie, W. Fraser, J. Kennett, A. Leventer, E.M. Thompson, S. Stammerjohn and M. Vernet. 1999. Marine ecosystem sensitivity to climate change. *Bio. Sci*., 49: 393-404.

Spiller, N.J., L. Koenders and W.F. Tjallingii. 1990. Xylem ingestion by aphids strategy for maintaining water balance. *Entomol. Exp. Appl*., 55: 101-104.

Tjallingii, W.F. 2006. Salivary secretions by aphids interacting with proteins of phloem wound responses. *J. Exp. Bot*., 57: 739-745.

Webster, J.A. and D.R. Porter. 2000. Plant resistance components of two green bug (Homoptera: Aphididae) resistant wheat. *J. Econ. Entomol*., 93: 1000-1004.

Wyatt, I.J. and P.F. White. 1977. Simple estimation of intrinsic increase rates for aphids and tetranychid mites. *J. Appl. Ecol*., 14: 757-766.

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