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Identification of *Aegilops* germplasm with multiple aphid resistance

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Summary

The greenbug, *Schizaphis graminum* (Rondani), the Russian wheat aphid, *Diuraphis noxia* (Mordvilko), and the bird cherry oat aphid, *Rhopalosiphum padi* (L.), annually cause several million dollars worth of wheat production losses in Europe and the United States. In this study, *Triticum* and *Aegilops* accessions from the Czech Research Institute of Crop Production and the Kansas State University Wheat Genetic Resource Center were evaluated for resistance to these aphids. Accessions with aphid cross-resistance were examined for expression of the antibiosis, antixenosis, and tolerance categories of resistance. *Aegilops neglecta* accession 8052 exhibited antibiotic effects toward all three aphids in the form of reduced intrinsic rate of increase (r_m). The r_m of greenbug (biotype I) on *Ae. neglecta* 8052 was significantly lower than that of greenbugs on plants of the susceptible U. S. variety Thunderbird. The r_m of Russian wheat aphids was significantly lower on foliage of both *Ae. neglecta* 8052 and *T. araraticum* accession 168 compared to Thunderbird. The r_m values of bird cherry oat aphids fed both *Ae. neglecta* 8052 and *T. araraticum* 168 were also significantly lower than those fed the susceptible accession *T. dicoccoides* 62. Neither *Ae. neglecta* 8052 or *T. araraticum* 168 exhibited tolerance to either greenbug biotype I or Russian wheat aphid. Preliminary data suggest that *T. araraticum* 168 may also possess tolerance to bird cherry oat aphid. New genes from *Ae. neglecta* 8052 and *T. araraticum* 168 expressing aphid antibiosis can be used to develop multiple aphid resistant wheat in the U. S. and Central Europe.

Introduction

Common wheat, *Triticum aestivum* L., is the most important cereal crop in the world, providing more nourishment for humans than any other source of nutrition (Johnson et al., 1978). However, various aphid pests damage wheat in all world wheat production areas. The greenbug, *Schizaphis graminum* (Rondani), is a major pest of wheat in North America. Several severe greenbug outbreaks have occurred since 1949 on wheat, barley, oats and sorghum. Annual losses to U. S. wheat production due to greenbug damage range from $60 million to more than $100 million (Webster et al., 2000). Significant yield reductions occur when aphids inject salivary enzymes into plants during feeding and remove plant nutrients (Webster & Kenkel, 1999). The Russian wheat aphid, *Diuraphis noxia* (Mordvilko), is a new pest of wheat in North America, South America, and South Africa since the mid-1980s. Significant wheat yield reductions related to Russian wheat aphid have been documented in Mexico, Lesotho, South Africa and the U.S. (Gilchrist et al., 1984, Morrison, 1988). Production losses in the U. S. and South Africa alone are approximately $90 million per year (Legg & Amosson, 1993). The Russian wheat aphid is also spreading into Central Europe (Stary, 1996), and a virulent biotype reported in Hungary is different from Russian wheat aphids in South Africa (Basky et al., 2001). The bird cherry oat aphid, *Rhopalosiphum padi* (L.), is the most economically significant aphid pest of European cereal crops (Papp & Mesterházy, 1993). Yield loss from bird cherry oat

aphid infestation results from aphid feeding during the seedling stage and from aphids serving as vectors for the barley yellow dwarf virus.

Twenty-two genes expressing resistance to greenbug have been characterized in various Gramineae. In wheat, these include resistance genes from rye, *Aegilops tauschii* and *Aegilops speltoides* (Castro et al., 1999; Dubcovsky et al., 1998; Flinn et al., 2001; Smith et al., 1999).

However, the development and use of aphid resistant wheat is an underdeveloped area of wheat integrated pest management that can greatly improve producer profitability. The first greenbug resistant wheat cultivar, 'TAM 110', was released by the Texas Agricultural Experiment Station in 1997 (Lazar et al., 1997). The yearly value of greenbug resistance in wheat in the U.S. states of Kansas, Texas, and Oklahoma alone is estimated to be more than $20 million (Webster & Kenkel, 1999).

