THE EFFECT OF THE A GENOME SPECIES (*Triticum monococcum* and *Triticum boeoticum*) ON THE FECUNDITY AND BEHAVIOUR OF *RHOPALOSIPHUM PADI* – BIRD CHERRY-OAT APHID

Henriett Elek [1*], Janet Martin [2], Shakoor Ahmad [2], Peter Werner [1], Angela Anda [3], John Pickett [2] and Lesley Smart[2]

[1]KWS UK Limited, Thriplow, UK
[2]Rothamsted Research, Harpenden, UK
[3]University of Pannonia Georgikon Faculty, Keszthely, Hungary
*henriett.elek@kws-uk.com

Abstract

Triticum monococcum is an A genome diploid species that is closely related to and cross-fertile with *T. uratu* which is now accepted as the donor of the A genome in the hexaploid bread wheat (*T. aestivum*). Ancestral A genome species present good potential sources for further crop improvement through synthetic polyploidisation and introgression into modern wheat cultivars. In this study we examined the antibiotic and antixenotic effect of different A genome diploid species and accessions on the aphid *Rhopalosiphum padi*. In choice tests most of the lines were less attractive to *R.padi* and showed reduction in aphid weight gain and fecundity compared to the hexaploid control. We found through HPLC studies that seedling leaf tissue of the A genome species *T. monococcum* and *T. boeoticum* did not contain the hydroxamic acids found in tetraploid and hexaploid wheats, but did produce two compounds present in the same retention range. Increased

production of both unknown compounds was recorded in the later seedling growth stage, which may have an effect on aphid development, but not as strongly as we have previously seen in the presence of high amount of hydroxamic acids in the B genome species *Aegilops speltoides*.

Keywords: *Triticum monociccum*, *Triticum boeoticum*, *Triticum aestivum*, *Rhopalosiphum padi*, aphid behaviour, aphid fecundity

Összefoglalás

Triticum monococcum az első emberek által termesztett búzafaj, amelynek vad alakja a *Triticum boeoticum*. A *T. monococcum* közeli rokonságban áll a modern hexaploid búza A genom donorjával, a *Triticum urartu*-val. A diploid fajok sok esetben a rezisztencia kutatások tárgyát képezik, mivel kitűnő forrásanyagot biztosítanak a modern búza fejlesztéséhez szintetikus poliploidizáción és introgresszión keresztül.

A fentiek keretében tanulmányoztuk a *Triticum monococcum* és *Triticum boeoticum* fajok néhány képviselőjének antibiotikus és antixenotikus hatását a *Rhopalosiphum padi* levéltetvekre.

Vizsgálataink során megállapítottuk, hogy a diploid fajták kevésbé atraktívak a táplálékválasztási tesztben, az utódszám csökkent és a súlygyarapodás is lassult a hexaploid kontrollon táplálkozó levéltetvekhez képest. HPLC-vel történő vizsgálat során megállapítotuk, hogy a diploid fajták nem tartalmaznak hidroxámsavakat, amiket előzőleg a tetraploid és hexaploid búzafajták levélmintáiban megtaláltunk. A hidroxámsavakkal azonos retenciós intervallumban két ismeretlen vegyület tűnt fel amelyek termelése a növekedési stádium előrehaladtával fokozódott. Vizsgálataink alapján arra következtethetünk, hogy ezek a vegyületek hatással lehetnek a levéltetvek viselkedésére, de mégsem olyan hatékonyak, mint előzőleg a magas hidroxámsav tartalmú B genomú *Aegilops speltoides* esetében voltak.

Introduction

Modern wheat belongs to two species the hexaploid *Triticum aestivum* (2n=42 chromosomes) and the tetraploid *T. turgidum* (2n=28 chromosomes). Polyploid wheat was developed under the influences of ancient human cultivation through amphiploidy between diploid *Triticum* species and *Aegilops* species (Nevo et al,. 2002).

