

This is the author's final, peer-reviewed manuscript as accepted for publication. The publisher-formatted version may be available through the publisher's web site or your institution's library.

Resistance to multiple cereal aphids in wheat-alien substitution and translocation lines

Leonardo A. Crespo-Herrera, C. Michael Smith, Ravi P. Singh, Inger Åhman

How to cite this manuscript

If you make reference to this version of the manuscript, use the following information:

Crespo-Herrera, L. A., Smith, C. M., Singh, R. P., & Åhman, I. (2013). Resistance to multiple cereal aphids in wheat-alien substitution and translocation lines. Retrieved from <http://krex.ksu.edu>

Published Version Information

Citation: Crespo-Herrera, L. A., Smith, C. M., Singh, R. P., & Åhman, I. (2013). Resistance to multiple cereal aphids in wheat-alien substitution and translocation lines. *Arthropod-Plant Interactions*, 7(5), 535-545.

Copyright: © Springer Science+Business Media Dordrecht 2013

Digital Object Identifier (DOI): doi:10.1007/s11829-013-9267-y

Publisher's Link: <http://link.springer.com/article/10.1007/s11829-013-9267-y>

This item was retrieved from the K-State Research Exchange (K-REx), the institutional repository of Kansas State University. K-REx is available at <http://krex.ksu.edu>

Resistance to multiple cereal aphids in wheat-alien substitution and translocation lines

Leonardo A. Crespo Herrera^{1*}, C. Michael Smith², Ravi P. Singh³, Inger Åhman¹

¹Department of Plant Breeding, Swedish University of Agricultural Sciences, P.O. Box 101, SE 23053 Alnarp, Sweden

²Department of Entomology, Kansas State University, Manhattan, KS 66506-4004, USA

³International Maize and Wheat Improvement Center (CIMMYT), Apdo. Postal 6-641, 06600, Mexico DF, Mexico

*Corresponding author: leo.crespo@slu.se, +46 40415512

Abstract

Rhopalosiphum padi, *Schizaphis graminum*, and *Sitobion avenae* are three of the most destructive aphid species of wheat (*Triticum aestivum* L.). They can significantly reduce wheat yields directly by feeding and indirectly by transmitting viruses. This study aimed to search for resistance to these aphid species among lines derived from different rye (*Secale cereale*) origins and from *Aegilops speltoides*, all in the genetic background of the wheat cultivar Pavon F76. Resistance was quantified as aphid weight (*R. padi*, *S. avenae*, *S. graminum*), and number of aphids and percentage of infested leaf area exhibiting chlorosis (*S. graminum*). The most resistant genotypes reduced *R. padi* and *S. avenae* weight by 24.2% and 34.3%, respectively, at the seedling stage, compared to Pavon F76 control plants. Strong *S. graminum* resistance was found only in *Ae. speltoides*-derived lines, the most resistant of which (7A.7S-L5) sustained just 3% chlorosis and reduced *S. graminum* colony weight by 67.7%. One line carrying the 1AL.1RS_{am} wheat-rye translocation from Amigo wheat (originally from Insave rye) reduced *S. avenae* weight by 23.2 and 21.8% in seedling and adult plants, respectively. Single genotypes carrying the complete 1R chromosome or the 1RS chromosome arm derived from E12165 wheat and Presto triticale proved to be resistant to both *R. padi* and *S. avenae* at the seedling stage. Further research should be conducted to unravel the genetic basis of resistance to these aphids in 1RS genotypes. The sources of resistance identified here may be useful for incorporating multiple aphid species resistance in wheat breeding programs, particularly for *R. padi* and *S. avenae*, to which no resistant wheats have been bred.

Keywords: *Rhopalosiphum pad*; *Schizaphis graminum*; *Sitobion avenae*; *Triticum aestivum*; *Secale cereale*; *Aegilops speltoides*

30 Introduction

31 Aphids are a major biotic constraint to wheat (*Triticum aestivum* L.) production because they inflict direct feeding
32 damage and can transmit viruses. Among those of particular importance are: the bird cherry – oat aphid
33 (*Rhopalosiphum padi* L.), the greenbug (*Schizaphis graminum* [Rondani]), and the English grain aphid (*Sitobion*
34 *avenae* [Fabricius]), which are widely distributed in wheat producing areas, with some differentiation depending on
35 the geographic region (Blackman and Eastop 2007). These aphids can reduce wheat yields by 30-40% (Kieckhefer
36 and Gellner 1992; Voss et al. 1997) and by up to 60% when direct damage is combined with losses from virus
37 infection (Riedell et al. 2003). Damage due to *R. padi* and *S. avenae* is evident mainly as reduced plant growth and
38 grain yields, whereas *S. graminum* feeding also causes plant chlorosis and necrotic spots at the feeding site (Voss et
39 al. 1997; Blackman and Eastop 2007; Franzen et al. 2008). Given that plant resistance to arthropod pests is an
40 environmentally and economically sound plant protection strategy, it is important to identify sources of aphid
41 resistance for developing aphid-resistant wheat cultivars (Berzonsky et al. 2003; Porter et al. 2009).

42 Wheat-alien chromosome translocations and substitutions have been successfully exploited in the development of
43 new wheat cultivars, and alien genetic sources such as rye (*Secale cereale* L.) were shown to enhance yield potential
44 and confer resistance to biotic stresses (Friebe et al. 1996; Kim et al. 2004). A widely utilized source is the 1R
45 chromosome from Petkus rye, which is deployed in hundreds of cultivars, such as Kavkaz wheat (Rabinovich 1998).
46 Resistance to various biotic stresses has also been found in *Aegilops speltoides* Tausch (Friebe et al. 1996).

47 Breeding for *S. graminum*-resistant wheat cultivars began in the 1950s (Porter et al. 1997; Berzonsky et al. 2003);
48 two of the resistance genes (*Gb2* and *Gb6*) originate from the 1RS chromosome arm of Insave rye (Sebesta and
49 Wood 1978; Lu et al. 2010), whilst *Gb5* was derived from the 7SL chromosome arm of *Ae. speltoides* (Tyler et al.
50 1985, 1987; Lukaszewski 1995). No *R. padi*-resistant wheat cultivars currently exist, but several triticale and rye-
51 wheat derived lines express resistance to *R. padi* (Hesler 2005; Hesler and Tharp 2005; Hesler et al. 2007). Similarly,
52 no wheat cultivars have been bred for resistance to *S. avenae*, though a resistance gene from durum wheat (*Triticum*
53 *turgidum* L. ssp. *durum* [Desf.]) was recently mapped (Liu et al. 2012). Previous studies have searched for *S. avenae*
54 resistance in several wild *Triticum* relatives, but not in wheat-rye derived germplasm (Di Pietro et al. 1998; Migui
55 and Lamb 2003; Migui and Lamb 2004). One exception is Amigo wheat (Lowe 1981; Hu et al. 2004), but *S. avenae*
56 resistance has not been attributed to the 1AL.1RS translocation carried by this cultivar.

57 Plant materials are rarely evaluated for resistance to more than two aphid species. However, Smith et al. (2004b)
58 evaluated 20 *R. padi*-resistant accessions, belonging to six *Aegilops* spp. and one *T. araraticum* Jakubz. They
59 reported that *Aegilops neglecta* Req. accession 8052 also possessed resistance to *S. avenae* and the Russian wheat
60 aphid, *Diuraphis noxia* (Kudjumov). Similarly, Migui and Lamb (2003) evaluated 19 wild wheat relatives for
61 resistance to *R. padi*, *S. avenae*, and *S. graminum*, and found resistance to *S. avenae* and *R. padi*, or *S. avenae* and *S.*
62 *graminum* in individual accessions, but none resistant to all three aphid species. Although diploid species appear to
63 have the highest frequency of resistance (Migui and Lamb 2003), introgressing resistance genes from multiple wild
64 resistance sources into adapted germplasm requires considerable pre-breeding efforts.