Genes in barley, rye, or wheat germplasm from various areas of the Middle East confer Russian wheat aphid resistance (Castro et al., 1999; Liu et al., 2001; Smith et al., 1999). Resistance attributable to at least three of these genes is based on plant tolerance of aphid-induced chlorophyll loss and antibiosis, expressed as reduced aphid population development (Smith et al., 1992). Russian wheat aphid resistant wheat cultivars are currently in use in the Republic of South Africa (Marasas et al., 1997; Prinsloo, 2000) and in the U.S. states of Colorado and Kansas (Martin et al., 2001; Quick et al., 1996). Initial savings to wheat producers in these countries from the use of resistant varieties is approximately $21 million (F. B. Peairs, personal communication).

Several sources of resistance to the bird cherry oat aphid have been identified in Europe (Havlícková, 1988; Havlícková & Holubec, 1995; Krivchenko & Radchenko 1990; Papp & Mesterhazy, 1993). Leaf phenolic content, phenolic acid content and phenylalanine ammonia-lyase activity have all been implicated as mechanisms of this resistance (Leszcynski, 1985; Havlícková et al., 1996). However, bird cherry oat aphid – resistant varieties are yet to be incorporated into European wheat production on a large scale, and there is a need to further develop this economically and ecologically valuable wheat pest management tactic. In addition, new genes expressing resistance to the bird cherry oat aphid, greenbug or Russian wheat aphid are necessary, in order to slow the development of virulent biotypes, such as those in the greenbug and the Russian wheat aphid.

Several new sources of greenbug and Russian wheat aphid resistance have been identified at the Kansas State University Wheat Genetic Resource Center (KSUWGRC) and the Czech Research Institute of Crop Production (CRICP) (Deol et al., 1995; Havlícková & Holubec, 1995) but this resistance is uncharacterized. The objectives of this study were to: evaluate cereal accessions from the CRICP Gene Bank for resistance to North American strains of the greenbug and Russian wheat aphid; evaluate cereal accessions from the KSUWGRC for resistance to bird cherry oat aphid in the Czech Republic; and determine the category(s) of resistance in accessions with aphid cross resistance.

Materials and methods

Germplasm evaluations

Twenty different accessions of *Aegilops* from the CRICP Gene Bank with resistance to bird cherry oat aphid (Havlícková & Holubec, 1995) were evaluated for greenbug and Russian wheat aphid resistance in a greenhouse at Kansas State University (KSU). Conditions were 14 h photoperiod, 26 °C (day), 20 °C (night) and 40–65% RH. The varieties 'Wichita' and 'Thunderbird' served as susceptible controls. The *Ae. tauschii* accession 1675 (Flinn et al., 2001) served as the greenbug resistant control and the *Triticum aestivum* wheat 'PI220127' (Liu et al., 2001) served as the Russian wheat aphid resistant control. Test and control plants were grown in greenhouse flats filled with Jiffy Mix® potting mixture. Each flat contained 10 rows of test entries as well as one resistant and one susceptible control. Rows contained approximately 10 seeds, depending on seed availability. Plants were infested at the two leaf stage of development, as described by Harvey et al. (1985), with approximately four aphids per seedling. Biotype I greenbugs originated from a colony collected on sorghum in Riley County, Kansas. Russian wheat aphids originated from a colony collected on wheat near Sharon Springs, Kansas.

Infested seedlings were observed daily, and when plants of the susceptible control varieties were dying or dead, the test plants were visually evaluated for greenbug resistance, using a 1–6 rating scale (Porter et al., 1982), where 1 = no injury, 2 = 0–25% chlorosis, 3 = 25–50% chlorosis, 4 = 50–75% chlorosis, 5 = $>$ 75% chlorosis, and 6 = most plants dead. Russian wheat aphid damage was determined as the sum of leaf folding, leaf rolling and chlorosis ratings. For each

damage symptom, plants were rated as 0 = no damage, 1 = < 50% symptoms, 2 = > 50% symptoms, and 3 = 100% of plants dead with both symptoms. Total plant damage ranged from 0 (no damage) to 9 (plant death). Although leaf folding has been evident in past measurements of Russian wheat aphid feeding damage (Smith et al., 1991), it occurred on only about one-third of all plants evaluated in the present study.

