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Responses of Resistant and Susceptible Barley and Wheat Genotypes to Simultaneous Infestation by Multiple Aphid Species

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Abstract

The bird cherry-oat aphid (BCOA, *Rhopalosiphum padi*) and the Russian wheat aphid (RWA, *Diuraphis noxia*) are major pests of barley and wheat, frequently posing a threat to crop yields. These aphids are managed through chemical pesticides and cultivars bred for resistance. This study evaluated morphological and physiological responses of susceptible and resistant barley (Morex, BCOR001) and wheat (Custer, Dn7) genotypes to individual and combined infestations of BCOA and two biotypes of RWA: RWA1, and RWA2. Plants were assessed weekly for one month to assess morphological responses, and CO_2 assimilation responses were measured using A/Ci curves 14 days after infestation. Aphid infestation significantly reduced several growth parameters from the first week of evaluation, with susceptible genotypes showing more pronounced reductions. In contrast, resistant genotypes—particularly BCOR001—maintained stability in root biomass and aerial dry weight under most aphid treatments. Gas-exchange analyses revealed that non-infested plants had the highest assimilation rates, while aphid-infested plants, especially under attack by aphids feeding on susceptible hosts exhibited notable declines. Interestingly, BCOR001 maintained high assimilation rates under BCOA infestation, suggesting potential tolerance mechanisms. Co-infestations by multiple aphid species did not produce additional damage, suggesting that thresholds based on aphid number are sufficient, regardless of species composition. These results emphasize the importance of evaluating host resistance under co-infestation scenarios and highlight physiological traits that may contribute to enhanced tolerance in resistant genotypes.

Keywords

Plant-Insect Interactions, Aphididae, Barley, Wheat, Host Plant Resistance, Feeding Guilds

1. Introduction

The bird cherry-oat aphid (BCOA), *Rhopalosiphum padi* L., and the Russian wheat aphid (RWA), *Diuraphis noxia* L., are considered important pests of both wheat and barley, frequently leading to substantial yield reductions [1] [2]. Both BCOA and RWA feed by inserting their sucking mouthparts into the sieve tubes of plants to acquire nutrients and water, and allows both species to indirectly transmit plant viruses [3].

The RWA has been reported as a destructive pest of grain plants since its arrival in the United States in 1986. Within less than a decade, RWA aphids caused an estimated $1 billion in economic losses to small grains [4]. Similarly, BCOA is an economically significant pest that can reduce wheat yield by up to 62% and is an effective vector for Barley Yellow Dwarf Virus (BYDV) in small grains [1] [3] [5] [6].

The RWA and BCOA feed on different locations of the plant and adopt distinct feeding strategies, which in turn elicit specific responses from their host plants [7] [8]. While BCOA feeding generally does not result in visible damage symptoms, RWA feeding can induce necrosis and chlorosis on leaves, leaf rolling, stunted growth, purple discoloration, and prostrate growth, especially in wheat [2] [9]-[11]. In addition to differences in feeding strategies between the two species, RWA also exhibits multiple biotypes. These biotypes are primarily characterized by the resistant or susceptible responses observed in a collection of wheat accessions believed to carry specific Dn-resistance genes [12]-[14].

The first RWA-identified biotype (RWA1) arrived in the US in 1986 [10]. In 2003, a RWA-resistant hard red winter wheat line Prairie Red, which contains the Dn4 resistance gene, was discovered to be infested with RWA in several areas of southeastern Colorado [13] [15]. This was later identified as a new biotype, RWA2 [10]. Among the tested accessions, only 94M370 (Dn7) showed resistance to RWA2. However, the Dn7 gene is often associated with negative impacts on the quality of leavened bread products [10].

Several wheat and barley genotypes have been identified and developed to resist BCOA and RWA biotypes [6] [14] [16] [17]. However, since these resistant genotypes were typically designed to target a single aphid species, and given that BCOA and RWA can concurrently infest the same host plant [8], it is crucial to investigate the interactions between these two species and their combined impact on resistant and susceptible barley and wheat responses.

The objectives of this work were to evaluate the morphological and physiological parameters of susceptible and resistant barley and wheat genotypes, under combinations of BCOA and RWA1 and RWA2 infestations.

2. Materials and Methods

2.1. Insect and Plant Materials

The BCOA colonies were initially collected in August 2017 from barley, *H. vulgare* fields in Payne County, Oklahoma, United States. Both RWA biotypes were initially collected in western Colorado. RWA1 colonies were found in wild grass species in 2007, while RWA2 was collected from wheat in 2003.

The aphid colonies for the experiments were reared on the "Aberdeen 812" susceptible barley for the BCOA population and susceptible wheat TAM 101 for the RWA1 and RWA2 biotypes. The plants were grown in 4.4 L pots fitted with 45 cm tall cylindrical Lexan® sleeve cages (SABIC Polymershapes, OK, USA). The cages were ventilated by covering them with an organdy cloth positioned atop each pot, which also prevented aphid escape. Seedlings were initiated every two weeks to ensure colony continuity. These plants were housed in growth chambers equipped with two T-8 fluorescent lights set to a photoperiod of 16 hours of light and 8 hours of darkness. The temperature was maintained within a range of 25 ± 2˚C. The seeds used in the experiments were sourced from the USDA-ARS in Stillwater, Oklahoma.

2.2. Plant Genotypes

The tested barley genotypes were "Morex," recognized as a susceptible six-rowed spring malting barley [18] [19] and the resistant "BCOR001" [20]. For wheat, we used "Custer", a susceptible winter wheat genotype [21] and "Dn7" that has shown resistance to RWA1 and RWA2 [22].

The seeds were planted in Cone-tainers™ (model SC10; S7S Greenhouse Supply, Tangent, OR) containing media consisting of three layers: potting soil for the first layer, then fitting clay and sand. Each Cone-tainer™ was fitted with an 8-cm-diameter Lexan sleeve which was ventilated with organdy cloth to prevent aphid escape and moisture buildup. Two seeds were planted in each Cone-tainer and grown in a chamber under two T-8 Florescent lights with a photoperiod of 16 hours of light, 8 hours of darkness, and a temperature of 25 ± 2˚C.