Einkorn wheat incorporates two related A genome species *Triticum monococcum* and *Triticum urartu*. By the early Bronze Age cultivation of tetraploid (AABB) emmer wheat in and around the Fertile Crescent had enabled agricultural societies to thrive (Zohary and Hopf, 1993). The cultivated tetraploid emmer wheat was probably developed from wild emmer wheat *T. turgidum ssp. dicoccoides,* which itself is a result of spontaneous but rare hybridisation between *T. uratu* (AA) and the B genome species *Aegilops speltoides* (Petersen et al., 2006). Although repeated and independent allopolyploidisation events may have occurred this process of speciation inevitably creates a severe evolutionary bottleneck. Modern bread wheat *T. aestivum* (AABBDD) has no wild representatives and certainly arose through a further incident(s) of amphidiploidisation under the influences of human cultivation. Throughout the history of human cultivation of wheat the crop gene pool has therefore always been relatively low in genetic diversity.

CIMMYT in particular has shown the value of resynthesising polyploid wheat in order to introduce greater genetic diversity from representatives of the ancestral diploid species. Most effort has been devoted to the more recent D genome progenitor *Ae. tauschii*. Recent strides in genetic marker technologies offer the potential for faster, targeted introgression of superior, but rare alleles from alien species and have encouraged a renewed interest in alternative compatible

sources in the A and B genomes. For further crop improvement by traditional breeding a wide range of A and B genome wild relatives exist that are cross compatible and could be used for introgression (Nevo et al., 2002).

Aphids, for example the bird cherry-oat aphid, *Rhopalosiphum padi*, are common pests in cereals and are able to cause serious wheat yield losses by direct feeding damage and by transferring plant pathogenic viruses such as barley yellow dwarf virus (BYDV) (Hand, 1989, Thackray et al., 2009). In mild winters in temperate regions the damage could increase under the influences of climate change if aphids are able to continue feeding and reproducing anholocyclically in the wheat crops (Leather, 1993) and therefore increase the risk of secondary spread of virus infection.

The alatae are responsible for the selection of the suitable host plants. The host-selection behaviour is affected by attraction to non-specific visual stimuli, the colour or the form of the host plant (Powell and Hardie, 2001) and plant volatiles, which are detected by antennal olfactory sensillae (Powell et al., 2006).Secondary metabolites can affect aphid behaviour and play an important role in insect resistance. For this reason in our previous work (unpublished data) we concentrated on the hydroxamic acids (HA), which are known as potential aphid resistance factors from the studies of Nicol et al (1992) and Givovich and Niemeyer (1991). However, our results did not support the hypothesis that HAs have antixenotic and antibiotic effects on *R. padi* for the hexaploid and tetraploid varieties tested.

In this study we investigated the antibiotic and antixenotic effects of different A genome diploid varieties on the behaviour and development of *R. padi*. The plants were also subjected to HPLC analysis to confirm the presence or absence of HAs in the leaf tissue.

Methods and material

Aphids

Rhopalosiphum padi was collected from volunteer wheat plants from the field in Thriplow, Herts, UK in August 2006 in September in 2007 and again (refreshing the colony) in 2008. The colony used in this study was established from one aphid using the mildew resistant spring wheat variety Tybalt as the culture plant. The colony was kept in a glasshouse in a temperature range of 12-25°C and light 16:8 L:D.

Plant material

Several accessions of the diploid species *Triticum monococcum* (MDR 002, MDR 037, MDR 043, MDR 044, MDR 049, MDR 050) and *T. boeoticum* (102, 8116, 8150, 8404) were provided by Rothamsted Research (RRes). For the HPLC analysis, the replicated experiment was set up in RRes in a controlled environment room at 20°C+/- 2°C, 16:8 L:D. Plants were grown in vermiculite.

For the fecundity and settling test plants were grown in a glasshouse (16:8 L:D, ≈ 20°C), in compost.

Fecundity test

This test was used to determine the intrinsic rate of population increase by recording how long it takes an aphid from birth to produce the first nymph and how many nymphs were produced over an equivalent time on the test varieties.