At the CRICP in Prague, bird cherry oat aphid resistance was assessed among 120 *Triticum* and *Aegilops* accessions from the KSUWGRC previously shown to exhibit greenbug and/or Russian wheat aphid resistance (Deol et al., 1995). Plants were grown in small field plots according to protocols developed by Havlícková & Holubec (1995). The numbers of bird cherry oat aphids per tiller on anthesis stage plants of each accession were counted as a measure of field resistance. Evaluations at two locations were arranged in randomized complete block designs with four replications. Data in all experiments were analyzed by ANOVA using the SAS (1995) GLM procedure. Accessions with high bird cherry oat aphid populations (>100 aphids per tiller) were omitted from further experiments.

Additional bird cherry oat aphid experiments were conducted on 67 accessions from the field evaluation to obtain preliminary information on antixenosis and antibiosis. Test plants (3 per pot) were cultivated in pots filled with a sand: soil: peat (1:1:1) mixture and grown in environmental chambers at 16 h photoperiod, 20 °C (continuous) and 60% RH. At the four-leaf stage, pots with test plants were uniformly arranged around Micherlich pots containing spring wheat plants infested with alate bird cherry oat aphids. Two pots of spring wheat were placed around each pot containing test accession plants. Each accession was replicated twice. The number of nymphs per plant was recorded 7 days after infestation with alate aphids. Fourteen days later (21 days post-infestation), aphids were removed, counted, killed by chloroform vapors, dried, and weighed. The numbers of nymphs present on plants 7 days after infestation (antixenosis), total bird cherry oat aphid production on each accession and dry weights of total surviving aphid populations (antibiosis) were used as criteria for establishing whether or not an accession was expressing antixenosis and/or antibiosis.

Resistance category assays

Entries expressing potential resistance were evaluated for their ability to tolerate aphid feeding or to express antibiosis to greenbug or Russian wheat aphid at KSU. Experiments were conducted in a greenhouse [14 h photoperiod, 26 °C (day), 20 °C (night), 40–65% RH]. In each experiment, 10 plants (replicates) of 'TAM 110' (resistant control), 'Thunderbird' (susceptible control), *Aegilops neglecta* 8052 (identified at KSU), and *Triticum araraticum* 168 (identified at CRICP) were grown in 10 cm diam plastic pots containing a Jiffy Mix® soil mixture. 'TAM 110' was chosen as a resistant control for both aphids because it is resistant to greenbug (Lazar et al., 1997) and has moderate resistance to Russian wheat aphid, manifested as delayed expression of damage symptoms (T. L. Harvey, personal communication). Separate antibiosis and tolerance experiments were conducted with different sets of plants to determine the effect of each category of resistance on each aphid.

Experiments to measure antibiosis and tolerance to bird cherry oat aphid were conducted at the CRICP in Prague. Genotypes evaluated included *Ae. neglecta* 8052, from a group of *Ae. neglecta* accessions with high levels of cereal aphid resistance (Havlícková & Holubec, 1999) and identified as resistant at KSU, *T. araraticum* 168, the highly susceptible *T. dicoccoides* 62, and the resistant 'Regina' and susceptible 'Zdar' wheat varieties (Havlícková et al., 1996) in antibiosis experiments, and *Ae. neglecta* 8052, *T. araraticum* 168 and *T. dicoccoides* 62 in tolerance experiments.

Antibiosis

Antibiosis was assessed at KSU by determining the intrinsic rate of increase (r_m) of single aphids on each replicate of each genotype, where $r_m = 0.738 (\log_e M_d)/d$; and d = time required for a newly emerged aphid (F_1) to produce its first offspring; M_d = total number of progeny produced by the mother of F_1 (p_1); and 0.738 = mean regression slope of M_d over d for four aphid species (Wyatt & White, 1977). At KSU, two-leaf stage plants were infested with one late instar greenbug or Russian wheat aphid (p_1) per plant. The plastic pot and plant were covered with a plastic cage with a mesh top and two side ventilation holes. When p_1 reproduction began, the first nymph produced (F_1) was moved to a different leaf of the same plant and caged in a drinking straw (Flinn et al., 2001). When aphid F_1 produced its first offspring,

d and M_d were determined. Differences in greenbug and Russian wheat aphid population growth were determined using PROC GLM and PROC MEANS (SAS Institute, 1985). Differences between treatment means were determined using LSD tests at $\alpha = 0.05$.