65 Burd and Porter (2006) studied *S. graminum* - host resistance interactions and found considerable intraspecific plant
66 variation and several *S. graminum* virulence patterns. A chronological review of the field deployment of *S.*
67 *graminum* resistant wheat cultivars and the detection of new *S. graminum* biotypes states that “The use of greenbug
68 [*S. graminum*]-resistant wheat cultivars could not have contributed to the development of new biotypes” (Porter et al.
69 1997). There is also variation in the virulence patterns of various *S. avenae* populations (Lowe 1981; Caillaud et al.
70 1995; Xu et al. 2011). However no *S. avenae* biotype designations have been made, since the biotype concept refers
71 to the specific outcomes from the aphid resistance/susceptibility interaction with germplasm differing in the
72 resistance genes they carry. No variation in *R. padi* virulence has been reported to date.

73 This study aimed to search for resistance to *R. padi*, *S. avenae*, and *S. graminum* in a set of wheat-alien translocation
74 and substitution lines previously developed in the same genetic background (spring wheat Pavon F76). Resistance to
75 multiple aphid species within a single genotype is a highly desired trait and can greatly facilitate the development of
76 new, aphid-resistant cultivars, particularly in regions where distributions of different aphid species overlap.

77

78 **Materials and methods**

79 *Plant materials*

80 We screened germplasm previously developed in the genetic background of the spring wheat cultivar Pavon F76 for
81 resistance to *R. padi*, *S. avenae*, and *S. graminum*. The material consisted of 54 wheat-rye centric translocation and
82 substitution lines, seven wheat-rye, and four *Ae. speltoides*-wheat recombinant derived lines (Table 1) from the
83 genetic resources program of the International Maize and Wheat Improvement Center (CIMMYT, Mexico). These
84 lines were second generation offspring from self-pollinated single plants that had been analyzed by C-banding to
85 confirm published substitution/translocation descriptions. Descriptions of the genotypes can be found in
86 Lukaszewski (1993, 1995, 1997, 2000, 2006, 2008), Brunell et al. (1999), Kumlay et al. (2003), Kim et al. (2004),
87 and Lukaszewski et al. (2004). This paper will follow the same nomenclature, where subscripts represent the origin
88 of wheat chromosomes (A, B, and D) and rye (R) genomes (L=chromosome long arm; S=chromosome short arm)
89 (Table 1).

90 *Aphid rearing*

91 Aphids were reared in cages under glasshouse conditions at ca. 22°C, with a minimum of 16 h of light, supplemented
92 when needed with 400W high-pressure sodium lamps. *R. padi* and *S. avenae* were maintained on oat plants in the
93 Department of Plant Breeding and Biotechnology at the Swedish University of Agricultural Sciences (SLU). Two
94 sources of Swedish *R. padi*, collected 600 km apart, were used in the screenings; they were collected from their
95 winter host (*Prunus padus* L.) in order to avoid infection by barley yellow dwarf virus. *S. avenae* adults were
96 collected from wheat fields around Alnarp, and a colony was started from nymphs born on a sugar diet to obtain
97 virus free aphids. Virus-free starter colonies of *S. graminum* biotype E were obtained from USDA-ARS and
98 maintained on Jagger wheat in the Department of Entomology at Kansas State University (KSU). Biotype E is

99 common and virulent to wheat carrying resistance genes *gb1* and *Gb2*, but non-virulent to genes *Gb3-Gb7* (Burd and
100 Porter 2006; Weng et al. 2005).

101 *Aphid resistance screening methods*

102 All experiments included a Pavon F76 control, since the alien material was transferred into that genetic background.
103 Screening consisted of two phases for each aphid species - Phase 1, where all lines were screened for resistance and
104 Phase 2, where genotypes selected in Phase 1 were screened three times with *R. padi* and *S. avenae*, or once with *S.*
105 *graminum*, to confirm or reject statistically significant results from Phase 1. Other lines from the stock having the
106 same alien origin as the statistically more resistant lines in Phase 1 were also included. *R. padi* tests preceded the *S.*
107 *avenae* tests and some lines resistant to *R. padi* were also included in the *S. avenae* Phase 2 test.

108 *Evaluation of seedling plant resistance to R. padi, S. avenae, and S. graminum*

109 Resistance to *R. padi* and *S. avenae* was measured as reduced growth rate at SLU. Candidate lines were divided into
110 subsets in Phase 1, as it was only possible to screen a maximum of 24 entries per week. Seeds of each genotype were
111 placed on humid filter paper in Petri dishes, cold treated at 5°C for three days, maintained at room temperature for
112 two days, and then individually planted in a plastic 10 cm-diameter pot (300 ml) in Weibull's Kronmull® potting soil
113 with Leca. Pots were arranged in a randomized complete block design with four replicates (one plant per replicate).
114 Seedlings were grown in a walk-in climate controlled chamber at 22°C, 80% RH, and 16 h light at the intensity of
115 250 µmol photons m⁻²s⁻¹ at plant level.

116 Seven to eight days after transplanting (second - third leaf stage), each plant was exposed to five nymphs born within
117 a time span of 24 hours. These nymphs were obtained from alate females in the rearing colony maintained overnight
118 on oat plants. Newborn nymphs were confined at the base of the test plants in transparent cylindrical acrylic cages (2
119 cm diameter, 5 cm length), sealed with cotton wool at the bottom and the top. Nymphs were individually weighed on
120 a Mettler M3 microbalance four days after infestation.

121 *S. graminum* experiments were performed under glasshouse conditions (ca. 22°C, 80% RH, and 16 h light) at KSU.
122 In Phase 1, 8-10 seeds of each genotype were sown in tufts in flats (36 cm x 51 cm) filled with Pro-mix® BX
123 (Hummert International), in a complete randomized experimental design with four replicates. The stock was divided
124 spatially in two subsets since there was only room for 35 genotypes per flat. The *S. graminum*-resistant control,
125 wheat line GRS-1201, containing the *Gb6* resistance gene from Insave rye (Lu et al. 2010) was included in each flat.
126 The plants were infested at the second to third leaf stage with previously infested leaves of Jagger wheat, at an
127 average density of 5 nymphs per tuft. We scored the percentage of chlorosis on infested leaves 14 days after
128 infestation. For Phase 2 screening, eight genotypes were selected based on their genetic background and a low
129 percentage of chlorosis in Phase 1. Seeds of each genotype were germinated in Petri dishes for six days, and
130 seedlings of each entry were then planted individually in 10 cm pots. Each pot was covered with a fine-mesh of
131 organdy fabric supported by wooden stakes allowing undisturbed growth of plants. Plants at the second to third leaf
132 stage were then infested with two third or fourth instar nymphs. The experiment was established as a randomized

133 complete block design with 10 replicates. Percentage of chlorosis, number of aphids, and weight of the colony were
134 recorded 10 days after infestation.

135 *Evaluation of adult plant resistance to S. avenae*

136 We selected and evaluated 12 genotypes for adult plant *S. avenae* resistance, using a similar rationale for selections
137 as for Phase 2 seedling tests. Screening additional lines was not possible due to space and management requirements
138 or lack of synchrony with the phenology of the control line.

