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# Resistance to Wheat Curl Mite in Arthropod-Resistant Rye-Wheat Translocation Lines

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**Abstract:** The wheat curl mite, *Aceria toschiella* (Keifer), and a complex of viruses vectored by *A. toschiella* substantially reduce wheat yields in every wheat-producing continent in the world. The development of *A. toschiella*-resistant wheat cultivars is a proven economically and ecologically viable method of controlling this pest. This study assessed *A. toschiella* resistance in wheat genotypes containing the *H13*, *H21*, *H25*, *H26*, *H18* and *Hdic* genes for resistance to the Hessian fly, *Mayetiola destructor* (Say) and in 94M370 wheat, which contains the *Dn7* gene for resistance to the Russian wheat aphid, *Diuraphis noxia* (Kurdjumov). *A. toschiella* populations produced on plants containing *Dn7* and *H21* were significantly lower than those on plants of the susceptible control and no different than those on the resistant control. *Dn7* resistance to *D. noxia* and *H21* resistance to *M. destructor* resulted from translocations of chromatin from rye into wheat (*H21*—2BS/2RL, *Dn7*—1BL/1RS). These results provide new wheat pest management information, indicating that *Dn7* and *H21* constitute resources that can be used to reduce yield losses caused by *A. toschiella*, *M. destructor*, *D. noxia*, and wheat streak mosaic virus infection by transferring multi-pest resistance to single sources of germplasm.

**Keywords:** wheat; *Mayetiola destructor*; *Diuraphis noxia*; resistance genes; antibiosis

## 1. Introduction

Wheat serves as a staple food vital nutritional source for one-third of the world's population [1], yet continues to suffer grain yield reductions of ~20% per year from arthropods and arthropod-vectored viruses [2]. The majority of these losses result from feeding damage by Hessian fly, *Mayetiola destructor*; Russian wheat aphid, *Diuraphis noxia* (Kurdjumov); and wheat curl mite, *Aceria toschiella* (Keifer) [3]; as well as from *A. toschiella* vectored transmission of Wheat streak mosaic virus [4], Wheat mosaic virus and Triticum mosaic virus [5–7].

Plant resistance to arthropods is a widely accepted method to manage pest populations and decrease wheat yield losses in a cost-effective and ecologically friendly manner [8]. Interactions between resistant plants and avirulent arthropods involve incompatible arthropod—plant interactions mediated by constitutively produced and arthropod-induced plant defense proteins synthesized by resistance gene products [9]. Several genes from barley, rye, wheat and wild wheat relatives provide functional resistance against *A. toschiella*, *D. noxia* or *M. destructor* [10–17]. However, the evolution

of virulence in each pest causes continual delays in the development of additional new resistant cultivars [18,19].

The *Cmc1* and *Cmc4* genes for resistance to *A. toschiella* were transferred to bread wheat from goatgrass, *Aegilops tauschii*, (Coss.) Schmal, the D genome donor of bread wheat [20,21] and *Cmc2* was transferred from *Agropyron elongatum* (Host.) Beauv. [22]. *Cmc3* [21] originated from the translocation of a segment of the short arm of rye chromosome 1 (1RS) onto the long arm of wheat chromosome 1A, resulting in 1AL/1RS translocation line used to create the wheat cultivar Amigo [23] which is also contains the *Gb2* and *Gb6* genes for resistance to the greenbug, *Schizaphis graminum* Rondani [24]. An *A. toschiella* virulent biotype rendered *Cmc3* ineffective within 5 years [18].

Over thirty genes from wheat and its relatives convey *M. destructor* resistance [17]. However, only plants containing *H13*, *H18*, *H21*, *H25*, *H26*, and *Hdic* are consistently effective against *M. destructor* populations in the Great Plains wheat production area of Texas, Oklahoma, and Kansas [25]. Resistance in *H13* and *H26* was derived from *Ae. tauschii* [26], in *H18* from durum wheat, *Triticum turgidum* L. var. *durum* [27], and in *Hdic* was transferred from Spelt wheat, *T. turgidum* ssp. *dicoccum* [16].