At CRICP, emerged seeds of tested plants were placed on moistened filter paper in 9 cm Petri dishes and grown at 16 h photoperiod, 24 °C (continuous), 60% RH. Plants with one fully expanded leaf were infested with one first instar larva produced by a female feeding on the relevant accession. Values for d and M_d were calculated as at KSU (see above) to calculate the intrinsic rate of increase (r_m). Each treatment was repeated six times and data were used to calculate values for the mean $r_m$and standard error of the mean.

Tolerance

Tolerance was assessed on entries tested at KSU as leaf chlorophyll loss due to feeding damage caused by each aphid. The relationship between SPAD (chlorophyll) unit values and actual chlorophyll loss is linear over a wide range of percent losses (Deol et al., 1997). In leaf chlorophyll loss experiments, a double-sided adhesive foam leaf cage (Converters, Inc., Huntington Valley, PA) was placed on the top of the middle of three fully expanded leaves and greenbugs or Russian wheat aphids were added (∼20) to cover the caged leaf surface area (0.5 cm diameter). Cages were then covered with a small piece of organdy cloth (2.5 × 2.5 cm) and aphids were allowed to feed for 4 days. Aphids were then removed, and differences in chlorophyll content of infested and non-infested leaf tissue were compared on each leaf. SPAD index values were calculated using the SPAD-502 chlorophyll meter (Minolta Camera Co., Ltd., Japan). Chlorophyll content data were then subjected to the formula: SPAD index = (C-T)/C (Deol et al., 1997), where C = SPAD unit value for control (non-infested) leaf tissue, and T = SPAD unit value for infested leaf tissue. Five representative SPAD unit measurements were taken at each leaf cage site and averaged, yielding a mean cage site SPAD unit measurement. These measurements were used to calculate a mean cage site SPAD index. The three cage site SPAD index measurements were then used to calculate a mean plant SPAD index, and each plant SPAD index value was used to calculate a mean genotype SPAD index. Data were subjected to analysis of variance using SAS GLM Procedure and PROC MEANS (SAS Institute, 1985). Differences between treatments in mean chlorophyll loss were determined using LSD tests at $\alpha = 0.05$. Percent chlorophyll loss values were calculated as [SPAD index values × 100]. Genotypes with a significantly lower chlorophyll loss than 'Thunderbird' were considered tolerant, based on results of Girma et al. (1999) and Flinn et al. (2001).

Tolerance to bird cherry oat aphid was determined at CRICP in plants of *Ae. neglecta* 8052, *T. araraticum* 168 and *T. dicoccoides* 62, grown in a sand: soil: peat (1:1:1) mixture in 15 cm diam pots in a greenhouse (3 plants per pot) at 16 h photoperiod, 24±4 °C (continuous), and 60% RH. At the third leaf stage, 12 plants of each accession were infested with 20 third instar bird cherry oat aphids from a pure line colony maintained on the susceptible winter wheat variety 'Samanta'. Infested plants and 12 uninfested control plants were then covered with nylon bags. Ten days after infestation, the length, fresh weight, dry matter of above ground parts and the root length and root dry matter of infested and un-infested control plants of each accession were determined.

Results

Germplasm evaluations

At KSU, *Ae. tauschii* accession 7096 from CRICP had a significantly lower greenbug feeding damage rating (3.4) than the susceptible control 'Wichita' (5.3) (Table 1), while *Ae. tauschii* accession 4062 was completely susceptible to greenbug feeding (6.0). Several *Aegilops* accessions sustained feeding damage similar to that of the resistant control, *Ae. tauschii* accession 1675 (3.8). These included *Ae. cylindrica* 4052 (4.0), *Ae. tauschii* 7153 (4.0), *Ae. neglecta* 8052 (4.2), and *Ae. markgrafi* 412 (4.2).