139 Seeds were germinated as described above, transplanted singly into 16 cm plastic pots (2 L), and grown under
140 glasshouse conditions (in a randomized complete block design with four replicates) until ear emergence (Zadoks
141 growth Scale GS: 53-55, Zadoks et al. 1974). Plants were then infested with five newborn *S. avenae* (obtained using
142 the above methods). Nymphs were confined to one emerging ear and its flag leaf with a perforated polyethylene bag
143 (18 x 26 cm, Baumann Saatzeitbedarf), and plants were then transferred to a walk-in climate controlled chamber
144 (22°C, 80% HR, and 16 h light at the intensity of 250 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ at plant level), where they were kept for
145 four days before the aphids were weighed on a Mettler M3 microbalance. The Pavon F76 control was sown weekly
146 three more times after the test plants, so that growth stages could be matched. Entries were grouped in two subsets
147 according to their phenology because they had different days until flowering.

148 *Statistical analysis*

149 We used SAS/STAT[®] software, version 9.2 (SAS Institute Inc. 2009) for all statistical analyses. Analysis of variance
150 for aphid weights from Phase 1 experiments with *R. padi* and *S. avenae* was conducted using the GLM procedure,
151 and least-square means per genotype were calculated. Statistical differences between genotype and control means
152 were tested by the comparatively less stringent *t*-test to identify all potentially resistant lines (type 1 error was not
153 anticipated, as Phase 2 experiments would correct). The ratio between the mean of aphids on a genotype and on a
154 control plant was calculated as aphid weight percentage of Pavon F76. Results from Phase 2 experiments were
155 analyzed with the MIXED procedure, where genotypes were included in the model as fixed effects, and blocks and
156 round of evaluation as random effects. Blocks were nested within round of evaluation. Least square means were
157 compared with those of Pavon F76 in a Dunnett's test. We analyzed both phases of the *S. graminum* evaluation with
158 the GLM procedure. In Phase 1, plant chlorosis was scored and means were compared with *t*-tests. In Phase 2,
159 numbers of *S. graminum* and the weight of the *S. graminum* colony were quantified and genotype means were
160 compared with those of Pavon F76 in a Dunnett's test. One degree of freedom contrasts were made between
161 genotypes 7A.7S-L7, 7A.7S-L5, 7A.7S-Gb5, and the resistant control (GRS-1201).

162

163 **Results**

164 In Phase 1 evaluations, *R. padi* weights ranged from 65.2 % to 119.3 % of the Pavon F76 control, though we
165 detected statistical differences between genotypes in only the first two subsets (subset 1: $df = 15, 213; F = 3.11;$
166 $p < 0.0001$; subset 2: $df = 17, 273; F = 3.63; p < 0.0001$; subset 3: $df = 17, 284; F = 1.55; p = 0.0771$; subset 4: $df = 16,$
167 $282; F = 1.41; p = 0.1428$). Eight genotypes carrying rye chromatin on chromosome 1 reduced *R. padi* weight

168 significantly ($p \leq 0.05$; t -test) compared to the Pavon F76 control (Table 1). These lines, six other lines carrying rye
169 chromatin of similar origin and the control (15 total genotypes) were selected for Phase 2 screening. Significant
170 differences were detected with the SAS MIXED procedure ($df = 14, 146$; $F = 3.29$; $p < 0.0001$). According to the
171 Dunnett's test, genotypes $1R_c(1A)$, $1R_c(1B)$, $1R_{pr}(1D)$, $1R_{pr-1D_{5+10-2}}(1D)$, $1AL.1RS_e$, and $1BL_v.1RS_e$ reduced *R. padi*
172 weight significantly (23.9-14.4%; Table 2), compared to the Pavon F76 control.

173 *S. avenae* mean weights in Phase 1 evaluations ranged from 69.8 % to 157.4 % of Pavon F76. However, statistical
174 differences ($p \leq 0.05$) between genotypes were observed only in subset 1 ($df = 18, 199$; $F = 3.63$; $p \leq 0.001$), where
175 only the $1BL_v.1RS_e$ genotype reduced *S. avenae* mean weight significantly more than Pavon F76 (Table 1). Selection
176 of genotypes for Phase 2 was therefore less strict than before ($p \leq 0.1$) and included some lines with rye chromosome
177 1 chromatin resistant to *R. padi*. *S. avenae* weights were significantly reduced on 11 genotypes ($df = 22, 211$; $F =$
178 3.52 ; $p \leq 0.0001$) compared to Pavon F76 (Table 2). The weight of *S. avenae* feeding on adult plants of the
179 $1AL.1RS_{am}$ genotype was significantly reduced ($p \leq 0.05$) compared to Pavon F76 (Table 3). This line also
180 significantly reduced *S. avenae* weight by 23.2% in the Phase 2 seedling test compared to Pavon F76).

181 Genotypes differed significantly for *S. graminum*-related leaf chlorosis (subset 1: $df = 34, 101$; $F = 1.84$; $p = 0.0105$;
182 subset 2: $df = 33, 65$; $F = 2.32$; $p = 0.0019$). However, only four lines with *Ae. speltoides* chromatin (7A.7S-L7,
183 7A.7S-L5, and 7A.7S-Gb5) were significantly less chlorotic than Pavon F76 (Table 1). Statistical differences were
184 observed for the numbers of *S. graminum* developing on the plants ($df = 9, 60$; $F = 2.37$; $p = 0.023$), weight of the
185 colony ($df = 9, 60$; $F = 3.24$; $p = 0.0029$) and chlorosis ($df = 9, 68$; $F = 7.74$; $p < 0.0001$). As in Phase 1, genotypes
186 7A.7S-L7, 7A.7S-L5, and 7A.7S-Gb5 showed less leaf chlorosis than Pavon F76 (Table 4), and 7A.7S-L5 also
187 significantly reduced *S. graminum* colony weight by 67.7 % and the number of *S. graminum*, compared to Pavon
188 F76. Genotypes 7A.7S-L7 and 7A.7S-Gb5 showed low average levels of chlorosis (0.9 % and 2.3%, respectively),
189 but did not significantly reduce *S. graminum* numbers or colony weight. Line 7A.7S-S3 showed a susceptible
190 response, as it also did in the Phase 1 test (Tables 1, 4). For the four genotypes with low chlorosis, one degree of
191 freedom contrasts showed differences in the number of *S. graminum* per plant between genotypes 7A.7S-L7 and
192 7A.7S-L5, and between 7A.7S-L7 and GRS-1201 ($p < 0.01$), but no significant differences were observed between
193 the four lines in terms of aphid colony weight or chlorosis (Table 5). Therefore these results indicate that genotypes
194 7A.7S-L5 and 7A.7S-Gb5 performed similarly to the resistant control GRS-1201 in all aspects studied (Table 5).