2.3. Aphid Infestations

For both experiments, one week old seedlings were thinned to one plant per plot and infested with a density of 10 aphids adults per plant or 5 aphids adults of each species for treatments of plants with two aphid species. The plants were infested with the following treatments: bcoa; rwa1; rwa2; bcoa + rwa1; rwa2 + bcoa; and a check (uninfested plants). A camel hairbrush was used to transfer aphids from the colony to the plant leaves.

2.4. Morphological Responses

Wheat and barley morphological responses were recorded weekly for four weeks, beginning one week after infestation. For this experiment, we adopted two genotypes (susceptible and resistant) for each crop (barley and wheat) and six treat-

ments (five aphid treatments and one control). The experiment was conducted using a completely randomized design, with five replications, with each plant considered an experimental unit.

Weekly, five plants of each treatment were used to assess plant growth by measuring plant height from the base of the soil to the tip of the plant (cm). The number of true leaves was counted, and the plant was clipped and weighed. The root system was also weighed after the soil was removed to determine aerial fresh weight (g) and root weight (g). The plant tissues were labeled, placed in envelopes, and dried in an oven at 50˚C for 72 hours before being reweighed, following the methods described by Limaje *et al.* [23]. Subsequently, aerial dry weight (g), root dry weight (g) were measured from the reweighed tissues [23], and total dry weight (g) were calculated.

2.5. Gas-Exchange Responses

We evaluated the CO_2 response (A/Ci curves) using one-month-old plants, 14 days after aphid introduction, with a portable photosynthesis system (model LI-6400, LI-COR, Lincoln, NE). We used the same treatments as above, with each treatment having four replications, conducted using a completely randomized design.

We measured CO_2 assimilation rates in barley and wheat genotypes subjected to aphid infestations. The measurements were conducted under CO_2 concentrations ranging from 50 to 1000 ppm, following the sequence: 400, 200, 100, 50, 400, 400, 600, 800, 1000, 2000, and 400 ppm. Light intensity was maintained at 1400 μmol photon $m^{-2} \cdot s^{-1}$, based on the methodology outlined by Paudyal *et al.* [24]. A stomatal ratio of one was adopted because the stomatal densities on both the abaxial and adaxial surfaces are similar [25].

2.6. Statistical Analysis

A two-way ANOVA was performed to analyze the effect of aphid infestation and plant genotypes on the morphological parameters of wheat and barley. The statistical analyses were conducted in the R computing environment, utilizing the "AgroR" package [26]. Before proceeding with the two-way ANOVA, we performed exploratory data analysis to assess the assumptions of normality of residuals [27] and homogeneity of variances [28]. When necessary, the data were transformed using Box-Cox transformation [29]. If, following transformation, the parameters did not meet the analysis assumptions (normality of residuals and homogeneity of variances), we conducted a non-parametric analysis using Kruskal-Wallis test followed by a Dunn post-hoc test ($\alpha = 0.05$) [29]. The A/Ci curves were plotted with a polynomial regression model of order 2 in Excel software (Microsoft Office; Version 2019).

3. Results

Both wheat and barley genotypes showed significant reductions in most evaluated parameters when infested with aphids, compared to the controls (**Tables 1-4**). Dur-

ing the first evaluation, significant interactions between genotype and aphid treatment were observed in barley for the number of leaves [$F(5, 48) = 8.75$, $p < 0.01$] and in wheat for root weight (g) [$F(5, 48) = 2.46$, $p < 0.01$] and total dry weight (g) [$F(5, 48) = 2.55$, $p < 0.01$]. Among these interactions, only the susceptible genotypes (Morex and Custer) showed reductions caused by the aphid treatments. Even when no significant interaction was observed, parameters such as aerial fresh weight (g) and aerial dry weight (g) for barley, as well as plant height (cm) and aerial fresh weight (g) for wheat, were impacted by the aphid treatments (Figure 1).

Table 1. Morphological parameters of barley genotypes "BCO R001" and "Morex" and wheat genotypes "Dn7" and "Custer" after one week of infestation under different aphid species treatments. Means ± 1SE or Median ± IQR followed by the same letter, lowercase in column (within aphid treatment) and uppercase in the line (between plant cultivars), do not differ from each other ($\alpha = 0.05$).

	Plant height (cm)		Number of leaves		Aerial fresh weight (g)		Root weight (g)		Aerial dry weight (g)		Root dry weight (g)[1]		Total dry weight (g)[1]	
Treatment	BCOR 001	Morex	BCOR 001	Morex	BCOR 001	Morex	BCOR 001	Morex	BCOR 001	Morex	BCOR 001	Morex	BCOR 001	Morex
check	41.14 ± 1.92 A	22.30 ± 1.16 B	4.00 ± 0.00 aA	4.20 ± 0.20 aA	0.88 ± 0.05 aA	0.40 ± 0.04aB	3.29 ± 0.35 A	1.15 ± 0.19 B	0.12 ± 0.01 aA	0.06 ± 0.00 aB	0.48 ± 0.08 A	0.12 ± 0.01 B	0.59 ± 0.09 A	0.18 ± 0.02 B
bcoa	39.48 ± 2.65 A	21.16 ± 1.35 B	3.80 ± 0.20 aA	2.80 ± 0.37 bB	0.70 ± 0.06 bA	0.21 ± 0.05bB	2.96 ± 0.29 A	0.88 ± 0.12 B	0.09 ± 0.01 aA	0.04 ± 0.01 abB	0.47 ± 0.04 A	0.10 ± 0.02 B	0.55 ± 0.05 A	0.14 ± 0.03 B
rwa1	35.46 ± 1.51 A	20.66 ± 2.03 B	4.20 ± 0.20 aA	2.00 ± 0.00 bB	0.69 ± 0.07 bA	0.06 ± 0.01bB	3.19 ± 0.90 A	0.65 ± 0.16 B	0.08 ± 0.01 aA	0.03 ± 0.00 abB	0.49 ± 0.17 A	0.07 ± 0.01 B	0.57 ± 0.18 A	0.10 ± 0.02 B
rwa2	36.00 ± 1.53 A	22.56 ± 2.26 B	3.80 ± 0.20 aA	2.20 ± 0.20 bB	0.81 ± 0.11 bA	0.09 ± 0.01bB	2.35 ± 0.19 A	0.74 ± 0.14 B	0.09 ± 0.01 aA	0.04 ± 0.01 abB	0.32 ± 0.03 A	0.16 ± 0.04 B	0.42 ± 0.03 A	0.19 ± 0.04 B
bcoa+rwa1	39.88 ± 0.82 A	16.02 ± 2.13 B	3.60 ± 0.24 aA	2.20 ± 0.20 bB	0.74 ± 0.06 bA	0.07 ± 0.01bB	2.36 ± 0.24 A	0.81 ± 0.18 B	0.09 ± 0.01 aA	0.03 ± 0.01 abB	0.33 ± 0.04 A	0.09 ± 0.02 B	0.42 ± 0.05 A	0.12 ± 0.02 B
bcoa+rwa2	35.94 ± 1.84 A	20.34 ± 0.88 B	4.00 ± 0.00 aA	2.20 ± 0.20 bB	0.70 ± 0.04 bA	0.07 ± 0.01 bB	2.33 ± 0.30 A	1.24 ± 0.39 B	0.08 ± 0.01 aA	0.02 ± 0.01 bB	0.35 ± 0.06 A	0.09 ± 0.01 B	0.43 ± 0.06 A	0.12 ± 0.01 B
$P_{aphid;\ cultivar;\ interaction}$	0.21; <0.01; 0.06		<0.01; <0.01; <0.01		<0.01; <0.01; 0.11		0.42; <0.01; 0.38		<0.00; <0.01; 0.96		0.65; <0.01; 0.38		0.49; 0.00; 0.47	
F	1.50; 296.32; 2.27		9.55; 126.75; 8.75		7.62; 425.83; 1.89		1.02; 81.76; 1.09		6.36; 172.98; 0.19		0.67; 67.93; 0.09		0.90; 84.74; 0.93	
$DF_{residuals}$	48		48		48		48		48		48		48	