Russian wheat aphid feeding damage scores for *Ae. neglecta* 8050 (3.2) and *Ae. neglecta* 8052 (3.6), *Ae. cylindrica* 4060 (3.4), 4058 (3.2) and *Ae. geniculata* 9086 (3.0) were not different from those for the PI 220127 resistant control, but were significantly lower than scores for the susceptible control Wichita and the remaining 15 accessions evaluated (Table 1). Of the Russian wheat aphid resistant lines, all but *Ae. neglecta* 8052 were highly susceptible to greenbug feeding damage. Only *Ae. neglecta* 8052 was subsequently used for resistance category experiments with both of these aphids.

At CRICP, bird cherry oat aphid, the grain aphid,*Sitobion avenae* (F.) and the rose grain aphid,

Table 1. Greenbug and Russian wheat aphid feeding damage to foliage of twenty one *Aegilops* accessions and wheat controls. Manhattan, KS, 1999

Aegilops species	Mean greenbug[1] damage score [2]	Mean Russian wheat aphid damage score[3]
tauschii 7096	3.4 a	5.6 c
tauschii 1675 (greenbug resistant control)	3.8 ab	4.6 d
cylindrica 4052	4.0 abc	9.0 f
tauschii 7153	4.0 abc	9.0 f
markgrafi 412	4.2 abc	9.0 f
neglecta 8052	4.2 abc	3.8 a
cylindrica 4057	4.6 abcd	9.0 f
cylindrica 4061	4.8 abcd	7.0 de
'Thunderbird' (greenbug and Russian wheat aphid susceptible control)	5.0 abcd	–
cylindrica 4060	5.0 abcd	3.4 a
geniculata 9086	5.0 abcd	3.0 a
cylindrica 4051	5.0 bcd	5.0 b
cylindrica 4058	5.2 bcd	3.2 a
tauschii 5162	5.2 bcd	9.0 f
tauschii 5160	5.2 bcd	9.0 f
umbellulata 912	5.3 bcd	9.0 f
'Wichita' (greenbug and Russian wheat aphid susceptible control)	5.3 bcd	6.4 cd
tauschii 7098	5.3 bcd	7.8 e
neglecta 8050	5.4 bcd	3.2 a
tauschii 7149	5.5 bcd	9.0 f
markgrafi 413	5.6 cd	9.0 f
cylindrica 4059	5.6 cd	9.0 f
cylindrica 4062	6.0 d	9.0 f
PI 220127 (Russian wheat aphid resistant control)	6.0 d	3.6 a
LSD (0.05)	1.75	0.89

[1] Biotype I; [2] 1 = no damage, 6 = heavy damage & plant death; [3] 1 = no damage, 9 = heavy damage & plant death.

Metopolophium dirhodum (Wlk.), were the three main aphid species infesting plants of *Aegilops* and *Triticum* accessions in the field evaluations (data not shown). The bird cherry oat aphid occurred in the greatest abundance. Similarly, there was great variation among the 67 accessions assessed for antixenosis and antibiosis in environmental chambers. Bird cherry oat aphid nymphs were found on 42 of the accessions only 7 days after release of alatae. The average infestation over all accessions was 2.8 nymphs per tiller. At the end of the 21 day infestation period, the maximum infestation was 64.8 aphids per tiller on the susceptible control *T. dicoccoides* 62. Five accessions sustained infestation of only one aphid per tiller (data not shown). *T. araraticum* 168 and *T. dicoccoides* 62 were chosen for resistance category experiments. On plants of *T. araraticum* 168, there were 0.29 aphids per tiller on plants in the field, 0 nymphs per plant 7 days post infestation, and 0.6 total aphids per plant 21 days post infestation (0.03 mg dry weight). On plants of *T. dicoccoides* 62, there were 24 aphids per tiller on plants in the field, 7 nymphs per plant 7 days post infestation, and 64.8 total aphids per plant 21 days post infestation (2.8 mg dry weight) (data not shown).