In contrast, resistance in *H21* and *H25* was derived from rye, *Secale cereale* L. [28,29]. *H21* in the winter wheat cultivar Hamlet resulted from the translocation of chromatin from the distal 20% segment of the long arm of rye chromosome 2 (2RL) onto the short arm of wheat B genome chromosome 2 (2BS), resulting in a 2BS/2RL translocation line [28,30]. The development of cultivars with *H25* involved the transfer of genetic material from the long arm of rye chromosome (6RL) to the long arm of either chromosome 4 of the wheat A genome, resulting in a 4AL/6RL translocation line, or chromosome 4 of the wheat B genome, resulting in either 4BL/6RL or 6BL/6RL translocation lines [23]. Chen et al. [31] used the breeding line KS92-WGRC20, which carries the 4AL/6RL translocation, to develop Cataldo spring wheat. Marais et al. [32] determined that the *Dn7* *D. noxia* resistance gene from rye was transferred into wheat from the long arm of rye chromosome 1 to form a 1BL/1RS translocation. *Dn7* in the cultivar 94M370 provides resistance against all U.S. and South African *D. noxia* biotypes [33–35].

Given the evidence of rye-based resistance in cultivars containing *H21*, *H25* and *Dn7*, we hypothesized was that rye genetic material may also confer *A. toschiella* resistance. Such resistance could provide opportunities to develop new cultivars with broad-based resistance to wheat arthropod pests. The objective of this study was to determine whether *A. toschiella* resistance exists in wheat cultivars carrying *Dn7*, *H13*, *H18*, *H21*, *H25*, *H26*, or *Hdic*.

## 2. Materials and Methods

### 2.1. Plant Materials

The *H13*, *H18*, *H21*, *H25*, *H26*, and *Hdic* *M. destructor* resistance genes and the *Dn7* *D. noxia* resistance were evaluated for *A. toschiella* resistance, using the *A. toschiella*-resistant control OK05312 that contains the *Cmc4* resistance gene, and susceptible controls 'Jagger' and 'Ike'. The USDA/ARS Plant Science Laboratory at Kansas State University provided seed of cultivars or breeding lines containing *H* genes. Seed of the wheat cultivars 94M370 containing the *Dn7* gene for *D. noxia* resistance, OK05312, and susceptible Ike and susceptible Jagger were obtained from the USDA/ARS Small Grains Repository, Aberdeen, ID; Dr. Brett Carver, Oklahoma State University; and the Kansas Crop Improvement Association, respectively. Plants in Experiment I were grown in pots containing Sungrow Metro-Mix 350 (Sun Gro Horticulture, Agawan, MA, USA) and plants in Experiments II and III were grown in pots containing Pro-Mix 'BX' (Premier ProMix, Lansing, MI, USA). All plants were fertilized once with 20-20-20 (N-P-K) and grown at 22 ± 2 °C, 40–50% relative humidity, and a photoperiod of 14:10 (light:dark) hours, which are optimum conditions for plant and mite growth and development [12,36,37]. Experiments I and III were conducted in a greenhouse and Experiment II was conducted in a growth chamber.

## 2.2. Biotype Origin and Verification

Experiment I used biotype 1 adults from a colony derived from a field collection in Hays, KS (voucher specimen no. 215, Kansas State University Museum of Entomological and Prairie Arthropod Research). Experiments II and III used biotype 1 originating from a field collection in Hughes County South Dakota supplied courtesy of Dr. Ada Szczepaniec, South Dakota State University, and biotype 2 originating from a field collection in Cheyenne County Nebraska. Both biotypes were collected in 2014. Prior to each experiment, the identity of each biotype was verified by DNA sequencing with an ITS1 marker developed by Reference [38].