Seven alatae were put in a cage with one plant of the test variety for 24 hours to produce pre-conditioned nymphs for the experiment after which the alatae were removed. Nymphs were allowed to develop on those plants for 3-4 days until they reached a reasonable size making them easier to transfer onto the 7 day old test plants. One experimental

3 day old nymph was placed on the middle part of the first leaf in a 2cm diameter clip cage. The developing aphids were monitored at the same time each day. From the first day of nymph production the new nymphs were removed and their numbers recorded daily. The experiment was carried out in a glasshouse at ≈20°C and 16:8 L:D.

From the data the intrinsic rate of population increase (r_m) was calculated using the formula by Wyatt and White (1977). Data were subjected to ANOVA and Student's t test (Microsoft Office Excel).

$$r_m = c (\log_e Md) / d$$

Where c is a constant = 0.74, d = pre-productive period (days) and Md = number of nymphs produced in the reproductive period equal to d

Settling test

Twenty alate *R. padi* were given a choice between a 7 day old seedling of the test plant and a 7 day old control plant, which was the hexaploid variety Solstice. Each cylindrical choice cage (12 cm diameter by 20 cm high) was made of clear acetate sheet and ventilated at the top. Each choice cage was replicated between 6 and 20 times depending upon the variety under test. To keep the humidity high, the experiment was set up on a wet sand tray. Alatae, which had settled on each of the two plants, were counted and recorded after 2, 5 and 24 hours from the beginning of the experiment. The test was conducted in the glasshouse at 20°C, 16:8 L:D. Data were analysed by Student's t test (Microsoft Office Excel).

Weight development and survival studies

Five apterous *R. padi* were put in a clip cage on the first leaf of replicated Solstice, MDR 037, and MDR 049 7-day old seedlings. After

24h, the adults were removed and the nymphs produced were counted. Numbers were reduced to about 10/cage to prevent overcrowding, and the nymphs were left in the clip cages to develop. After 7 days nymphs were recounted and weighed in their batches. Survival rates and average nymph weights were calculated and compared. A mean relative growth rate could not be calculated since birth weights were not taken due to the high mortality incurred during the weighing process in a preliminary trial. Data were analysed by Student's t test (Microsoft Office Excel).

Sample analysis by high pressure liquid chromatography (HPLC)

The leaf tissue of two varieties, MDR 037 and MDR 049, was sampled for the HPLC analysis at the 7, 19 and 28 day growth stage. The middle area was removed from each leaf, weighed into an Eppendorf tube containing three metal beads, which break the tissue during grinding. The Eppendorf tube was frozen in liquid nitrogen and stored on -80 °C until processing. The tissue was ground using a Qiagen tissuelyser for two minutes on 30/s frequency. This equipment enabled 48 samples to be ground at the same time. Before the samples were processed, the plates of the tissuelyser were frozen in liquid nitrogen to ensure that the samples remained frozen during grinding. The buffer, 0.5ml of a methanol 98% and acetic acid 2% mixture, was added to the sample after grinding. It was then sonicated for 10 minutes and centrifuged for 10 minutes at 16000rpm at 4 °C. Supernatant was transferred into a glass vial and analysed by HPLC. The HPLC method was adapted from Baumeler *et al*., (2000). A thermal hypersil C-18 5µ, 250 x 4.6mm column was used, mobile phase (A) HPLC grade water (B) methanol/isopropanol (95/5) + 0.025% acetic acid. The gradient profile of solvent A and B was 0-2 min 10% B; 2-11min 10-50% B; 11-16min 50% B; 16-17min 50 to 10% B. Injection volume 20µl, the flow rate was 1ml/min and the run time 17 minutes.

HA levels were compared and analysed by Student's t test (Microsoft Office Excel).