Antibiosis assays

The intrinsic rate of increase (r_m) of greenbugs confined on *Ae. neglecta* accession 8052 (0.106) was significantly lower than that for greenbugs on the susceptible control 'Thunderbird' (0.142) or the test line *T. araraticum* 168 (0.131) (Figure 1A). As expected, there was no significant difference in the rate of increase between greenbugs confined to *Ae. neglecta* 8052 and the resistant control 'TAM 110' (0.126). The lack of differences in r_m between TAM110 and 'Thunderbird' was unexpected, as 'Thunderbird' plants die under high levels of infestation in the greenhouse and

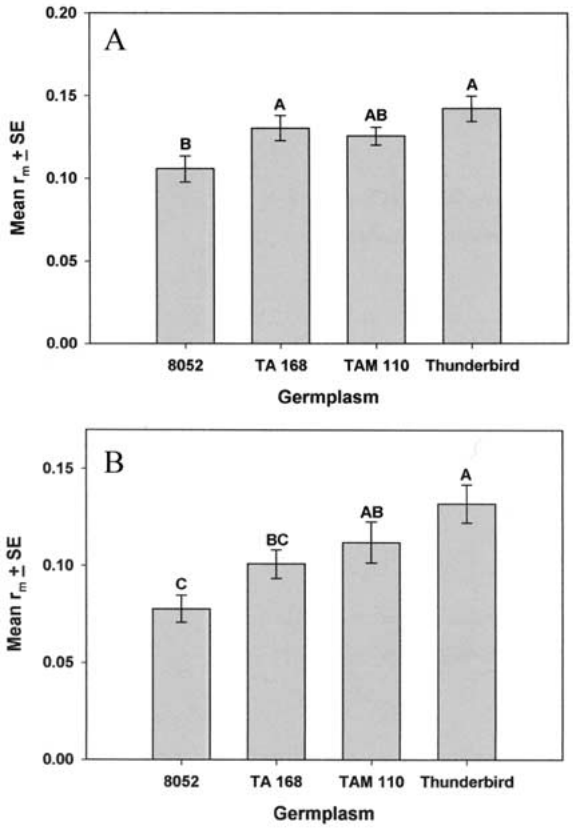

Figure 1. Mean intrinsic rate of increase (r_m) (± standard error) of aphids confined to foliage of *Aegilops neglecta* accession 8052 and *Triticum araraticum* accession 168. (A.) greenbug biotype I, (B.) Russian wheat aphid. Resistant control = 'TAM 110', susceptible control = 'Thunderbird'. ABC – Means followed by the same letter are not significantly different ($p > 0.05$, PROC GLM), LSD = 0.0208 (greenbug), 0.0254 (Russian wheat aphid).

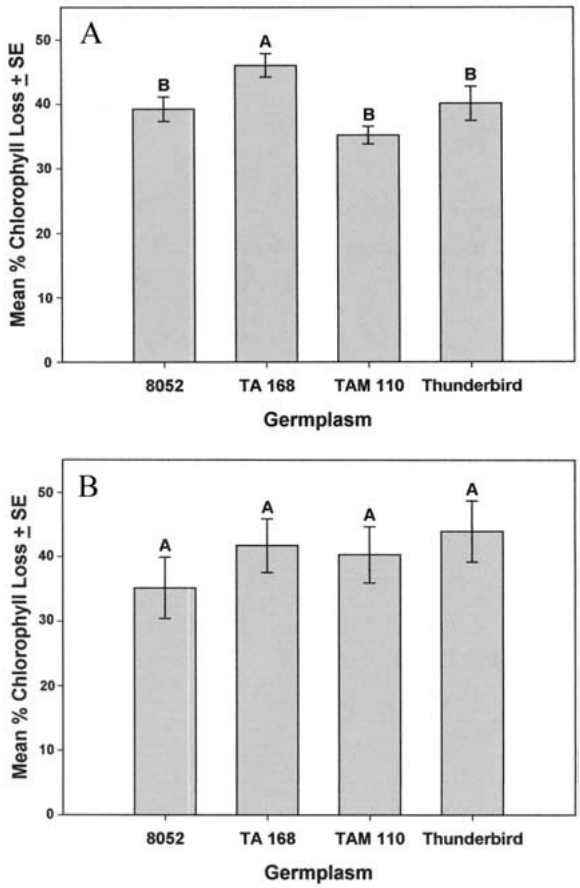

Figure 2. Mean relative per cent chlorophyll loss (± standard error) of foliage from *Aegilops neglecta* accession 8052 and *Triticum araraticum* accession 168 fed upon by caged: (A.) greenbug biotype I, (B.) Russian wheat aphid. Resistant control = 'TAM 110', susceptible control = 'Thunderbird'. ABC – Means followed by the same letter are not significantly different ($p > 0.05$, PROC GLM), LSD = 0.057 (greenbug), 0.1323 (Russian wheat aphid).

weights of greenbugs reared on 'Thunderbird' are significantly greater than those reared on the greenbug resistant germplasm 'Largo' (Smith & Starkey, 2003). Nevertheless, the r_m of greenbugs confined on *Ae. neglecta* accession 8052 was significantly lower than those for greenbugs on 'Thunderbird' or *T. araraticum* 168.