## 2.3. Experiment I. Response of *A. toshiella* to *M. destructor* Resistance Genes in No-Choice Tests

The reaction of plants containing *H13*, *H18*, *H21*, *H25*, *H26* or *Hdic* were compared to susceptible Ike and resistant OK05312 controls (Table 1) for *A. toshiella* susceptibility. Seeds were sown in  $6 \times 6 \times 5.5$  cm plastic pots. At the two-leaf stage, 10 pairs of plants of each genotype of similar height were selected to test for antibiosis and tolerance to *A. toshiella*. One plant of each pair was infested with a leaf piece containing ~30 *A. toshiella* biotype 1 adults. The second plant of each pair served as an un-infested control. The infested plant of each genotype was randomly placed in each of 10 cages covered with  $36 \mu$  mite-proof screen. Plants were arranged at random in each cage and separated to prevent plant-to-plant contact. Un-infested plants were similarly placed in 10 additional mite-proof cages. The experiment was arranged in a randomized complete block design with 10 replicates (cages), where cages were the blocking factor.

At 14 days post-infestation, plant height was measured in infested and un-infested plants and the presence or absence of leaf folding was determined in infested plants. All plants were then cut at the soil level, and leaves of un-infested plants were placed in individual aluminum foil pouches and dried at  $60^\circ\text{C}$  for 12 days. Leaves of infested plants were individually placed on each of two  $3.9 \times 7.5$  cm sheets of sticky tape attached to each of two sheets of gridded blue paper. The two sheets of gridded paper, tape and leaves were placed in each of two 50 mL centrifuge tubes, labeled by treatment and replication and stored uncapped for 8 days. As leaves dried, mites moved off leaves and were trapped on the sticky tape. Leaves of infested plants were then removed from tape, placed in an aluminum bag and dried for 4 more days at  $60^\circ\text{C}$ . Leaf dry weights of all plants were then measured with an XS-310D analytical balance (1 mg sensitivity, Denver Instrument Company, Bohemia, NY, USA). Trapped total numbers of *A. toshiella* adults and nymphs were estimated as a measure of antibiosis using a Nikon SMZ645 stereoscope (Nikon Instruments Inc., Melville, NY, USA) at  $10\times$  magnification, by combining the counts on each pair of blue gridded paper sheets.

Percent proportional plant height change (% PHC), percent proportional plant dry weight change (% DWT) and plant tolerance index (TI) were measured to estimate tolerance to mite feeding [36]. % PHC was calculated as  $[(\text{height of un-infested plant} - \text{height of a paired infested plant at the time of cutting the plants}) / \text{height of un-infested plant}] \times 100$ . % DWT was calculated as  $[(\text{dry weight of un-infested plant} - \text{dry weight of a paired infested plant}) / \text{dry weight of un-infested plant}] \times 100$  [39]. TI was calculated as % DWT/total number of *A. toshiella* biotype 1 produced on infested plants at the end of the experiment. TI values calculated from plants with no mites were considered missing values.

## 2.4. Experiment II. Response of *A. toshiella* to *M. destructor* Resistance Genes in Choice Tests

In August and September 2013, each of 10 plastic pots (replicates) with the dimensions  $10 \times 10 \times 7$  cm, each housing, one plant each containing *H13*, *H18*, *H21*, *H25*, *H26*, *Hdic*, OK05312 or Jagger were arranged at random around the periphery of the pot to avoid leaves touching throughout the experiment. The emerging second leaf of each plant was infested with a piece of wheat leaf containing a mixture of 30–35 *A. toshiella* biotype 1 adults and nymphs. The 10 replicate pots were arranged at random in a screen-ventilated plastic cage. The experiment was arranged in a randomized complete block design where pots were the blocking factor. Leaf folding and mean total *A. toshiella* biotype 1 per plant at 7 days post-infestation

were determined as in Experiment I. The 7-day post-infestation interval was selected based on previous results [36], which demonstrated that adults reach and feed on plants within 7 days in antixenosis (choice) experiments.