195

196 Discussion

197 Certain translocation and substitution lines with rye chromatin in chromosome 1 were resistant to both *R. padi* and *S.*
198 *avenae*, but no 1R or 1RS line was strongly resistant to *S. graminum* biotype E. However, previous studies have
199 shown that the 1RS chromosome arm carries the *Gb2* and *Gb6* *S. graminum* resistance genes from Insave rye
200 (Sebesta and Wood 1978; Porter et al. 1991; Lu et al. 2010). Chromosome 1R from the E12165 wheat substitution
201 line incorporated into Pavon F76 carries gene(s) for *R. padi* and *S. avenae* resistance. Most genotypes with this $1R_c$
202 source reduced the weights of *R. padi* or *S. avenae*, and genotypes $1R_c(1A)$, $1R_c(1B)$, $1AL.1RS_e$, and $1BL_v.1RS_e$
203 significantly reduced the weights of both aphid species. These genetic stocks were produced by Lukaszewski (1997)

204 from the wheat line E12165 with a 1R(1D) substitution from a cross between Panda triticale and a CIMMYT wheat
205 line. Both sources of resistance are very useful in breeding programs, since 1RS has previously been reported to
206 increase yield in the Pavon F76 background (Kim et al. 2004). Of the two lines resistant to both *R. padi* and *S.*
207 *avenae* (1AL.1RS_e and 1BL_v.1RS_e), 1AL.1RS_e is preferred because it has less of a negative effect on baking quality
208 (Kumlay et al. 2003). Genotypes 1R_{pr}(1D) and 1R_{pr}.1D₅₊₁₀₋₂(1D), from Presto triticale, also expressed resistance to *R.*
209 *padi* and *S. avenae*. The 1R chromosome from Presto was engineered to improve bread-making quality in hexaploid
210 triticale, and later, genotypes 1R_{pr}(1D) and 1R_{pr}.1D₅₊₁₀₋₂(1D) were produced in the Pavon F76 background
211 (Lukaszewski 2006).

212 The 1RS chromosome was present in all of the lines that significantly reduced the weight of *R. padi* and/or *S. avenae*
213 in Phase 2 tests, suggesting that gene(s) for resistance are located on 1RS. Interestingly, the genotype carrying the
214 1RS translocation from Amigo wheat (1AL.1RS_{am}) showed resistance to *S. avenae* both at seedling and adult stages.
215 Other studies have reported that Amigo wheat is resistant to *S. avenae* (Lowe 1981; Hu et al. 2004), and our results
216 suggest that this resistance is conferred by 1RS from Insave rye.

217 Unlike *R. padi* and *S. graminum*, which cause significant damage at early plant stages, *S. avenae* normally has a
218 higher reproductive rate when feeding on flowering plants (Watt 1979) and causes more deleterious damage to plants
219 in later developmental stages (Voss et al. 1997). It is therefore important to screen for *S. avenae* resistance in adult
220 plants, but the challenges of keeping plants free of other pests and diseases, and the difficulty in matching the
221 phenology of test plants, make adult plant testing much more demanding than seedling tests. This is perhaps the
222 reason why few studies of adult plant resistance to *S. avenae* have been conducted under controlled conditions.

223 Of the lines expressing *S. avenae* resistance as seedlings, only the 1AL.1RS_{am} translocation line from Amigo wheat
224 significantly reduced *S. avenae* weights on adult plants. Similar results were obtained by Migui and Lamb (2004)
225 when they compared resistance effects on *S. avenae* from seedlings and adult plants of *Triticum monococcum* L.
226 accessions. They hypothesized that the lower resistance of adult plants was due to a reduction in hydroxamic acids
227 (Hx) during wheat plant growth and development (Migui and Lamb 2004). However, Hx concentration is still
228 relatively high in the young tissues of mature plants, such as in emerging flag leaves (Thackray et al. 1990; Copaja et
229 al. 1999). Several studies have shown a strong negative correlation between cereal aphid performance, including *S.*
230 *avenae*, and Hx concentrations in young wheat plants (Thackray et al. 1990; Leszczynski and Dixon 1992; Givovich
231 and Niemeyer 1994; Hansen 2006), whereas other have shown no correlation between Hx concentration and *S.*
232 *avenae* performance (Nicol and Wratten 1997; Castaneda et al. 2010).

233 Hx concentrations are unlikely to explain the resistance patterns that we found in lines with a Pavon F76 background.
234 In Australia, this wheat cultivar is called Hartog (Skovmand et al. 1997) and is known to have low Hx levels (Nicol
235 et al. 1992). Furthermore, the genes governing the accumulation of Hx are located on hexaploid wheat chromosome
236 groups 4 and 5, and on rye chromosomes 5R and 7R (Niemeyer and Jerez 1997; Nomura et al. 2002; Nomura et al.
237 2003), whereas rye resistance to *S. avenae*, and *R. padi* was only found in chromosome 1 substitution and
238 translocation lines. Since the plant genotypes into which the alien material was incorporated have low Hx
239 concentrations, both seedling and adult plant resistance is most likely due to other physiological processes, unless

240 there are epistatic effects of resistance genes on Hx genes. Further investigation is needed to elucidate the causes of
241 seedling resistance and the lack of resistance in the adult stage.

242 Tyler et al. (1985) identified a source of resistance to *S. graminum* originating from *Ae. speltoides* in several wheat
243 substitution lines. The resistance gene in those lines was later designated as *Gb5* (Tyler et al. 1987). One substitution
244 line (CI17884) was utilized to derive the KS90H445 wheat translocation line. This line lacked suitable agronomic
245 performance (Friebe et al. 1991). Therefore, Lukaszewski (1995) transferred the *Gb5* chromatin from KS90H445 to
246 Pavon 76 through homoeologous recombination with the *ph1b* mutation, resulting in, among others, the lines 7A.7S-
247 S3, 7A.7S-L7, 7A.7S-L5, and 7A.7S-Gb5. Dubcovski et al. (1998) characterized 7A.7S-S3 and 7A.7S-Gb5 as
248 carrying the *Lr47* and *Gb5* resistance genes, respectively. The substitution line CI17884 demonstrates moderate
249 antibiotic effects on *S. graminum* biotype E (Tyler et al. 1985), and in our experiments, genotype 7A.7S-Gb5 gave
250 similar results combined with low chlorosis levels. Genotype 7A.7S-L7 had significantly lower levels of chlorosis
251 compared to Pavon F76, but significantly higher numbers of *S. graminum* per plant when compared to 7A.7S-L5 and
252 the resistant check GRS-1201. The differences between these recombinant derived lines suggest that genotype
253 7A.7S-L7 is predominantly tolerant to *S. graminum* feeding, and that the chromatin transferred to Pavon F76 has
254 more than one locus responsible for *S. graminum* resistance, which may have been separated during its transfer to
255 Pavon F76. Further evaluations are needed to investigate the two categories of resistance in 7A.7S-L7, 7A.7S-L5,
256 and 7A.7S-Gb5 to better clarify such differences.

257 As expected, the line 1AL.1RS_{am} containing *Gb2* was susceptible to *S. graminum* biotype E, whereas the GRS-1201
258 control containing *Gb6* was resistant. Both genotypes are derived from the rye cultivar Insave. Lu et al. (2010) used
259 molecular mapping to show that they are non-allelic genes and that Insave must be genetically heterogeneous.

260 The usefulness of the resistant genotypes we found, especially those with resistance to *R. padi* and *S. avenae*, will be
261 largely determined by the genetic variation in aphid populations. It is well known that aphid virulence can vary in
262 different geographic areas, as demonstrated for the Russian wheat aphid (Haley et al. 2004; Smith et al. 2004a;
263 Weiland et al. 2008). Several unique *S. graminum* biotypes in the USA differ in virulence patterns to resistant
264 cultivars (Burd and Porter 2006), and were present before resistant cultivars were grown (Porter et al. 1997).
265 Variation in host responses also exist in different *S. avenae* populations (Lowe 1981; Caillaud et al. 1995; Xu et al.
266 2011), but to our knowledge, no studies have compared virulence patterns among different *R. padi* populations.