The pattern of results for r_m values of Russian wheat aphids was different and more marked between treatment and control germplasm. Russian wheat aphid r_m values were significantly lower on *Ae. neglecta* 8052 (0.078), than on the resistant 'TAM 110' control (0.112) or the susceptible 'Thunderbird' control (0.132) (Figure 1B). However, the r_m values for Russian wheat aphids on *T. araraticum* 168 (0.101) were not different from those on *Ae. neglecta* 8052 or 'TAM 110' but significantly less than those for 'Thunderbird'. The r_m values of Russian wheat aphids reared on 'TAM110' were numerically less than those reared on 'Thunderbird', but there were no significant differences between the two r_m values.

In general, bird cherry oat aphids produced large numbers of offspring in antibiosis experiments at CRICP (Table 2). When these data were used to compute life tables, the r_m values of aphids on *T. araraticum* 168, *Ae. neglecta* 8052, 'Regina' and 'Zdar' were lower than those on *T. dicoccoides* 62 (Table 2).

Tolerance assays

As with the results of the antibiosis assays, plants of *Ae. neglecta* 8052 were significantly more tolerant to greenbug-related chlorophyll loss (39.3%) than plants

Table 2. Mean intrinsic rate of increase (r_m) ± standard error of the mean (SEM) of bird cherry oat aphid confined to seedlings of three *Triticeae* species, and the wheat cultivars 'Regina' and 'Zdar'. Prague, Czech Republic, 2000

Triticeae species or wheat variety	Mean days to first progeny (d)	Mean total progeny (M_d)	Mean [1] intrinsic rate of increase (r_m) ± SEM
T. araraticum 168	7.0	36.7	0.3707 ± 0.0314
Ae. neglecta 8052	6.9	52.4	0.4241 ± 0.0043
'Regina' (resistant)	6.0	40.2	0.4564 ± 0.0132
'Zdar' (susceptible)	6.0	51.0	0.4817 ± 0.0126
T. dicoccoides 62	6.0	72.7	0.5268 ± 0.0063

[1] Mean of six replications.

of *T. araraticum* 168 (46.0%). However, chlorophyll loss on *Ae. neglecta* 8052 was no different than on 'TAM 110' (35.2%), or 'Thunderbird' (40.1%) (Figure 2A). The latter can be explained by the fact that recent results indicate that 'Thunderbird' possesses a level of tolerance equivalent to 'Largo', as well as TA1675, a highly greenbug tolerant *Ae. tauschii* accession (Flinn et al., 2001; Smith & Starkey, 2003).

There were no differences in chlorophyll loss between test accessions or the controls for Russian wheat aphid feeding tolerance (Figure 2B). Although tolerance to both Russian wheat aphid (Smith et al., 1992) and greenbug (Flinn et al., 2001), has been demonstrated in cultivated wheat, tolerance was not expressed by the *Triticeae* species evaluated in the present study. The chlorotic streaks produced by plants susceptible to Russian wheat aphid are very different from the general leaf chlorosis produced by greenbug feeding. The difference in these two types of plant tissue damage expression may be such that SPAD meter measurements do not detect differences in wheat tolerance to Russian wheat aphid.

Bird cherry oat aphid feeding on *Ae. neglecta* 8052, *T. dicoccoides* 62, and *T. araraticum* 168 plants increased leaf length but decreased the fresh and dry weights of all plant parts (data not shown). The retarded development of shoots and roots of infested plants indicated that bird cherry oat aphid feeding affected the growth of all accessions. However, root dry matter was reduced less on *T. araraticum* 168 (9.1%) than on *T. dicoccoides* 62 or *Ae. neglecta* 8052 (17.0%). The above-ground dry matter of *T. araraticum* 168 was also reduced less (7.3%) than *T. dicoccoides* 62 (9.0%) or *Ae. neglecta* 8052 (8.8%). Limited amounts of seed of all the accessions evaluated did not allow determination of statistically significant levels of tolerance.