### 2.5. Experiment III. Response of *A. toshiella* to the *Dn7* Gene in No-Choice Tests

The reaction of *Dn7* to *A. toshiella* in comparison to the OK05312-resistant control and the susceptible control Jagger was assessed in May and June 2014. Independent assays were performed for *A. toshiella* biotypes 1 and 2. Each assay consisted of four plants of each cultivar, evenly distributed in each of three cages covered with 36  $\mu$  mite-proof screen, for a total of nine cages in the experiment. The emerging second leaf of each plant was infested with leaf pieces containing ~30 adult *A. toshiella* biotype 1 or biotype 2 reared on Jagger plants before infestation. Cages were placed on greenhouse benches in a random fashion and were considered the blocking factor in each experiment. At 14 days post-infestation, plants were cut, placed on sticky tape and gridded paper, and counted to determine numbers of mites present as described previously. A cage mean was calculated from the four plants of each variety in a cage.

## 3. Statistical Analysis

In Experiment I, numbers of *A. toshiella*, % PHC, % DWT and TI were analyzed using a generalized linear mixed model where wheat genotype was the fixed effect and cages were the random effect. The assumption of normality and homogeneity of variances was checked using studentized residuals and the Kolmogorv-Smirnov test for each response variable [40,41]. The Kenward-Rogers method was used to estimate the degrees of freedom [42]. The number of *A. toshiella* was fitted using a Poisson distribution to account for skewness of the data, and over dispersion was assessed based on a maximum-likelihood Pearson  $\chi^2$ /degrees of freedom statistic [43]. The model for % proportional plant height change, % proportional plant dry weight change and plant tolerance index was modified to account for heterogeneous residual variances. Variance groups were made with treatment combinations having similar residual dispersion for each response variable. The choice of model with the best fitting heterogeneous variance specification was based on the Bayesian information criterion [44].

Fisher's Least Significant Difference (LSD) and Tukey-Kramer tests were used for multiple comparisons when the type III test of fixed effect was significant ( $p < 0.05$ ). Fisher's LSD test was used for the numbers of *A. toshiella* since this was an exploratory experiment, and the Tukey-Kramer test was too conservative to detect differences between the treatments [45]. Fisher's exact test was used to make paired comparisons of leaf-folding between controls and infested genotypes. Analyses were performed using PROC GLIMMIX and PROC FREQ in SAS software v.9.4 [46,47].

No transformations were necessary for data in Experiment II. Where F-tests were significant at  $\alpha = 0.05$ , the mean numbers of *A. toshiella* on different plant genotypes were separated at ( $p < 0.05$ ) by the Tukey's Studentized Range HSD test. Pearson  $\chi^2$  tests were performed to detect differences between genotypes with different *H* genes to *A. toshiella*-induced leaf folding. When significant, paired comparisons of leaf folding between control and test cultivars were performed using a  $\chi^2$  Fisher's exact test.

In Experiment III, biotype 1 no-choice data with *Dn7* fit a normal distribution and were not transformed for analysis. Biotype 2 data were transformed to natural logarithms for ANOVA and back-transformed for the presentation of mean numbers of mites. Where F-tests were significant at  $\alpha = 0.05$ , the mean numbers of *A. toshiella* on cultivars were separated at ( $p < 0.05$ ) by the Tukey-Kramer test. Data in Experiments II and III were analyzed by two-way analysis of variance (ANOVA) using PROC GLIMMIX and PROC FREQ in SAS software v.9.4 [46,47].

## 4. Results

### 4.1. Response of *A. toschella* to *M. destructor*-Resistance Genes in No-Choice Tests

There were significant differences in the numbers of *A. toschella*, % proportional plant dry weight change and tolerance index between genotypes ( $F = 2.65$ ,  $df = 7$ ,  $34.7$ ,  $p < 0.05$ ;  $F = 4.82$ ,  $df = 7$ ,  $48.04$ ,  $p < 0.05$ ; and  $F = 2.55$ ,  $df = 7$ ,  $31.15$ ,  $p < 0.05$ , respectively) (Table 1). Differences between genotypes for % proportional plant height change were non-significant ( $F = 2.22$ ,  $df = 7$ ,  $40.42$ ,  $p > 0.05$ ). Mean *A. toschella* numbers were significantly lower on plants of the cultivar Hamlet, containing the *H21* gene from rye, in comparison to plants of all other genotypes containing *H* genes or the susceptible control cultivar Jagger, and *A. toschella* numbers on *H21* plants were not significantly different than those on OK05312 resistant control plants (Table 1).