267 Our results indicate that resistance to *R. padi* and *S. avenae* can be transferred from rye into wheat depending on the
268 chromosome arm of wheat that is substituted and the source of the alien chromatin. We used reduced aphid growth in
269 no-choice assays as an estimate of resistance levels. How this translates into reduced population growth remains to
270 be determined. There may also be a component of antixenosis that would be expressed more if aphids were not
271 confined. Future field evaluations need to be conducted to determine the degree of aphid infestation and to estimate
272 yield losses due to aphid damage in this resistant germplasm.

273 Chromosome arm-specific mapping populations are being developed to unravel the genetic basis of resistance to *R.*
274 *padi* and *S. avenae* in 1AL.1RS_e and 1AL.1RS_{am} translocation lines. The high levels of *S. graminum* resistance found
275 in the *Ae. speltoides* recombinant-derived lines require further investigation, since it is advantageous to combine

276 resistance mechanisms to reduce damage levels and to improve the durability of resistance. The sources of resistance
277 to *R. padi*, *S. avenae*, and *S. graminum* identified in this study have great potential for use by breeding programs
278 aiming to transfer aphid resistance into cultivars. Resistance is already present in adapted spring wheat germplasm,
279 and, in the case of *R. padi* and *S. avenae*, single genotypes can be used as resistance sources.

280
281 **Acknowledgments:** We thank the Monsanto's Beachell-Borlaug International Scholars Program for financing this
282 research project. The Swedish Foundation for Strategic Environmental Research (Mistra) through the
283 PlantComMistra program is acknowledged for support. We would also like to thank for technical support provided
284 by Dr. Vehbo Hot (SLU) and Ms. Lina Aguirre (KSU) and to Dr. Jan-Eric Englund (SLU) for his valuable advice on
285 the statistical analyses. E. Quilligan provided editing assistance.

286
287 **References**

- 288 Berzonsky WA, Ding H, Haley SD, Harris MO, Lamb RJ, McKenzie RIH, Ohm HW, Patterson FL, Peairs FB,
289 Porter DR, Ratcliffe RH, Shanower TG (2003) Breeding wheat for resistance to insects. *Plant Breeding*
290 *Reviews* 22:221-296
- 291 Blackman RL, Eastop VF (2007) Taxonomic Issues. In: Van Emden HF and Harrington R (eds) *Aphids as Crop*
292 *Pests*. pp 1-29
- 293 Brunell MS, Lukaszewski AJ, Whitkus R (1999) Development of arm specific RAPD markers for rye chromosome
294 2R in wheat. *Crop Sci* 39 (6):1702-1706
- 295 Burd JD, Porter DR (2006) Biotypic diversity in greenbug (Hemiptera: Aphididae): characterizing new virulence and
296 host associations. *J Econ Entomol* 99 (3):959-965
- 297 Caillaud CM, Dedryver CA, DiPietro JP, Simon JC, Fima F, Chaubet B (1995) Clonal variability in the response of
298 *Sitobion avenae* (Homoptera: Aphididae) to resistant and susceptible wheat. *Bull Entomol Res* 85 (2):189-
299 195
- 300 Castaneda LE, Figueroa CC, Nespolo RF (2010) Do insect pests perform better on highly defended plants? Costs and
301 benefits of induced detoxification defences in the aphid *Sitobion avenae*. *J Evol Biol* 23 (11):2474-2483.
302 doi:10.1111/j.1420-9101.2010.02112.x
- 303 Copaja SV, Nicol D, Wratten SD (1999) Accumulation of hydroxamic acids during wheat germination.
304 *Phytochemistry* 50 (1):17-24. doi:10.1016/s0031-9422(98)00479-8
- 305 Di Pietro JP, Caillaud CM, Chaubet B, Pierre JS, Trottet M (1998) Variation in resistance to the grain aphid, *Sitobion*
306 *avenae* (Sternorhynca : Aphididae), among diploid wheat genotypes: Multivariate analysis of agronomic
307 data. *Plant Breeding* 117 (5):407-412. doi:10.1111/j.1439-0523.1998.tb01964.x

308 Dubcovsky J, Lukaszewski AJ, Echaide M, Antonelli EF, Porter DR (1998) Molecular characterization of two
309 *Triticum speltoides* interstitial translocations carrying leaf rust and greenbug resistance genes. *Crop Sci* 38
310 (6):1655-1660

311 Franzen LD, Gutsche AR, Heng-Moss TM, Higley LG, Macedo TB (2008) Physiological responses of wheat and
312 barley to Russian wheat aphid, *Diuraphis noxia* (Mordvilko) and bird cherry-oat aphid, *Rhopalosiphum*
313 *padi* (L.) (Homoptera: Aphididae). *Arthropod-Plant Inte* 2 (4):227-235. doi:10.1007/s11829-008-9048-1

314 Friebe B, Jiang J, Raupp WJ, McIntosh RA, Gill BS (1996) Characterization of wheat-alien translocations conferring
315 resistance to diseases and pests: Current status. *Euphytica* 91 (1):59-87

316 Friebe B, Mukai Y, Dhaliwal HS, Martin TJ, Gill BS (1991) Identification of alien chromatin specifying resistance to
317 wheat streak mosaic and greenbug in wheat germ plasm by C-banding and in situ hybridization. *Theor Appl*
318 *Genet* 81 (3):381-389. doi:10.1007/bf00228680

319 Givovich A, Niemeyer HM (1994) Effect of hydroxamic acids on feeding behaviour and performance of cereal
320 aphids (Homoptera: Aphididae) on wheat. *Eur J Entomol* 91:371-374

321 Haley SD, Peairs FB, Walker CB, Rudolph JB, Randolph TL (2004) Occurrence of a new Russian wheat aphid
322 biotype in Colorado. *Crop Sci* 44 (5):1589-1592

323 Hansen LM (2006) Effect of 6-methoxybenzoxazolin-2-one (MBOA) on the reproduction rate of the grain aphid
324 (*Sitobion avenae* F.). *J Agric Food Chem* 54 (4):1031-1035. doi:10.1021/jf0509005

325 Hesler LS (2005) Resistance to *Rhopalosiphum padi* (Homoptera: Aphididae) in three triticale accessions. *J Econ*
326 *Entomol* 98 (2):603-610

327 Hesler LS, Haley SD, Nkongolo KK, Peairs FB (2007) Resistance to *Rhopalosiphum padi* (Homoptera: Aphididae)
328 in triticale and triticale-derived wheat lines resistant to *Diuraphis noxia* (Homoptera: Aphididae). *J Entomol*
329 *Sci* 42 (2):217-227

330 Hesler LS, Tharp CI (2005) Antibiosis and antixenosis to *Rhopalosiphum padi* among triticale accessions. *Euphytica*
331 143 (1-2):153-160. doi:10.1007/s10681-005-3060-7

332 Hu X, Zhao H, Heimbach U, Thieme T, Li J, Zhang Y, Liu B, Li D, Hu Z (2004) Study on cereal aphid resistance on
333 three winter wheat cultivars introduced into China. *Acta Bot Boreali-Occidentalis Sinica* 24 (7):1221-1226

334 Kieckhefer RW, Gellner JL (1992) Yield losses in winter wheat caused by low-density cereal aphid populations.
335 *Agron J* 84 (2):180-183