Results

Settlement choice assay

In the settling test the antixenotic effect of the diploid varieties was tested (Figure 1). Accessions of both *T. monococcum* (MDR 002, MDR 043, MDR 044, MDR 050) and *T. boeoticum* (8404, 8116, 8150) showed reduced attraction for *R. padi* alatae, and for 8404 and MDR 043 the differences were significant (P<0.02). However, the other *T. monococcum* varieties MDR 040 (*P*=0.004) and MDR 037 (*P*=0.0002) had three times more alatae settled on the plants compared to the Solstice control.

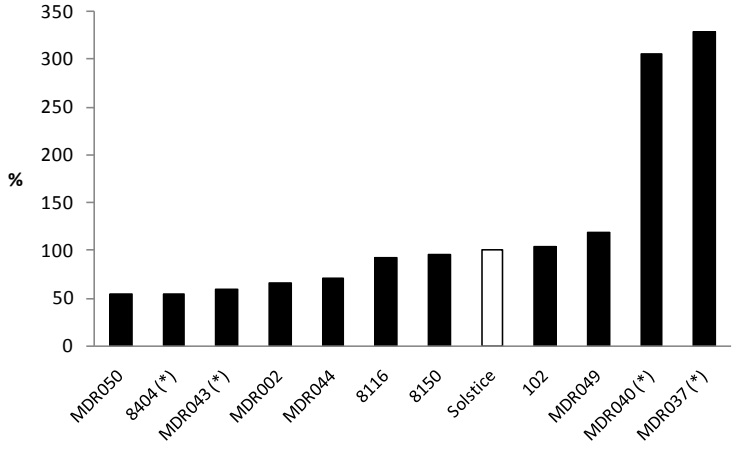

Figure 1. Settlement of *R. padi* on the diploid varieties as a percentage of settlement on Solstice (hexaploid control), which =100%* significantly different to the Solstice control

No choice development assay

The intrinsic rate of population increase (r_m) study showed differences in aphid development rate and fecundity between the varieties (Figure 2). The r_m was reduced on all of the diploid accessions (4-15% lower) compared to the Solstice control. On most of the varieties (MDR 044, MDR 049, 8116, 8150 and 8404) nymph production was significantly reduced (P<0.02) compared to Solstice. On MDR 043, MDR 050, 102, where there were fewer replicates, the differences were not significant (P>0.05).

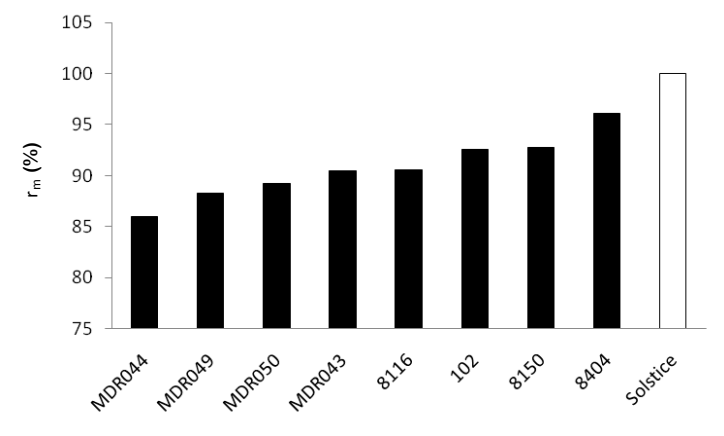

Figure 2. Intrinsic rate of population increase (rm) of *R. padi* on diploid varieties as a percentage of the rm on the hexaploid control (Solstice), which = 100%.

The *R. padi* nymphs on the diploid varieties took 9-10 days to produce the first nymphs. However; nymphs feeding on the hexaploid variety only took 7-9 days from birth. This slower maturation was also reflected in the aphid size. On the diploid varieties aphids were smaller and produced fewer offspring than on the hexaploid variety. The average daily nymph production was 5.9 on Solstice and 4.4 on the diploid varieties.

These results indicate that the diploid varieties may contain attributes that could be important in the resistance breeding against aphids in the future.