**Table 1.** Mean (Lower, Upper 95% CI) total *A. toschella* biotype 1 adults and nymphs, percent proportional plant dry weight change <sup>a</sup> and plant tolerance index <sup>b</sup> of *M. destructor* resistant wheat cultivars, the susceptible cultivar Ike and the *A. toschella* resistant cultivar OK05312 at 14 days post-*A. toschella*-infestation in no-choice antibiosis and tolerance tests.

Genotype	Resistance Gene	Mean (Lower, Upper 95% CI)		
		Mean Number of <i>A. toschella</i> <sup>c</sup>	% Dry Weight Change <sup>c</sup>	Tolerance Index <sup>d</sup>
OK05312	<i>Cmc4</i>	4.7 (1.7, 13.2) <sup>a</sup>	8 (−4.7, 20.7) <sup>a</sup>	3.6 (−0.9, 8.1) <sup>b,c</sup>
Hamlet	<i>H21</i>	5.8 (2.1, 16.1) <sup>a</sup>	2 (−38.2, 42.2) <sup>a,b</sup>	5.4 (0.9, 10) <sup>c</sup>
KSWGRC26	<i>H26</i>	61.7 (23, 165.4) <sup>b</sup>	−3.3 (−16, 9.4) <sup>a,b</sup>	−1.4 (−3.9, 1.1) <sup>a</sup>
Molly	<i>H13</i>	68 (25.2, 183.7) <sup>b</sup>	4 (−8.7, 16.7) <sup>b</sup>	−1.3 (−3.8, 1.2) <sup>a,b</sup>
Ike	None	94.7 (35.8, 250.5) <sup>b</sup>	11.8 (−0.9, 24.4) <sup>b</sup>	0.1 (−0.05, 0.3) <sup>a,b</sup>
KS92WGRC20	<i>H25</i>	125.5 (47.4, 332) <sup>b</sup>	15.8 (3.1, 28.5) <sup>b</sup>	0.3 (0.1, 0.4) <sup>a,b</sup>
KS99WGRC42	<i>Hdic</i>	151.5 (57.3, 400.2) <sup>b</sup>	−8.3 (−21, 4.4) <sup>a,b</sup>	−0.03 (−0.2, 0.1) <sup>a,b</sup>
Redland	<i>H18</i>	177.7 (67.2, 469.6) <sup>b</sup>	−25.5 (−38.2, −12.8) <sup>a</sup>	−0.5 (−1.5, 0.4) <sup>a,b</sup>

<sup>a</sup> % plant dry weight change = [(weight of un-infested plant − weight of paired infested plant/weight of un-infested plant] × 100. <sup>b</sup> Tolerance index = % plant dry weight change/total number of *A. toschella* biotype 1 produced on infested plants. <sup>c</sup> Means followed by a different letter within a column are significantly different based on Tukey-Kramer mean separation test ( $p < 0.05$ ). <sup>d</sup> Means followed by a different letter within a column are significantly different based on Fisher's LSD mean separation test ( $p < 0.05$ ).

*H18* plants had a significantly lower mean % proportional plant dry weight change than plants of either control, *H25* plants, or *H13* plants (Table 1). In contrast, *H26* plants exhibited a significantly lower mean plant tolerance index than plants of the OK05312 control or plants containing *H21*. Mean *A. toschella*-induced leaf folding was significantly greater on plants of all *H* genotypes compared to the resistant OK05312 control, with the exception of *H21*, which sustained only 10% leaf folding compared to 0% folding on resistant control plants (Table 2).

