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

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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 \( t_{mr} \) 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, *Schizaphis 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 Clandwell). 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 & Sperner, 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-
chlorophyll content of infested (caged area) and days of feeding, aphids were collected and destroyed. The of green bugs, but allowing some exchange of air. After 5 adhesive top surface of each cage, to prevent the escape piece of 1.5-3.5 cm organdy cloth, attached to the cover the exposed leaf area inside each cage with green bugs (Girma et al., 1998). 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 20-25°C, 50-70 R.H and a photoperiod of 14:10 [L: D] h). 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 line 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.

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 °C, 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 $10^7$ 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, number 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<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 Schizaphis 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 (rm) for S. graminum, where the genotype, ‘Molly’ (0.124), had the minimum rate of intrinsic increase (rm) 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 (rm) on different wheat lines

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

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, Schizaphis graminum stayed for a significant longer period on susceptible ‘jagger’ plants compared to the other entries; ‘Molly, ‘Caldwell and ‘Mumnum’ (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

<table>
<thead>
<tr>
<th>Feeding behavior parameters</th>
<th>Jagger</th>
<th>Magnum</th>
<th>Caldwell</th>
<th>Molly</th>
<th>X²</th>
<th>DF</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Time from start of recording to first probe</td>
<td>16.67±2.33</td>
<td>12.50±0.89A</td>
<td>10.92±2.87A</td>
<td>11.50±2.67A</td>
<td>8.05</td>
<td>3</td>
<td>0.0449</td>
</tr>
<tr>
<td>2. Time from start of recording to first xylem phase</td>
<td>119.83±8.15A</td>
<td>117.25±3.01A</td>
<td>136.67±4.00A</td>
<td>127.17±4.54A</td>
<td>9.68</td>
<td>3</td>
<td>0.0215</td>
</tr>
<tr>
<td>3. Time from start of recording to first sieve element phase</td>
<td>107.92±4.15C</td>
<td>183.17±3.96B</td>
<td>208.00±4.18A</td>
<td>194.58±3.67AB</td>
<td>33.60</td>
<td>3</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>4. No of potential drops</td>
<td>69.83±3.70A</td>
<td>53.75±3.45B</td>
<td>49.25±2.38B</td>
<td>58.00±4.49AB</td>
<td>12.71</td>
<td>3</td>
<td>0.0053</td>
</tr>
<tr>
<td>5. No of pathway phase</td>
<td>6.08±1.73B</td>
<td>10.33±1.01AB</td>
<td>9.17±1.24A</td>
<td>12.00±1.70AB</td>
<td>13.51</td>
<td>3</td>
<td>0.0037</td>
</tr>
<tr>
<td>6. No of xylem phase</td>
<td>1.43±0.05A</td>
<td>1.18±0.13A</td>
<td>1.32±0.06A</td>
<td>1.26±0.07A</td>
<td>3.73</td>
<td>3</td>
<td>0.2924</td>
</tr>
<tr>
<td>7. No of sieve element phase</td>
<td>2.24±0.14A</td>
<td>0.19±0.01B</td>
<td>0.20±0.01B</td>
<td>0.23±0.01B</td>
<td>27.94</td>
<td>3</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>8. Total duration pathway phase</td>
<td>168.67±4.86B</td>
<td>200.33±4.51A</td>
<td>201.92±5.63A</td>
<td>203.75±4.31A</td>
<td>19.22</td>
<td>3</td>
<td>0.0002</td>
</tr>
<tr>
<td>9. Total duration xylem phase</td>
<td>44.58±2.91A</td>
<td>42.17±2.16A</td>
<td>49.50±6.35A</td>
<td>39.83±2.27A</td>
<td>1.23</td>
<td>3</td>
<td>0.7456</td>
</tr>
<tr>
<td>10. Total duration non-probing</td>
<td>121.58±4.42C</td>
<td>154.50±3.05B</td>
<td>169.67±3.90A</td>
<td>160.75±3.69AB</td>
<td>28.75</td>
<td>3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>11. Percentage of time left available after first sieve element phase that was spent in the sieve element phase</td>
<td>15.81±1.41A</td>
<td>7.10±0.57B</td>
<td>3.93±0.07C</td>
<td>3.21±0.31C</td>
<td>39.08</td>
<td>3</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>

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 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, K590WGRC4 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 ‘Mumnum 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 that than 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 intercellular factors that may delay the establishment of stylist 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., 1991) 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 aphids 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 RW1-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 (Tjallingii, 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


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