336 Kim W, Johnson JW, Baenziger PS, Lukaszewski AJ, Gaines CS (2004) Agronomic effect of wheat-rye
337 translocation carrying rye chromatin (1R) from different sources. *Crop Sci* 44 (4):1254-1258

338 Kumlay AM, Baenziger PS, Gill KS, Shelton DR, Graybosch RA, Lukaszewski AJ, Wesenberg DM (2003)
339 Understanding the effect of rye chromatin in bread wheat. *Crop Sci* 43 (5):1643-1651

340 Leszczynski B, Dixon AFG (1992) Resistance of cereals to aphids - the interaction between hydroxamic acids and
341 glutathione S-transferases in the grain aphid *Sitobion avenae* (F) (Hom., Aphididae). J Appl Entomol 113
342 (1):61-67

343 Liu X, Yang X, Wang C, Wang Y, Zhang H, Ji W (2012) Molecular mapping of resistance gene to English grain
344 aphid (*Sitobion avenae* F.) in *Triticum durum* wheat line C273. Theor Appl Genet 124 (2):1-7

345 Lowe H (1981) Resistance and susceptibility to colour forms of the aphid *Sitobion avenae* in spring and winter
346 wheats (*Triticum aestivum*). Ann Appl Biol 99 (1):87-98

347 Lu H, Rudd JC, Burd JD, Weng Y (2010) Molecular mapping of greenbug resistance genes *Gb2* and *Gb6* in
348 T1AL.1RS wheat-rye translocations. Plant Breeding. doi:10.1111/j.1439-0523.2009.01722.x

349 Lukaszewski AJ (1993) Reconstruction in wheat of complete chromosomes 1B and 1R from the 1RS.1BL
350 translocation of 'Kavkaz' origin. Genome 36 (5):821-824

351 Lukaszewski AJ (1995) Physical distribution of translocation breakpoints in homoeologous recombinants induced by
352 the absence of the Ph1 gene in wheat and triticale. Theor Appl Genet 90 (5):714-719

353 Lukaszewski AJ (1997) Further manipulation by centric misdivision of the 1RS.1BL translocation in wheat.
354 Euphytica 94 (3):257-261. doi:10.1023/a:1002916323085

355 Lukaszewski AJ (2000) Manipulation of the 1RS.1BL translocation in wheat by induced homoeologous
356 recombination. Crop Sci 40 (1):216-225

357 Lukaszewski AJ (2006) Cytogenetically engineered rye chromosomes 1R to improve bread-making quality of
358 hexaploid triticale. Crop Sci 46 (5):2183-2194. doi:10.2135/cropsci2006.03.0135

359 Lukaszewski AJ (2008) Unexpected behavior of an inverted rye chromosome arm in wheat. Chromosoma 117
360 (6):569-578. doi:10.1007/s00412-008-0174-4

361 Lukaszewski AJ, Rybka K, Korzun V, Malyshev SV, Lapinski B, Whitkus R (2004) Genetic and physical mapping
362 of homoeologous recombination points involving wheat chromosome 2B and rye chromosome 2R. Genome
363 47 (1):36-45. doi:10.1139/g03-089

364 Migui SM, Lamb RJ (2003) Patterns of resistance to three cereal aphids among wheats in the genus *Triticum*
365 (Poaceae). Bull Entomol Res 93 (4):323-333. doi:10.1079/ber2003246

366 Migui SM, Lamb RJ (2004) Seedling and adult plant resistance to *Sitobion avenae* (Hemiptera: Aphididae) in
367 *Triticum monococcum* (Poaceae), an ancestor of wheat. Bull Entomol Res 94 (1):35-46.
368 doi:10.1079/ber2003278

369 Nicol D, Copaja SV, Wratten SD, Niemeyer HM (1992) A screen of worldwide wheat cultivars for hydroxamic acid
370 levels and aphid antixenosis. Ann Appl Biol 121 (1):11-18. doi:10.1111/j.1744-7348.1992.tb03982.x

371 Nicol D, Wratten SD (1997) The effect of hydroxamic acid concentration at late growth stages of wheat on the
372 performance of the aphid *Sitobion avenae*. Ann Appl Biol 130 (3):387-396. doi:10.1111/j.1744-
373 7348.1997.tb07669.x

374 Niemeier HM, Jerez JM (1997) Chromosomal location of genes for hydroxamic acid accumulation in *Triticum*
375 *aestivum* L. (wheat) using wheat aneuploids and wheat substitution lines. Heredity 79 (1):10-14

376 Nomura T, Ishihara A, Imaishi H, Ohkawa H, Endo TR, Iwamura H (2003) Rearrangement of the genes for the
377 biosynthesis of benzoxazinones in the evolution of Triticeae species. Planta 217 (5):776-782.
378 doi:10.1007/s00425-003-1040-5

379 Nomura TN, Ishihara AI, Imaishi HI, Endo TE, Ohkawa HO, Iwamura HI (2002) Molecular characterization and
380 chromosomal localization of cytochrome P450 genes involved in the biosynthesis of cyclic hydroxamic
381 acids in hexaploid wheat. Mol Genet Genomics 267 (2):210-217. doi:10.1007/s00438-002-0653-x

382 Porter DR, Burd JD, Shufran KA, Webster JA, Teetes GL (1997) Greenbug (Homoptera: Aphididae) biotypes:
383 selected by resistant cultivars or preadapted opportunists? J Econ Entomol 90 (5):1055-1065

384 Porter DR, Harris MO, Hesler LS, Puterka GJ (2009) Insects which challenge global wheat production. In: Carver
385 BF (ed) Wheat Science and Trade. Wiley-Blackwell, Iowa, USA, pp 189-201

386 Porter DR, Webster JA, Burton RL, Puterka GJ, Smith EL (1991) New sources of resistance to greenbug in wheat.
387 Crop Sci 31 (6):1502-1504

388 Rabinovich SV (1998) Importance of wheat-rye translocations for breeding modern cultivars of *Triticum aestivum* L.
389 (Reprinted from Wheat: Prospects for global improvement, 1998). Euphytica 100 (1-3):323-340

390 Riedell WE, Kieckhefer RW, Langham MAC, Hesler LS (2003) Root and shoot responses to bird cherry-oat aphids
391 and barley yellow dwarf virus in spring wheat. Crop Sci 43 (4):1380-1386

392 SAS Institute Inc. (2009) SAS/STAT[®] 9.2 User's guide. SAS Institute Inc, Cary, NC, USA

393 Sebesta E, Wood E (1978) Transfer of greenbug resistance from rye to wheat with X-rays. Agronomy abstracts
394 70:61-62

395 Skovmand B, Villareal RL, van Ginkel M, Rajaram S, Ortiz-Ferrera G (1997) Semidwarf bread wheats: names,
396 parentages, pedigrees and origins. CIMMYT, Mexico, D. F.