**Table 2.** Percent *A. toschella* biotype 1-induced folding in wheat plants with *M. destructor* resistance genes, the OK05312 (*Cmc4*) resistant control and the susceptible Ike control at 14 days post-*A. toschella* infestation in a no-choice test.

Genotype	<i>H</i> Gene	% Folded Leaf Plants	$\chi^2$ Fisher's Exact Test	
			Ike	OK05312
OK05312	<i>Cmc4</i>	0	ns	-
Hamlet	<i>H21</i>	10	ns	ns
KSWGRC26	<i>H26</i>	50	ns	*
Molly	<i>H13</i>	60	ns	*
KS99WGRC42	<i>Hdic</i>	80	ns	**
KS92WGRC20	<i>H25</i>	90	*	**
Redland	<i>H18</i>	80	ns	**
Ike	None	30	-	ns

ns: not significant at  $p > 0.05$ ; \* significant at  $p < 0.05$ ; \*\* significant at  $p < 0.01$ .

#### 4.2. Response of *A. toschella* to *M. destructor*-Resistance Genes in Choice Tests

There were significant differences in the mean total number of *A. toschella* biotype 1 adults produced in choice tests using the OK05312 resistant control, the Jagger susceptible control, and plants with different *H* genes ( $F = 4.9$ ;  $df = 7.57$ ;  $p < 0.01$ ). The percentage of plants with folded leaves also differed significantly between plants with *H* genes and controls (Pearson  $\chi^2 = 42.7$ ;  $df = 7$ ;  $p < 0.01$ ). Plants containing *H21*, *H25*, and *Hdic* produced significantly lower *A. toschella* populations than those on susceptible Jagger control plants and were no different from the population on OK05312 resistant control plants (Table 2). *H21*, *H25*, and *Hdic* plants also displayed significantly fewer folded leaves than Jagger susceptible control plants (Table 3).

**Table 3.** Mean  $\pm$  SE number *A. toschella* biotype 1 and *A. toschella*—induced folding in plants of *M. destructor* resistant cultivars, the resistant control OK05312 and the susceptible control Jagger at 7 d post-infestation in a choice test.

Genotype	Resistance Gene	Mean $\pm$ SE Number of <i>A. toschella</i> Adults	% Leaf Folding	$\chi^2$ Fisher's Exact Test	
				Jagger	OK05312
OK05312	<i>Cmc4</i>	32.4 $\pm$ 60.8 <sup>a</sup>	0	**	-
Hamlet	<i>H21</i>	88.1 $\pm$ 64.1 <sup>a,b</sup>	0	**	ns
KS99WGRC42	<i>Hdic</i>	89.7 $\pm$ 55.9 <sup>a,b</sup>	20	**	ns
KS92WGRC20	<i>H25</i>	96.0 $\pm$ 58.2 <sup>a,b</sup>	0	**	ns
KSWGRC26	<i>H26</i>	218.2 $\pm$ 55.9 <sup>a,b,c</sup>	60	ns	*
Redland	<i>H18</i>	246.5 $\pm$ 55.9 <sup>a,b,c</sup>	100	ns	**
Molly	<i>H13</i>	261.8 $\pm$ 55.9 <sup>b,c</sup>	50	ns	*
Jagger	None	328.1 $\pm$ 55.9 <sup>c</sup>	90	-	**

Means followed by a different letter differ significantly based on Tukey-HSD mean separation test ( $p < 0.05$ ). ns: not significant at  $p > 0.05$ ; \* significant at  $p < 0.05$ ; \*\* significant at  $p < 0.01$ .

#### 4.3. Response of *A. toschella* to the *D. noxia*-Resistant *Dn7* Genes in No-Choice Tests

The mean number of *A. toschella* biotype 1 mites on Jagger, 93M370 and OK05312 differed significantly in assay 1 ( $F = 101.4$ ;  $df = 2, 6$ ;  $p < 0.05$ ) and in assay 2 ( $F = 50.4$ ;  $df = 2, 6$ ;  $p < 0.05$ ). In both assays, *Dn7* plants produced significantly lower biotype 1 populations than those on susceptible Jagger plants, but no different than those on *Cmc4* (OK05312) resistant control plants (Table 4). The mean number of biotype 2 mites on all three genotypes also differed significantly in assay 1 ( $F = 18.6$ ;  $df = 2, 6$ ;  $p < 0.05$ ) and in assay 2 ( $F = 6.3$ ;  $df = 2, 6$ ;  $p < 0.05$ ). However, in assay 2, biotype 2 populations were significantly lower on *Dn7* plants than on Jagger plants.