[1]Data transformed using Box-cox method for analysis.

	Plant height (cm)		Number of leaves[2]		Aerial fresh weight (g)		Root weight (g)		Aerial dry weight (g)		Root dry weight (g)[1]		Total dry weight (g)	
	Dn7	Custer	Dn7	Custer	Dn7	Custer	Dn7	Custer	Dn7	Custer	Dn7	Custer	Dn7	Custer
check	19.92 ± 0.57 aA	18.26 ± 0.82 aB	3.00 ± 0.00	3.00 ± 0.00	0.19 ± 0.02 a	0.22 ± 0.03 a	0.34 ± 0.03 aA	0.32 ± 0.02 aA	0.02 ± 0.00	0.02 ± 0.00	0.03 ± 0.00 A	0.03 ± 0.00 B	0.05 ± 0.01 aA	0.05 ± 0.00 aA
bcoa	19.64 ± 0.51 aA	16.36 ± 0.49 bB	3.00 ± 1.00	3.00 ± 0.00	0.18 ± 0.02 ab	0.14 ± 0.03 ab	0.28 ± 0.03 aA	0.17 ± 0.03 bB	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.00 A	0.02 ± 0.00 B	0.05 ± 0.01 aA	0.03 ± 0.01 bB
rwa1	17.56 ± 1.22 bA	16.70 ± 0.80 bB	3.00 ± 0.00	3.00 ± 1.00	0.13 ± 0.01 b	0.10 ± 0.02 b	0.36 ± 0.03 aA	0.21 ± 0.02 abB	0.02 ± 0.01	0.01 ± 0.01	0.05 ± 0.01 A	0.03 ± 0.00 B	0.07 ± 0.01 aA	0.04 ± 0.00 abB
rwa2	21.20 ± 0.87 aA	18.10 ± 0.53 aB	3.00 ± 0.00	3.00 ± 0.00	0.21 ± 0.03 a	0.16 ± 0.02 a	0.39 ± 0.02 aA	0.27 ± 0.03 abB	0.03 ± 0.01	0.02 ± 0.00	0.04 ± 0.01 A	0.03 ± 0.00 B	0.07 ± 0.01 aA	0.04 ± 0.01 abB

Continued

bcoa + rwa1	18.84 ± 1.65 abA	15.30 ± 1.34 bB	3.00 ± 1.00	3.00 ± 0.00	0.18 ± 0.03 ab	0.12 ± 0.02 ab	0.38 ± 0.05 aA	0.30 ± 0.02 aA	0.02 ± 0.00	0.01 ± 0.04	0.04 ± 0.00 A	0.03 ± 0.00 B	0.06 ± 0.01 aA	0.04 ± 0.00 abB
bcoa + rwa2	17.20 ± 1.43 bA	16.80 ± 0.40 bB	3.00 ± 0.00	3.00 ± 0.00	0.13 ± 0.01 b	0.15 ± 0.01 ab	0.31 ± 0.03 aA	0.33 ± 0.02 aA	0.02 ± 0.00	0.01 ± 0.01	0.03 ± 0.01 A	0.03 ± 0.00 B	0.06 ± 0.01 aA	0.05 ± 0.00 aA
$P_{aphid;\ cultivar;\ interaction}$	0.03; <0.01; 0.45		0.35	0.11	<0.01; 0.12; 0.24		<0.01; <0.01; 0.04		0.38	0.13	0.11; <0.01; 0.05		0.32; <0.01; 0.04	
F/ Kruskal-Wallis	2.76; 14.50; 0.96		5.58	8.90	4.21; 2.56; 1.40		4.02; 20.55; 2.46		5.30	8.53	1.93; 10.33; 2.28		1.20; 19.59; 2.55	
$DF_{residuals}$	48		5	5	48		48		5	5	48		48	

[1]Data transformed using Box-cox method.[2] Kruskal-Wallis performed; median ± IQR.

Table 2. Morphological parameters of barley genotypes "BCO R001" and "Morex" and wheat genotypes "Dn7" and "Custer" after two weeks of infestation under different aphid species treatments. Means ± 1SE or Median ± IQR followed by the same letter, lowercase in column (within aphid treatment) and uppercase in the line (between plant cultivars), do not differ from each other ($\alpha = 0.05$).