397 Smith CM, Belay T, Stauffer C, Sary P, Kubeckova I, Starkey S (2004a) Identification of Russian wheat aphid
398 (Homoptera: Aphididae) populations virulent to the *Dn4* resistance gene. J Econ Entomol 97 (3):1112-1117.
399 doi:10.1603/0022-0493(2004)097[1112:iorwah]2.0.co;2

400 Smith CM, Havlickova H, Starkey S, Gill B, Holubec V (2004b) Identification of *Aegilops* germplasm with multiple
401 aphid resistance. Euphytica 135 (3):265-273

402 Thackray DJ, Wratten SD, Edwards PJ, Niemeyer HM (1990) Resistance to the aphids *Sitobion avenae* and
403 *Rhopalosiphum padi* in Gramineae in relation to hydroxamic acid levels. *Ann Appl Biol* 116 (3):573-582.
404 doi:10.1111/j.1744-7348.1990.tb06640.x

405 Tyler JM, Webster JA, Merkle OG (1987) Designations for genes in wheat germplasm conferring greenbug
406 resistance. *Crop Sci* 27 (3):526-527

407 Tyler JM, Webster JA, Smith EL (1985) Biotypic diversity in WSMV resistant wheat germplasm lines.
408 *Crop Sci* 25 (4):686-688

409 Voss TS, Kieckhefer RW, Fuller BW, McLeod MJ, Beck DA (1997) Yield losses in maturing spring wheat caused
410 by cereal aphids (Homoptera: Aphididae) under laboratory conditions. *J Econ Entomol* 90 (5):1346-1350

411 Watt A (1979) The effect of cereal growth stages on the reproductive activity of *Sitobion avenae* and *Metopolophium*
412 *dirhodum*. *Ann Appl Biol* 91 (2):147-157

413 Weiland AA, Peairs FB, Randolph TL, Rudolph JB, Haley SD, Puterka GJ (2008) Biotypic diversity in Colorado
414 Russian wheat aphid (Hemiptera: Aphididae) populations. *J Econ Entomol* 101 (2):569-574.
415 doi:10.1603/0022-0493(2008)101[569:bdicrw]2.0.co;2

416 Weng Y, Li W, Devkota RN, Rudd JC (2005) Microsatellite markers associated with two *Aegilops tauschii*-derived
417 greenbug resistance loci in wheat. *Theor Appl Genet* 110:462-469

418 Xu ZH, Chen JL, Cheng DF, Sun JR, Liu Y, Francis F (2011) Discovery of English grain aphid (Hemiptera:
419 Aphididae) biotypes in China. *J Econ Entomol* 104 (3):1080-1086

420 Zadoks JC, Chang TT, Konzak CF (1974) A decimal code for the growth stages of cereals. *Weed Res* 14 (6):415-421
421

422 **Table 1.** *R. padi* and *S. avenae* weights, and percent leaf chlorosis response to *S. graminum* relative to the control
 423 Pavon F76 in Phase 1 seedling experiments, and subset number in which lines were tested for each aphid species.

Genotype [†]	<i>R. padi</i>		<i>S. avenae</i>			<i>S. graminum</i>	
	% Pavon F76 [‡]	Subset	% Pavon F76 [‡]	Subset	%Pavon F76 [¶]	Subset	
SUBSTITUTION LINES							
1R _e (1A)	81.8	1	127.8	(+)	1	89.5	1
1R _e (1B)	105.7	1	69.8	+	1	121.3	1
1R _e (1D)	91.5	1	128.4	(+)	1	110.2	1
MA1S.1RL _e (1A)	88.9	1	119.4		1	111.7	1
MA1S.1RL _e (1B)	90.9	1	130.4	(+)	1	83.6	1
MA1S.1RL _e (1D)	87.3	1	90.7		1	120.6	1
1R _{pr} (1D)	74.2	**	89.2		1	160.5	1
1R _{pr} .1D ₅₊₁₀₋₂ (1D)	71.2	**	73.8		1	90.2	1
1R _{rec} (1A)	105.1	1	108.5		1	84.3	1
1R _{rec} (1B)	115.7	1	137.7	(+)	1	85.1	1
1R _{rec} (1D)	85.6	1	107.0		1	114.6	1
1R _{inv} (1A)	83.4	1	80.7		1	56.2	1
2R _{rec} (2B)	91.6	1	137.8	(+)	1	156.1	1
TRANSLOCATION LINES							
1AL.1RS _e	75.7	**	79.5	+	2	116.9	1
1AL.1RS _{am}	104.1		107.7		1	95.4	1
1AL.1RS _v	95.6		82.0		2	117.6	1
1AL.1RS _{rh}	85.5		77.0	+	2	100.6	1
1BL.1RS _e	70.6	**	78.8	+	2	124.3	1
1BL _v .1RS _e	71.5	**	73.0	*	2	85.8	1
1BL.1RS _{cim}	117.4		133.2	(*)	1	162.7	1
1BL.1RS _{gnr}	92.4		130.1	(+)	1	62.1	1
1BL.1RS _v	95.6		103.2		2	85.1	1
1BL.1RS _i	92.3		96.7		2	91.0	1
1BL.1RS _i	101.2		86.7		3	125.1	2
MA1	76.8	**	100.4		2	123.1	1
MA2	84.7		110.1		2	105.0	1
Te1	91.6		81.9		2	81.4	1
Te2	90.3		82.0		2	99.1	1
1B _i	99.0		86.7		3	101.8	2
1R _i (1B)	110.8		125.5	(+)	1	86.5	1

1DL.1RS _{bb}	85.7		2	77.5	+	2	97.6		1
1DL.1RS _w	65.2	**	2	86.4		2	90.2		1
1DL.1RS _e	90.2		2	92.1		2	101.3		1
1DL.1RS _v	101.2		2	103.4		2	118.3		1
1AS.1RL _e	95.0		3	86.2		2	84.90		2
1BS.1RL _e	88.8		3	93.0		2	69.71		2
1DS.1RL _e	75.6	**	3	96.7		2	130.4		2
1DS.1RL _{bb}	89.9		3	92.0		2	80.4		2
1AS.#2L	96.8		4	89.2		3	58.1		2
1AL.1RS+1DL.1RS	96.0		4	104.6		3	76.8		2
2BL.2RS _{cs}	94.2		3	103.6		2	103.6		2
2AS.2RL _{cs}	115.4		4	111.7		4	105.4		2
2BS.2RL _{cs}	96.8		3	107.4		2	50.0		2
2BS.2RL _{bl}	84.3		3	103.5		2	113.4		2
2BS.2RL _{cs}	102.7		4	109.4		3	88.4		2
3DL.3RS _{rh}	103.0		3	111.5		3	84.0		2
3DL.3RS _{cs}	92.7		3	86.5		3	62.5		2
3DS.3RL _{cs}	81.1		3	105.2		3	120.6		2
4A _{ril}	106.0		1	157.4	(**)	1	150.1		1
5AL.5RS _{cs}	98.8		3	95.6		3	85.7		2
5BL.5RS _e	90.8		3	114.1		3	114.3		2
5DL.5RS _{rh}	111.3		4	108.3		3	133.1		2
6BS.6RL _{bb}	97.3		4	115.2		3	114.0		2
7DS.4RL _m	94.3		3	111.3		3	95.6		2
RECOMBINANT DERIVED LINES									
1D+9	107.8		4	136.5	(*)	4	44.6		2
T-9	119.3	(*)	4	117.0		4	108.1		2
1D+4	94.6		4	98.4		4	96.5		2
2D(s)+2	95.8		3	97.6		3	67.0		2
2D(s)+4	92.5		3	93.2		3	108.1		2
2B.2R	104.6		4	123.6	(+)	4	75.0		2
5D.5R-1	92.7		3	103.1		3	77.7		2
7A.7S-S3	101.3		4	107.1		3	113.4		2
7A.7S-L7	98.1		4	98.7		3	31.2	*	2
7A.7S-L5	92.3		4	90.2		3	0.0	**	2
7A.7S-Gb5	110.3		4	88.5		3	31.2	*	2

424 †Subscripts for genotypes: e=E12165; pr=Presto triticale; rec=Reconstructed from Kavkaz; inv=Inverted E12165
 425 long arm; am=Amigo wheat; v=Veery; cim=E12169; gnr=Genaro; i=inserted segment; bb=BH1146/Blanco rye;
 426 w=Wheaton, cs=Chinese Spring; ril=reduced internode length mutation in cs; bl=Blanco; rh=Rhino; cim=CIMMYT
 427 E12169 m=*Secale montanum* Guss.