Survival and weight development studies

The survival rate of *R. padi* was studied on two *T. monococcum* lines (MDR 037, selected because of the high preference in the settling test and MDR 049 which was selected because it had a reduced r_m value in the fecundity test) and the reference *T. aestivum* (Solstice) cultivar. Differences were noticeable between the species. After 7 days feeding on the hexaploid variety, significantly more nymphs survived (99.4%) than on the two diploid varieties ($P<0.05$) (Figure 3).

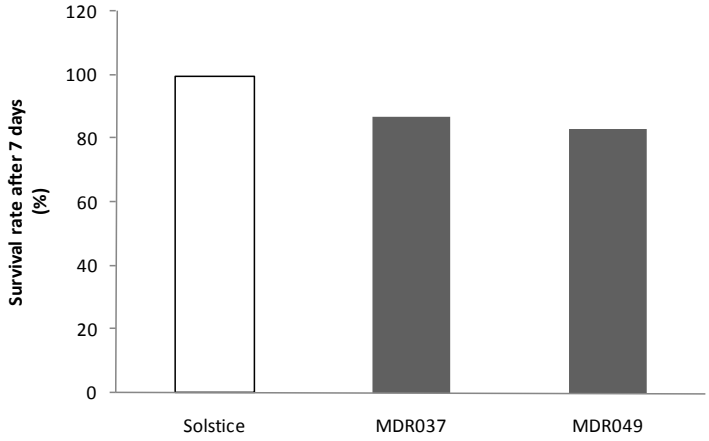

Figure 3. The survival rate of *R. padi* after 7 days on two diploid varieties compared to the hexaploid control (Solstice)

On the hexaploid variety, nymphs gained weight faster than on the diploid varieties (Figure 4). On line MDR 037 nymphs showed significantly lower survival rates, but the weight gain was only slightly reduced compared to aphids feeding on the hexaploid variety. For MDR

049, which was less attractive in the settling test and showed reduced intrinsic rate of development and survival rate, the weight gain was very significantly lower than on the hexaploid control ($P<0.001$).

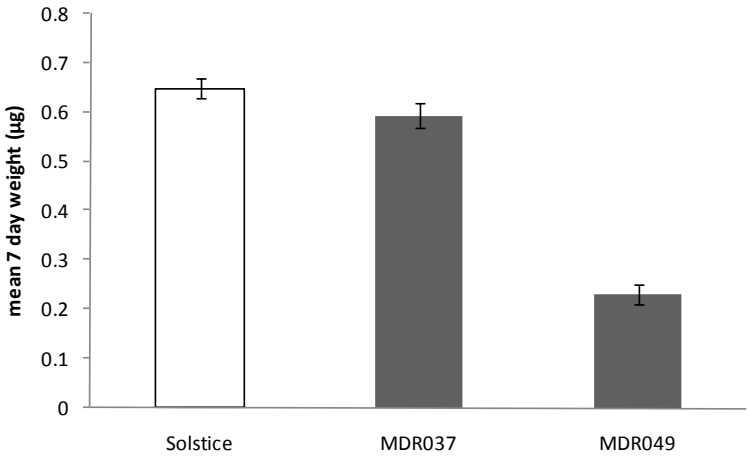

Figure 4. Weight gain of *R. padi* after 7 days on the hexaploid control and two diploid varieties

Result of HPLC analysis

In the previous experiments (Figures 1-4) *R. padi* reproduction and settlement was reduced on the diploid varieties compared to the control. Analysis of the leaf tissue showed that the A genome diploid varieties did not contain known HA related compounds, but two unknown peaks appeared on the HPLC trace at 13.4 (compound I.) and 13.8 minutes (compound II.) which will be investigated further (Figure 5).