	Plant height (cm)[1]		Number of leaves[1]		Aerial fresh weight (g)[2]		Root weight (g)[1]		Aerial dry weight (g)[2]		Root dry weight (g)[2]		Total dry weight (g)	
	BCOR 001	Morex	BCOR 001	Morex	BCOR 001	Morex	BCOR 001	Morex	BCOR 001	Morex	BCOR 001	Morex	BCOR 001	Morex
check	45.58 ± 3.44 aA	33.14 ± 2.88 aB	4.80 ± 0.37 abA	4.00 ± 0.45 aB	1.42 ± 0.20 a	0.92 ± 0.38 a	2.83 ± 0.73 aA	4.36 ± 0.42 aA	0.20 ± 0.05 a	0.14 ± 0.05 a	0.64 ± 0.17 aB	0.99 ± 0.10 aA	0.81 ± 0.19 aB	1.12 ± 0.11 aA
bcoa	46.24 ± 2.54 abA	22.16 ± 1.35 abB	4.80 ± 0.20 abA	3.40 ± 0.24 aB	1.16 ± 0.42 a	0.20 ± 0.18 ab	2.91 ± 0.57 aA	1.70 ± 0.23 abB	0.15 ± 0.04 a	0.04 ± 0.02 b	0.49 ± 0.10 aA	0.36 ± 0.05 bA	0.66 ± 0.10 abA	0.40 ± 0.06 bA
rwa1	35.60 ± 5.20 bA	23.20 ± 1.72 bB	4.20 ± 0.20 bA	2.00 ± 0.00 bB	0.59 ± 0.45 b	0.06 ± 0.02 b	2.15 ± 0.45 aA	1.06 ± 0.26 bA	0.09 ± 0.01 b	0.04 ± 0.01 b	0.36 ± 0.08 aA	0.23 ± 0.05 bA	0.45 ± 0.07 abA	0.27 ± 0.05 bA
rwa2	37.84 ± 1.63 bA	21.26 ± 2.42 bB	4.00 ± 0.00 bA	2.00 ± 0.00 bB	0.22 ± 0.17 b	0.04 ± 0.02 b	1.52 ± 0.43 aA	0.67 ± 0.33 bB	0.09 ± 0.02 b	0.02 ± 0.00 b	0.27 ± 0.08 aA	0.16 ± 0.09 bA	0.36 ± 0.08 bA	0.19 ± 0.09 bA
bcoa + rwa1	43.68 ± 2.86 abA	21.78 ± 1.92 abB	5.20 ± 0.20 aA	2.00 ± 0.00 bB	1.22 ± 0.33 a	0.05 ± 0.02 b	2.86 ± 0.38 aA	0.89 ± 0.18 bB	0.15 ± 0.06 a	0.02 ± 0.01 b	0.49 ± 0.07 aA	0.22 ± 0.05 bB	0.64 ± 0.08 abA	0.31 ± 0.09 bB
bcoa + rwa2	35.84 ± 6.87 bA	20.84 ± 1.08 bB	4.20 ± 0.20 bA	1.80 ± 0.20 bB	0.23 ± 0.05 b	0.04 ± 0.03 b	2.22 ± 0.77 aA	0.83 ± 0.17 bB	0.08 ± 0.00 b	0.03 ± 0.02 b	0.40 ± 0.15 aA	0.18 ± 0.04 bA	0.49 ± 0.15 abA	0.26 ± 0.07 bA
$P_{aphid;\ cultivar;\ interaction}$	0.01; 0.00; 0.36		<0.01; <0.01; <0.01		0.00	0.00	<0.01; <0.01; 0.02		0.01	0.07	<0.01; 0.12; 0.02		<0.01; 0.02; 0.04	
F/Kruskal-Wallis	3.22; 82.40; 1.13		14.67; 240.00; 6.88		18.09	20.78	6.28; 13.36; 3.04		15.79	10.10	10.63; 2.58; 6.96		11.49; 5.81; 2.51	
$DF_{residuals}$	48		48		5	5	48		5	5	48		48	

[1]Data transformed using Box-cox method. [2]Kruskal-Wallis performed; median ± IQR.

	Plant height (cm)[1]		Number of leaves[2]		Aerial fresh weight (g)[2]		Root weight (g)		Aerial dry weight (g)[2]		Root dry weight (g)[2]		Total dry weight (g)[2]	
	Dn7	Custer	Dn7	Custer	Dn7	Custer	Dn7	Custer	Dn7	Custer	Dn7	Custer	Dn7	Custer
check	35.56 ± 1.79	34.38 ± 2.87	4.00 ± 0.00 a	4.00 ± 0.00 a	0.46 ± 0.09 a	0.40 ± 0.02 a	0.42 ± 0.05 a	0.42 ± 0.04 a	0.05 ± 0.02 a	0.04 ± 0.02 a	0.04 ± 0.04	0.04 ± 0.01	0.10 ± 0.03 a	0.09 ± 0.03 a
bcoa	29.78 ± 2.77	23.50 ± 0.71	3.00 ± 0.00 b	3.00 ± 0.00 ab	0.19 ± 0.07 b	0.18 ± 0.01 a	0.30 ± 0.02 b	0.23 ± 0.03 b	0.02 ± 0.01 b	0.02 ± 0.01ab	0.03 ± 0.02	0.02 ± 0.01	0.05 ± 0.02 ab	0.04 ± 0.01 a

Continued

rwa1	19.30 ± 1.58	17.24 ± 0.51	3.00 ± 0.00 b	3.00 ± 1.00 b	0.02 ± 0.01 b	0.11 ± 0.03 b	0.27 ± 0.04 b	0.19 ± 0.05 b	0.03 ± 0.01 ab	0.02 ± 0.01ab	0.03 ± 0.02	0.03 ± 0.02	0.05 ± 0.04 ab	0.04 ± 0.03 a
rwa2	24.22 ± 2.22	19.22 ± 2.55	3.00 ± 0.00 b	3.00 ± 0.00 ab	0.04 ± 0.01 ab	0.17 ± 0.05 ab	0.27 ± 0.04 b	0.25 ± 0.05 b	0.03 ± 0.01 ab	0.02 ± 0.01ab	0.02 ± 0.02	0.01 ± 0.02	0.05 ± 0.01 ab	0.04 ± 0.05 a
bcoa + rwa1	17.88 ± 0.95	17.74 ± 2.19	3.00 ± 0.00 b	3.00 ± 1.00 b	0.05 ± 0.05 ab	0.11 ± 0.03 b	0.21 ± 0.04 b	0.24 ± 0.03 b	0.02 ± 0.01 b	0.01 ± 0.01b	0.02 ± 0.04	0.01 ± 0.01	0.04 ± 0.01 b	0.05 ± 0.01 a
bcoa + rwa2	19.46 ± 0.60	20.32 ± 1.06	3.00 ± 0.00 b	3.00 ± 0.00 ab	0.10 ± 0.03 a	0.13 ± 0.06 ab	0.21 ± 0.02 b	0.26 ± 0.02 b	0.02 ± 0.01 b	0.02 ± 0.01ab	0.02 ± 0.03	0.01 ± 0.01	0.04 ± 0.01 ab	0.05 ± 0.01 a
$P_{aphid;\ cultivar;\ interaction}$	0.07; 0.90; 0.10		<0.01	0.03	<0.01	<0.01	<0.01; 0.57; 0.47		0.01	0.03	0.18	0.31	0.04	0.17
F/Kruskal-Wallis	2.18; 0.02; 1.96		18.11	12.51	18.26	21.48	7.60; 0.32; 0.93		14.15	12.55	7.66	5.94	11.90	7.76
$DF_{residuals}$	48		5	5	5	5	48		5	5	5	5	5	5

[1]Data transformed using Box-cox method for analysis. [2]Kruskal-Wallis performed; median ± IQR.