428 ‡Mean aphid weight on the genotypes divided by the mean aphid weight on Pavon F76 times 100

429 ¶Mean % leaf chlorosis of the genotypes relative to the mean % chlorosis of Pavon F76 times 100

430 + P≤0.1, * P≤0.05; ** P≤0.01 significance levels compared to control Pavon F76 in *t*-tests, () = significantly
 431 increased aphid weight relative to the control

432

433 **Table 2.** *R. padi* or *S. avenae* weights relative to the control Pavon F76 and mean *R. padi* or *S. avenae* weight ±SE in
 434 Phase 2 seedling experiments.

Genotype [†]	<i>R. padi</i>				<i>S. avenae</i>			
	% Pavon	Weight	±SE	*	% Pavon	Weight	±SE	*
	F76 [‡]	(mg)			F76 [‡]	(mg)		
1R _e (1A)	85.3	0.251	0.0402	*	73.7	0.166	0.0169	**
1R _e (1B)	83.3	0.245	0.0403	**	73.7	0.166	0.0168	**
1R _e (1D)	87.4	0.257	0.0402		70.6	0.159	0.0170	***
MA1S.1RL _e (1A)	-	-	-		85.3	0.192	0.0167	
MA1S.1RL _e (1B)	-	-	-		79.1	0.178	0.0170	
MA1S.1RL _e (1D)	-	-	-		90.2	0.203	0.0166	
1R _{pr} (1D)	77.9	0.229	0.0404	***	65.7	0.148	0.0178	***
1R _{pr} .1D ₅₊₁₀₋₂ (1D)	80.3	0.236	0.0402	**	75.5	0.170	0.0170	**
1R _{inv} (1A)	-	-	-		68.4	0.154	0.0170	***
1AL.1RS _e	81.0	0.238	0.0402	**	74.2	0.167	0.0168	**
1AL.1RS _{am}	-	-	-		76.8	0.173	0.0170	*
1AL.1RS _{rh}	-	-	-		69.7	0.157	0.0166	***
1BL.1RS _e	89.7	0.264	0.0403		71.1	0.160	0.0170	***
1BL _v .1RS _e	75.8	0.223	0.0404	***	69.7	0.157	0.0170	***
MA1	99.3	0.292	0.0404		84.4	0.190	0.0238	
1DL.1RS _{bb}	-	-	-		90.2	0.203	0.0169	
1DL.1RS _w	102.3	0.301	0.0403		91.1	0.205	0.0258	
1DL.1RS _e	94.2	0.277	0.0404		95.5	0.215	0.0170	
1AS.1RL _e	91.5	0.269	0.0403		87.1	0.196	0.0171	
1BS.1RL _e	92.5	0.272	0.0402		91.5	0.206	0.0170	
1DS.1RL _e	95.5	0.281	0.0401		84.0	0.189	0.0172	
1DS.1RL _{bb}	-	-	-		82.6	0.186	0.0170	
Pavon F76	100.0	0.294	0.0403		100.0	0.225	0.0169	

435 †Subscripts for genotypes: e=E12165; pr= Presto triticale; inv=Inverted E12165 long arm; am=Amigo wheat;
 436 v=Veery; bb=BH1146/Blanco rye; w=Wheaton; rh=Rhino.

437 ‡Mean aphid weight on the genotypes divided by the mean aphid weight on Pavon F76 times 100

438 * = $P \leq 0.05$; ** = $P \leq 0.01$; *** = $P \leq 0.0001$ significance levels compared to Pavon F76 in Dunnett's test

439

440 **Table 3.** *S. avenae* weight relative to the control Pavon 76 in the adult plant test.

Genotype [†]	% Pavon F76 [‡]
1R _e (1A)	99.1
1R _e (1B)	92.3
1R _{rec} (1A)	119.9
1R _{rec} (1B)	125.7
1AL.1RS _e	104.0
1AL.1RS _{am}	78.2*
1AL.1RS _v	108.1
1BL.1RS _e	100.6
1BL _v .1RS _e	119.2
1BL.1RS _v	130.0
1AS.1RL _e	107.4
1BS.1RL _e	131.3

441 †Subscripts for genotypes: e=E12165; rec=Reconstructed; am=Amigo wheat; v=Veery

442 ‡Mean aphid weight on the genotypes divided by the mean aphid weight on Pavon F76 times 100

443 * $P \leq 0.05$ significance level compared to Pavon F76 in *t*-tests

444

445 **Table 4.** Resistance to *S. graminum* measured as number of *S. graminum* developing on plants, percentage of colony
 446 weight relative to Pavon F76, colony weight, and percent leaf chlorosis in response to *S. graminum* feeding.

Genotype [†]	No. <i>S. graminum</i>	±SE	% Pavon F76 [‡]	Colony weight (mg)	±SE	Chlorosis (%)	±SE
1R _{rec} (1A)	39.7	8.04	62.9	5.1	0.94	60.6	8.44
1R _{inv} (1A)	44.5	8.66	64.8	5.2	1.01	61.2	8.45
2BS.2RL _{cs}	47.2	7.53	82.0	6.6	0.88	43.0	7.97
1D+9	43.6	9.43	77.3	6.2	1.10	84.5	9.02
7A.7S-S3	45.4	7.09	75.3	6.1	0.83	61.0	7.97

7A.7S-L7	48.6	8.05		59.5	4.8	0.94		0.9	8.46	***
7A.7S-L5	22.7	7.09	**	32.3	2.6	0.83	**	3.0	7.97	***
7A.7S-Gb5	31.9	8.05		54.6	4.4	0.94		2.3	9.02	***
GRS-1201	20.3	8.67	**	32.3	2.6	1.01	**	1.4	9.70	***
Pavon F76	62.9	9.42		100.0	8.1	1.10		57.6	9.70	

447 [†]Subscripts for genotypes: inv= Inverted E12165 long arm; rec=Reconstructed from Kavkaz; cs=Chinese Spring

448 [‡]Weight percentage of Pavon F76 obtained by dividing the mean colony weight of genotypes by the mean colony
449 weight of the control times 100

450 ** = P≤0.01; *** = P≤0.0001 significance levels compared to Pavon F76 in Dunnett's test

451
452 **Table 5.** One degree of freedom contrasts for number of *S. graminum* per plant, aphid colony weight and leaf
453 chlorosis between genotypes carrying *Ae. speltoides* chromatin and between the resistant control GRS-1201 carrying
454 the *Gb6* resistance gene.

Contrast	No. <i>S.</i> <i>graminum</i>	Colony Weight	Chlorosis
7A.7S-L7 vs. 7A.7S-L5	**	NS	NS
7A.7S-L7 vs. 7A.7S-Gb5	NS	NS	NS
7A.7S-L7 vs. GRS-1201	**	NS	NS
7A.7S-L5 vs. 7A.7S-Gb5	NS	NS	NS
7A.7S-L5 vs. GRS-1201	NS	NS	NS
7A.7S-Gb5 vs. GRS-1201	NS	NS	NS

455 ** P≤0.01; NS = Not significantly different