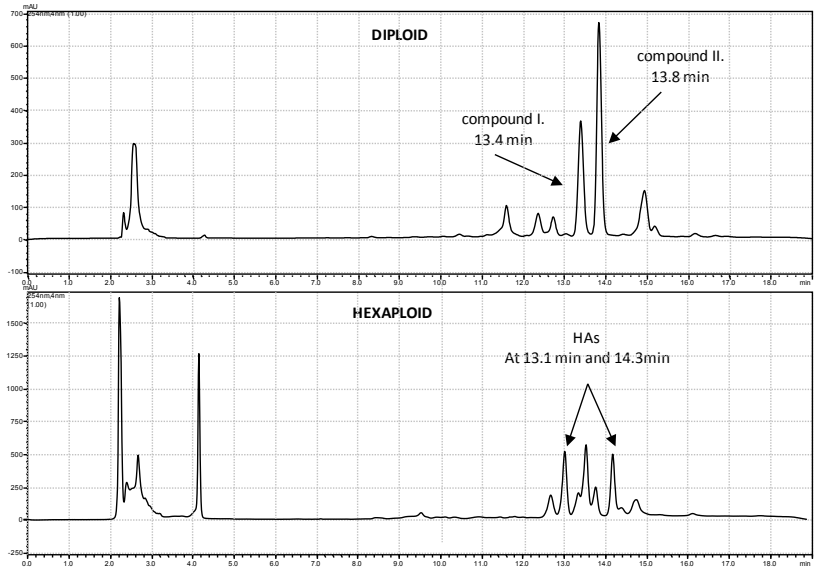

Figure 5. HPLC traces of leaf tissue extracts of an A genome diploid (*T. monococcum* MDR049) and a hexaploid variety (Tybalt). The relative quantities of the peaks are not directly comparable because the sample sizes are not identical.

Leaf samples were taken from two *T. monococcum* varieties MDR 049 and MDR 037 at different growth stages to follow the changes in the levels of the unknown compounds during the early growth of the plants.

Results for compound I. suggest a significant difference between the two lines in the pattern of production. In the case of MDR 037 the compound is absent from the very young leaf, builds to a concentration of 8.0E+05 as the leaf matures and then remains constant in the maturing leaf. Each successive leaf gives similar expression levels. MDR 049 shows a continuing build up of the compound as the leaf ages leading to significantly higher concentrations than for MDR 037 in the later stages of the leaf. Furthermore the 2nd leaf shows significantly higher levels of the compound than the 1st leaf.

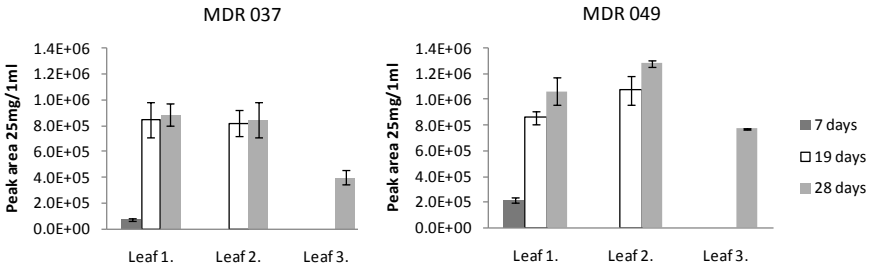

Figure 6. Concentration of compound I. (HPLC retention time 13.4 minutes) in leaf tissue sampled from the first 3 seedling leaves of *T. monococcum* lines after 7, 9 and 28 days. Leaf 2 appeared between days 7 and 19 and leaf 3 between days 19 and 28.

The second unknown peak (HPLC retention time 13.8 minutes), showed a different pattern of production to the other compound. For this compound, the two lines give similar results although MDR 037 may be producing a slightly higher concentration than MDR 049. In each case concentrations increase in subsequent leaves and do not build with time within a leaf suggesting that production of this compound occurs during the early genesis of each leaf.