Table 3. Morphological parameters of barley genotypes "BCO R001" and "Morex" and wheat genotypes "Dn7" and "Custer" after three weeks of infestation under different aphid species treatments. Means ± 1SE or Median ± IQR followed by the same letter, lowercase in column (within aphid treatment) and uppercase in the line (between plant cultivars), do not differ from each other ($\alpha = 0.05$).

	Plant height (cm)[1]		Number of leaves[1]		Aerial fresh weight (g)[1]		Root weight (g)[1]		Aerial dry weight (g)[1]		Root dry weight (g)[1]		Total dry weight (g)[1]	
	BCOR 001	Morex	BCOR 001	Morex	BCOR 001	Morex	BCOR 001	Morex	BCOR 001	Morex	BCOR 001	Morex	BCOR 001	Morex
check	51.80 ± 1.00 a	31.40 ± 1.80 a	6.00 ± 0.00 a	6.00 ± 0.00 a	2.20 ± 0.08 a	0.88 ± 0.39 a	4.85 ± 1.42 a	2.46 ± 0.34 a	0.33 ± 0.06 a	0.12 ± 0.01 a	1.15 ± 0.06 a	0.40 ± 0.05 a	1.50 ± 0.17 a	0.54 ± 0.06 a
bcoa	49.50 ± 4.20 a	21.00 ± 2.70 b	6.00 ± 1.00 a	3.00 ± 1.00 ab	1.64 ± 0.42 ab	0.10 ± 0.12 ab	4.16 ± 0.90 a	0.75 ± 0.30 ab	0.23 ± 0.02 ab	0.04 ± 0.01 ab	0.64 ± 0.26 ab	0.12 ± 0.01 ab	0.88 ± 0.17 ab	0.16 ± 0.02 ab
rwa1	42.20 ± 4.80 a	22.00 ± 4.70 b	4.00 ± 1.00 b	2.00 ± 0.00 b	0.14 ± 0.05 b	0.04 ± 0.01 b	2.07 ± 2.12 b	0.23 ± 0.14 b	0.10 ± 0.06 b	0.02 ± 0.02 b	0.33 ± 0.44 b	0.04 ± 0.05 b	0.43 ± 0.50 b	0.06 ± 0.07 b
rwa2	52.80 ± 14.90 a	25.20 ± 1.40 a	6.00 ± 0.00 a	2.00 ± 0.00 b	2.32 ± 0.54 a	0.04 ± 0.01 b	6.22 ± 2.60 a	0.42 ± 0.06 ab	0.36 ± 0.09 a	0.03 ± 0.01 b	1.26 ± 0.76 a	0.08 ± 0.03 ab	1.63 ± 0.68 a	0.11 ± 0.03 ab
bcoa + rwa1	44.60 ± 8.00 a	22.00 ± 6.00 b	4.00 ± 1.00 b	2.00 ± 0.00 b	0.66 ± 0.74 ab	0.04 ± 0.01 b	2.63 ± 1.55 b	0.44 ± 0.19 ab	0.12 ± 0.04 b	0.03 ± 0.01 b	0.38 ± 0.11 b	0.09 ± 0.03 ab	0.50 ± 0.22 b	0.11 ± 0.03 ab
bcoa + rwa2	48.00 ± 13.50 a	22.50 ± 3.90 b	4.00 ± 1.00 b	2.00 ± 1.00 b	0.28 ± 1.04 ab	0.04 ± 0.02 b	2.67 ± 0.15 b	0.43 ± 0.07 b	0.15 ± 0.03 ab	0.03 ± 0.01 b	0.46 ± 0.09 ab	0.08 ± 0.02 ab	0.61 ± 0.14 ab	0.12 ± 0.02 ab
$P_{aphid;\ cultivar;\ interaction}$	0.34	0.02	0.01	<0.01	<0.01	0.01	0.01	0.01	<0.01	<0.01	<0.01	0.01	<0.01	0.01
F/Kruskal-Wallis	5.68	13.38	14.52	14.52	18.42	16.00	15.72	16.37	19.88	17.69	16.84	15.10	18.36	15.42
$DF_{residuals}$	5.0	5	5	5	5	5	5	5	5	5	5	5	5	5

[1]Kruskal-Wallis performed; median ± IQR.

	Plant height (cm)[1]		Number of leaves[2]		Aerial fresh weight (g)		Root weight (g)		Aerial dry weight (g)		Root dry weight (g)		Total dry weight (g)	
	Dn7	Custer	Dn7	Custer	Dn7	Custer	Dn7	Custer	Dn7	Custer	Dn7	Custer	Dn7	Custer
check	40.50 ± 1.40 a	43.70 ± 1.60 a	6.00 ± 1.00 a	5.00 ± 0.00 a	0.73 ± 0.05 a	0.64 ± 0.25 a	0.56 ± 0.05 a	0.46 ± 0.03 a	0.05 ± 0.05 a	0.07 ± 0.03	0.02 ± 0.01	0.05 ± 0.01 a	0.07 ± 0.06 a	0.12 ± 0.05 a
bcoa	31.10 ± 3.80 a	22.90 ± 2.20 a	4.00 ± 0.00 ab	3.00 ± 0.00 b	0.29 ± 0.02 ab	0.21 ± 0.09 ab	0.25 ± 0.04 b	0.24 ± 0.04 b	0.02 ± 0.01 ab	0.03 ± 0.01	0.02 ± 0.01	0.03 ± 0.01 ab	0.04 ± 0.02 ab	0.06 ± 0.02 ab