Additional experiments will be necessary to determine if the lower reduction of dry matter reflects tolerance in *T. araraticum* 168 to bird cherry oat aphid.

Discussion

The reduced chlorosis caused by Russian wheat aphid and greenbug on *Ae. neglecta* 8052 resulted from antibiotic factor(s) in this germplasm to both aphids. The intrinsic rate of increase of each aphid was significantly reduced when they were confined to the foliage of *Ae. neglecta* 8052. In comparison, the rate of increase of both aphids on the susceptible control 'Thunderbird' was significantly greater. These results are similar to those of Havlícková & Holubec (1995) who observed very low or no population development of bird cherry oat aphid in field plantings of several *Ae. neglecta* accessions in the Czech Republic. In general, the population rate of increase of greenbugs and Russian wheat aphids in our experiments was much lower than the rate of increase of bird cherry oat aphid populations on several wheat cultivars (Havlícková, 1996). The trend in susceptibility of *T. dicoccoides* 62 and resistance of *Ae. neglecta* 8052 and *T. araraticum* 168 in the present study are in accordance with results of Havlícková & Holubec (1999) who demonstrated a greater susceptibility of ancestral wheat to bird cherry oat aphid than to improved wheat cultivas.

The effect of *T. araraticum* 168 on aphid growth differed between aphid species. In general, *T. araraticum* 168 had a greater negative effect on bird cherry oat aphids and Russian wheat aphids than on greenbugs. The rate of increase of Russian wheat aphids confined to *T. araraticum* 168 was equivalent to that of those confined to the resistant control 'TAM 110', and was significantly lower than that of Russian wheat aphids feeding on the susceptible control

'Thunderbird' (Figure 1B). However, *T. araraticum* 168 had no detrimental effect on greenbug development (Figure 1A). Bird cherry oat aphids reared on *T. araraticum* 168 also appeared to exhibit antibiotic effects, as they produced fewer nymphs than aphids reared on the susceptible control *T. dicoccoides* 62, *Ae. neglecta* 8052 or the cultivars 'Regina' and 'Zdar' (Table 2). Deol et al. (1995) noted significant reductions in Russian wheat aphid-induced leaf rolling and leaf chlorosis among several *T. araraticum* accessions from Iran.

In the experiments we conducted, tolerance was not expressed as a functional category of resistance to either the Russian wheat aphid in either *Ae. neglecta* 8052 or *T. araraticum* 168. Tolerance to greenbug was noted in *Ae. neglecta* 8052 at a level comparable to the tolerance in both 'TAM110' and 'Thunderbird'. To determine the accuracy of the tolerance data in the present study, we compared the SPAD chlorophyll loss indices of 'Thunderbird' to those in previous experiments with other sources of greenbug resistance (Flinn et al., 2001). The SPAD indices for 'Thunderbird' exposed to Russian wheat aphids (0.440) and greenbugs (0.400) in the present study were comparable to the SPAD index of 'Thunderbird' exposed to greenbugs in experiments conducted by Flinn et al. (2001) (0.383). The increased level of tolerance to bird cherry oat aphids in *T. araraticum* 168 is not unusual, as moderate levels of tolerance to bird cherry oat aphid have been detected in several winter wheat cultivars in the Czech Republic (Havlícková, 1997). Tolerance is an important trait for breeding resistance in wheat to aphids, as well as arthropod pests in general (Smith et al., 1999).

It is possible that antixenotic (aphid nonpreference) factors in *Ae. neglecta* 8052 and *T. araraticum* 168 also contributed to the results of the present study, as well as the field screening results of Havlícková & Holubec (1995). However, antixenosis is much less useful in crop monoculture, and as such, was not considered a viable category of resistance for investigation.

The *Ae. neglecta* and *T. araraticum* resistance identified in this research represent potential new sources of genes for antibiosis resistance to three important aphid pests of cereals. These sources can now be used to develop multiple aphid resistant cereal germplasm for use in the midwestern U.S. and Central Europe.

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