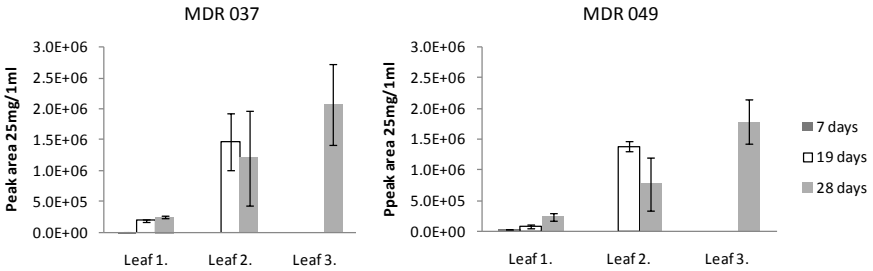

Figure 7. Concentration of compound II. (HPLC retention time 13.8 minutes) in leaf tissue sampled from the first 3 seedling leaves of *T. monococcum* lines after 7, 9 and 28 days. Leaf 2 appeared between days 7 and 19 and leaf 3 between days 19 and 28.

Discussion

In our previous work we have investigated the effect on *Rhopalosiphum padi* of the hydroxamic acids (HAs) in the hexaploid, tetraploid and the B genome diploid species (manuscripts in preparation/ submitted). The importance of this group of secondary metabolites was highlighted in one of the B genome species where a high concentration of HAs, observed in the leaf tissue, had a significant effect on the development of *R. padi*. The defence mechanism in the B genome species could be an option to improve the insect resistance in modern hexaploid wheat.

In the current work, good evidence for reduced fecundity and aphid weight gain has been demonstrated on A genome species, with no corresponding link with increased levels of the known HAs. Indeed, the HPLC analyses failed to find any known HAs. Similar studies by Nomura et al. (2007), found no HAs in *T. boeoticum*.

Of the two novel compounds, the expression pattern of compound II. is similar between the two lines tested and would suggest that this is not responsible for any differences in aphid growth between the lines MDR 037 and MDR 049. Compound I. on the other hand is expressed differently between the lines, with the more aphid resistant line MDR 049 showing higher concentrations in progressively older leaves. Furthermore compound II builds continuously in the leaf. Unfortunately it is not possible to take the conclusion any further than to note the existence of these unidentified compounds and to look to future work to determine if either may have a role as a protective mechanism against aphids.

These results do identify potentially useful A genome accessions that show some degree of resistance to aphid development. The settlement test is based on physical and chemical differences between the

test plant and the control plant (the hexaploid wheat variety Solstice), providing the aphids with a simple choice. Most of the A genome varieties tested were less attractive to *R. padi* alatae. In particular, significantly fewer aphids settled on line 8404 (*T. boeoticum*) and MDR043 (*T. monococcum*) than on the control. MDR040 and MDR037 were highly attractive to *R. padi* and 2.5-3 times more aphids settled on those varieties than on Solstice. For this reason they were not selected for the intrinsic rate of population increase assays.

The intrinsic rate of population increase values for *R. padi* on the A genome varieties were 4-15% lower than on the hexaploid control. On the diploid varieties, the nymph development and maturation was slower; the average daily nymph production on the diploid varieties was 4.4 and on the hexaploid variety 5.2.

The negative effect of some of the diploids on *R. padi* was also demonstrated in the survival and the weight gain studies on lines MDR037 and MDR049. On the diploids, 13-16% fewer aphids survived, which was significantly lower than on the hexaploid control. The weight gain study also provided very interesting results. Weight gain on MDR 037, which was highly attractive in the settlement choice test, was not significantly different from Solstice, and the aphids developed on that diploid variety just as well as on Solstice. On the other hand, aphid settlement on MDR 049 was similar to the control, but the offspring production was significantly reduced in the intrinsic rate of population increase assays. This observation was supported by the weight gain study where aphid development was significantly reduced compared to the hexaploid control.

Although we found a reduction in fecundity and development on some of the diploid lines, it was not as effective as we had seen previously on B genome species *Aegilops speltoides*. The absence of the HAs in *T. monococcum* indicates that the negative effects on the aphid

are caused by a different defence mechanism, which could be combined with the mechanism found in *Ae. speltoides* to provide a pre breeding objective, which could be tested using a *de novo* resynthesis of the allotetraploid (AABB).

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