Continued

rwa1	16.50 ± 2.80 b	15.50 ± 2.30 b	3.00 ± 0.00 b	3.00 ± 0.00 b	0.03 ± 0.06 b	0.02 ± 0.01 b	0.13 ± 0.03 b	0.12 ± 0.01 b	0.03 ± 0.01 ab	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01 b	0.04 ± 0.01ab	0.04 ± 0.01 b
rwa2	18.00 ± 10.70 b	19.00 ± 2.40 a	3.00 ± 0.00 b	3.00 ± 0.00 b	0.08 ± 0.02 b	0.03 ± 0.01 b	0.16 ± 0.03 b	0.13 ± 0.02 b	0.02 ± 0.01 b	0.02 ± 0.01	0.01 ± 0.01	0.02 ± 0.01 b	0.03 ± 0.02 b	0.05 ± 0.02 ab
bcoa + rwa1	22.60 ± 5.20 b	18.00 ± 2.30 b	3.00 ± 0.00 b	3.00 ± 0.00 b	0.08 ± 0.06 b	0.02 ± 0.01 b	0.10 ± 0.02 b	0.14 ± 0.02 b	0.03 ± 0.01 ab	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01 b	0.05 ± 0.02 ab	0.04 ± 0.01 b
bcoa + rwa2	20.60 ± 4.00 b	14.30 ± 2.30 b	3.00 ± 0.00 b	3.00 ± 0.00 b	0.04 ± 0.08 b	0.03 ± 0.02 b	0.12 ± 0.02 b	0.14 ± 0.01 b	0.01 ± 0.01 b	0.02 ± 0.01	0.06 ± 0.02	0.02 ± 0.01 b	0.08 ± 0.01 a	0.04 ± 0.01 b
$P_{aphid;\ cultivar;\ interaction}$	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01; 0.29; 0.21	0.02	<0.01	0.06	0.01	0.03	0.01	<0.01
F/Kruskal-Wallis	18.00	21.50	20.82	21.46	20.32	21.77	58.37; 1.12; 1.49	13.38	19.14	10.45	14.28	12.71	15.32	18.00
$DF_{residuals}$	5	5	5	5	5	5	48	5	5	5	5	5	5	5

[1]Data transformed using Box-cox method for analysis. [2]Kruskal-Wallis performed; median ± IQR.

Table 4. Morphological parameters of barley genotypes "BCO R001" and "Morex" and wheat genotypes "Dn7" and "Custer" after four weeks of infestation under different aphid species treatments. Means ± 1SE or Median ± IQR followed by the same letter, lowercase in column (within aphid treatment) and uppercase in the line (between plant cultivars), do not differ from each other ($\alpha = 0.05$).

	Plant height (cm)[1]		**Number of leaves[2]**		**Aerial fresh weight (g)[1]**		**Root weight (g)[2]**		**Aerial dry weight (g)[2]**		**Root dry weight (g)**		**Total dry weight (g)**	
	BCOR 001	Morex	BCOR 001	Morex	BCOR 001	Morex	BCOR 001	Morex	BCOR 001	Morex	BCOR 001	Morex	BCOR 001	Morex
check	58.48 ± 1.62 aA	35.66 ± 2.52 abB	6.00 ± 1.00 a	7.00 ± 2.00 a	1.73 ± 0.48 aA	0.87 ± 0.20 aA	3.48 ± 1.72 a	1.64 ± 0.66 a	0.44 ± 0.03 a	0.18 ± 0.09 a	1.52 ± 0.58 aA	0.25 ± 0.05 aB	1.93 ± 0.62 aA	0.41 ± 0.08 aB
bcoa	55.26 ± 2.19 abA	22.54 ± 1.80 bB	5.00 ± 0.00 ab	2.00 ± 1.00 b	0.53 ± 0.14 abA	0.05 ± 0.01 bB	1.53 ± 1.20 ab	0.40 ± 0.24 ab	0.19 ± 0.05 ab	0.03 ± 0.01 a	0.60 ± 0.13 bA	0.07 ± 0.03 bB	0.79 ± 0.15 bA	0.10 ± 0.03 bB
rwa1	43.24 ± 1.82 cA	23.36 ± 1.96 bB	4.00 ± 0.00 b	2.00 ± 0.00 b	0.12 ± 0.02 cdA	0.05 ± 0.00 bB	1.05 ± 0.30 b	0.21 ± 0.05 b	0.08 ± 0.01 ab	0.03 ± 0.01 a	0.27 ± 0.04 bA	0.10 ± 0.04 bB	0.37 ± 0.04 bA	0.12 ± 0.04 bB
rwa2	43.78 ± 4.29 cA	19.60 ± 2.21 bB	4.00 ± 1.00 b	2.00 ± 1.00 b	0.13 ± 0.04 cdA	0.05 ± 0.01 bB	0.86 ± 0.12 b	0.17 ± 0.11 b	0.08 ± 0.06 ab	0.03 ± 0.02 a	0.37 ± 0.16 bA	0.05 ± 0.01 bB	0.48 ± 0.15 bA	0.08 ± 0.01 bB
bcoa + rwa1	44.76 ± 4.94 bcA	24.88 ± 1.34 abB	5.00 ± 1.00 ab	2.00 ± 1.00 b	0.40 ± 0.18 bcA	0.06 ± 0.01 bB	1.23 ± 0.48 ab	0.30 ± 0.17 b	0.13 ± 0.07 ab	0.03 ± 0.01 a	0.46 ± 0.05 bA	0.05 ± 0.01 bB	0.60 ± 0.07 bA	0.08 ± 0.01 bB
bcoa + rwa2	36.68 ± 2.83 cA	22.72 ± 1.65 bB	4.00 ± 1.00 b	2.00 ± 0.00 b	0.08 ± 0.01 dA	0.05 ± 0.01 bA	0.84 ± 0.38 b	0.37 ± 0.02 ab	0.07 ± 0.01 b	0.03 ± 0.01 a	0.38 ± 0.07 bA	0.09 ± 0.01 bB	0.45 ± 0.07 bA	0.12 ± 0.01 bB
$P_{aphid;\ cultivar;\ interaction}$	<0.01; <0.01; 0.03		0.03	<0.01	<0.01; <0.01; 0.04		0.02	<0.01	<0.01	0.32	<0.01; <0.01; 0.22		<0.01; <0.01; 0.15	
F/Kruskal-Wallis	11.26; 211.07; 2.75		12.32	18.59	24,90; 62.32; 4.18		13.17	18.45	19.23	5.89	8.07; 137.62; 1.46		13.20; 181.08; 1.70	
$DF_{residuals}$	48		5	5	48		5	5	5	5	48		48	