**Table 4.** Mean  $\pm$  SE number of *A. toschella* biotype 1 and 2 on wheat genotypes with genes for resistance to *A. toschella* (*Cmc4*) or *D. noxia* (*Dn7*), and the susceptible control Jagger at 14 d post-infestation in a no-choice test.

Genotype	Resistance Gene	Mean $\pm$ SE Number of <i>A. toschella</i>			
		Biotype 1		Biotype 2	
		Assay 1	Assay 2	Assay 1	Assay 2
OK05315	<i>Cmc4</i>	21.4 $\pm$ 7.1 <sup>b</sup>	22.8 $\pm$ 17.1 <sup>b</sup>	36.1 $\pm$ 30.2 <sup>b</sup>	83.6 $\pm$ 34.0 <sup>a,b</sup>
93M370	<i>Dn7</i>	41.9 $\pm$ 7.1 <sup>b</sup>	16.6 $\pm$ 17.1 <sup>b</sup>	28.6 $\pm$ 30.2 <sup>b</sup>	14.3 $\pm$ 34.0 <sup>b</sup>
Jagger	none	153.8 $\pm$ 7.1 <sup>a</sup>	133.5 $\pm$ 17.1 <sup>a</sup>	257.3 $\pm$ 30.2 <sup>a</sup>	163.1 $\pm$ 34.0 <sup>a</sup>

Means in each column followed by a different letter differ significantly, Tukey—Kramer mean separation test ( $p < 0.05$ ).

## 5. Discussion

The ability of the *A. toschella*-virus complex to reduce wheat yields is continuously driven by the immense reproductive potential of *A. toschella* on numerous wild and cultivated grass hosts [48].

Attempts to manage *A. toshiella* with acaracides or delayed planting continue to fail [49,50], and the most effective management tactic to date continues to be *A. toshiella*-resistant cultivars. Although *A. toshiella* biotype 2 is virulent to *Cmc3* [18], *Cmc4* remains an effective tool for wheat cultivar improvement [51].

Results of the present study demonstrate that *A. toshiella* biotype 1 populations are significantly reduced by the rye-based *M. destructor* resistance in plants containing *H21* compared to plants of the susceptible control Jagger in both choice- and no-choice experiments (Tables 1 and 2). Furthermore, the level of *A. toshiella* population reduction by *H21* was not significantly different from that in plants of the *Cmc4* mite resistant control. *H21* plants also exhibited a level of *A. toshiella* leaf folding similar to *Cmc4* plants, but significantly less of than that in Jagger plants in both choice- and no-choice experiments. *A. toshiella* resistance in *H21* appears to be based primarily on properties that limit mite population increases, as tolerance measurements detected no differences between plants containing *H21*, *Cmc4* or the susceptible Jagger control.

*A. toshiella* resistance in *Dn7* and *H21* may be related to a dual effect of each gene on more than one pest or the interplay of one or more rye genes from each rye-wheat translocation. *D. noxia* is a phloem feeder, while *A. toshiella* and *M. destructor* feed on and within epidermal tissue cells, respectively, suggesting that *Dn7* may provide resistance to epidermal tissue cell feeders as well. This hypothesis was beyond the scope of the current study and will be tested in additional future experiments.

The results of the current experiments provide useful management information to producers about wheat cultivar selection in areas of chronic yield reduction due to *A. toshiella*, *M. destructor*, *D. noxia*, and wheat streak mosaic virus infection. Further research is in progress to identify gene(s) conferring *A. toshiella* resistance.

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