[1]Data transformed using Box-cox method for analysis. [2]Median ± IQR

	Plant height (cm)[1]		**Number of leaves[1]**		**Aerial fresh weight (g)[1]**		**Root weight (g)[1]**		**Aerial dry weight (g)[1]**		**Root dry weight (g)[1]**		**Total dry weight (g)[1]**	
	Dn7	**Custer**	**Dn7**	**Custer**	**Dn7**	**Custer**	**Dn7**	**Custer**	**Dn7**	**Custer**	**Dn7**	**Custer**	**Dn7**	**Custer**
check	35.60 ± 5.30 a	32.40 ± 5.10 a	5.00 ± 0.00 a	4.00 ± 0.00 a	0.29 ± 0.14 a	0.41 ± 0.13 a	0.16 ± 0.07 a	0.16 ± 0.08 a	0.04 ± 0.01 a	0.04 ± 0.01 a	0.03 ± 0.03	0.04 ± 0.03	0.06 ± 0.01 a	0.06 ± 0.01 a

Continued

bcoa	19.00 ± 2.70 b	16.50 ± 2.00 b	2.00 ± 1.00 b	3.00 ± 0.00 b	0.03 ± 0.02 ab	0.03 ± 0.01 b	0.07 ± 0.02 ab	0.06 ± 0.02 ab	0.02 ± 0.00 ab	0.01 ± 0.00 a	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01 b	0.02 ± 0.00 b
rwa1	18.60 ± 1.00 b	17.30 ± 1.30 b	2.00 ± 0.00 b	2.00 ± 1.00 b	0.02 ± 0.00 b	0.02 ± 0.01 b	0.04 ± 0.02 b	0.05 ± 0.05 b	0.02 ± 0.00 ab	0.02 ± 0.00 a	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.01 b	0.02 ± 0.00 b
rwa2	18.00 ± 0.80 b	17.50 ± 4.70 b	3.00 ± 0.00 b	3.00 ± 0.00 b	0.02 ± 0.01 b	0.02 ± 0.01 b	0.04 ± 0.01 b	0.07 ± 0.01 ab	0.01 ± 0.00 b	0.02 ± 0.01a	0.01 ± 0.01	0.02 ± 0.01	0.03 ± 0.01 b	0.03 ± 0.01 b
bcoa + rwa1	20.50 ± 1.80 b	16.00 ± 0.00 b	3.00 ± 0.00 b	3.00 ± 1.00 b	0.03 ± 0.00 ab	0.02 ± 0.00 b	0.03 ± 0.02 b	0.03 ± 0.02 b	0.02 ± 0.00 ab	0.01 ± 0.00 a	0.02 ± 0.01	0.01 ± 0.01	0.03 ± 0.01 b	0.02 ± 0.00 b
bcoa + rwa2	20.00 ± 2.00 b	13.50 ± 1.00 c	3.00 ± 0.00 b	2.00 ± 0.00 b	0.02 ± 0.00 b	0.02 ± 0.01 b	0.05 ± 0.00 b	0.03 ± 0.02 b	0.02 ± 0.00 ab	0.01 ± 0.00 a	0.02 ± 0.01	0.01 ± 0.01	0.03 ± 0.01 b	0.02 ± 0.00 b
$P_{aphid;\ cultivar;\ interaction}$	0.01	<0.01	<0.01	<0.01	0.01	<0.01	<0.01	<0.01	0.01	0.07	0.76	0.09	<0.01; 0.50; 0.61	
F/ Kruskal-Wallis	16.20	21.12	18.32	16.98	16.66	18.74	17.48	19.69	14.79	10.12	2.58	9.51	7.33; 0.47; 0.73	
$DF_{residuals}$	5	5	5	5	5	5	5	5	5	5	5	5	48	

[1]Kruskal-Wallis test performed; median ± IQR.

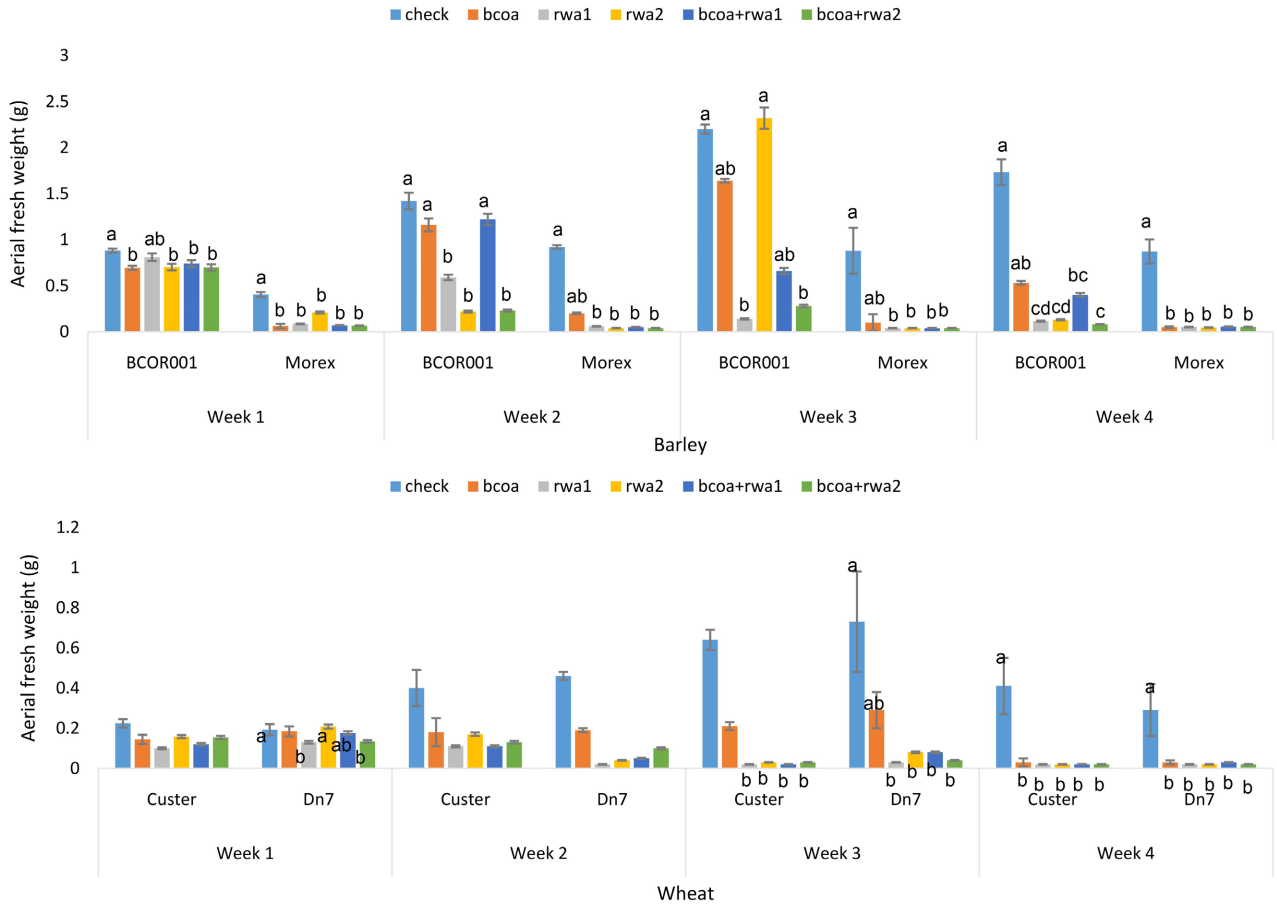

Figure 1. Growth response [aerial fresh weight in (g)] of barley and wheat cultivars to aphid infestation during 4 weeks of infestation. Means ± 1SE followed by the same letter do not differ from each other ($\alpha = 0.05$).

For these parameters, barley aerial fresh weight (g) was equally impacted by

aphid infestations, regardless of the type of treatment or barley genotype, with the reductions ranging up to 20% of the mass for BCOR001 and up to 80% for Morex. A reduction in aerial dry weight (g) was observed only in the susceptible Morex, with the plants under BCOA + RWA2 infestation showing a significant reduction of about 66%. For wheat, both genotypes showed reductions in plant height (cm) and aerial fresh weight (g) under rwa1 infestation; however, they were not impacted by infestations of rwa2.

In the second evaluation, conducted two weeks after infestation, significant interactions were observed only in barley, for the number of leaves [$F(5, 48) = 6.88$, $p < 0.001$], root weight (g) [$F(5, 48) = 3.04$, $p = 0.02$], root dry weight (g) [$F(5, 48) = 2.96$, $p = 0.02$], and total dry weight (g) [$F(5, 48) = 2.51$, $p = 0.04$]. In general, Morex plants had a significant reduction of about 50% in the number of leaves, when infested with either RWA biotypes and their combination with BCOA. This reduction also affected other parameters, such as aerial fresh weight (g) and root weight (g), in which plants had reduction up to 95% and 85%, respectively. However, in the resistant genotype BCOR001, the same pattern was not observed, showing no reduction in root fresh or dry weight (g) when infested with any aphid treatment.

In wheat, although no significant interactions were observed, the aphid effect was noted on almost all other morphological parameters in both genotypes, except for plant height [cm; $F(5) = 2.18$, $p = 0.07$] and root dry weight [$\chi^2(5) = 7.66$, $p = 0.18$ and $\chi^2(5) = 5.94$, $p = 0.31$ for Dn7 and Custer, respectively].

On the third evaluation, no two-way ANOVA tests were performed because assumptions were not met except for root weight (g) on wheat. The aphid treatments, however, impacted all evaluated parameters. Differential responses between genotypes were observed in the plant height for barley, where the resistant genotype BCOA001 was similar to the control ($\chi^2(5) = 15.68$, $p = 0.34$), and very different than susceptible Morex ($\chi^2(5) = 13.38$, $p = 0.02$). Differential responses were also observed in the wheat root dry weight (g) where the resistant Dn7 showed reductions but no significant differences ($\chi^2(5) = 10.45$, $p = 0.06$) compared to its counterpart Custer ($\chi^2(5) = 14.28$, $p = 0.01$).

After four weeks of infestation, significant interactions were observed in barley for plant height (cm, $F(5, 48) = 2.75$, $p = 0.03$] and aerial fresh weight [g, $F(5, 48) = 4.18$, $p = 0.04$]. Interestingly, aerial dry weight (g) was not significantly reduced in either barley or wheat resistant genotypes [$\chi^2(5) = 10.12$, $p = 0.07$ and $\chi^2(5) = 9.50$, $p = 0.09$ for Morex and Custer respectively]; while root dry weight (g) was not impacted in either wheat genotypes [$\chi^2(5) = 2.58$, $p = 0.06$ and $\chi^2(5) = 9.50$, $p = 0.09$ for Dn7 and Custer respectively].

Assimilation rates (μmol CO_2 $m^{-2} \cdot s^{-1}$) generally increased with higher intercellular CO_2 concentration (Ci) across all treatments. However, the susceptible and resistant barley and wheat genotypes exhibited varying efficiencies in CO_2 assimilation, as reflected by their distinct curves (**Figure 2** and **Figure 3**). As expected, the non-infested treatment (check) consistently showed the highest assimilation rates at all CO_2 levels in both genotypes for barley and wheat, while aphid infesta-

tion reduced the assimilation rate. However, the magnitude of reduction differed significantly.

For wheat, the susceptible genotype Custar experienced the strongest reductions in assimilation rate under single-aphid infestations, RWA1 and RWA2, followed by BCOA + RWA2 and BCOA (**Figure 2(A)**). Plants under BCOA + RWA1 showed a moderate reduction. Interestingly, in the resistant genotype Dn7, the lowest curve was observed in the BCOA treatment, whereas the other treatments fell in the middle range, with no distinct differences between them (**Figure 2(B)**).

The susceptible barley Morex had the lowest curve under BCOA + RWA1 infestation, while all other aphid treatments impacted the assimilation rate similarly, especially under high CO_2 concentrations. Notably, in the resistant genotype BCOR001, the response of plants under the BCOA treatment was similar to the check, followed by RWA2, BCOA + RWA2, BCOA + RWA1, and RWA1.

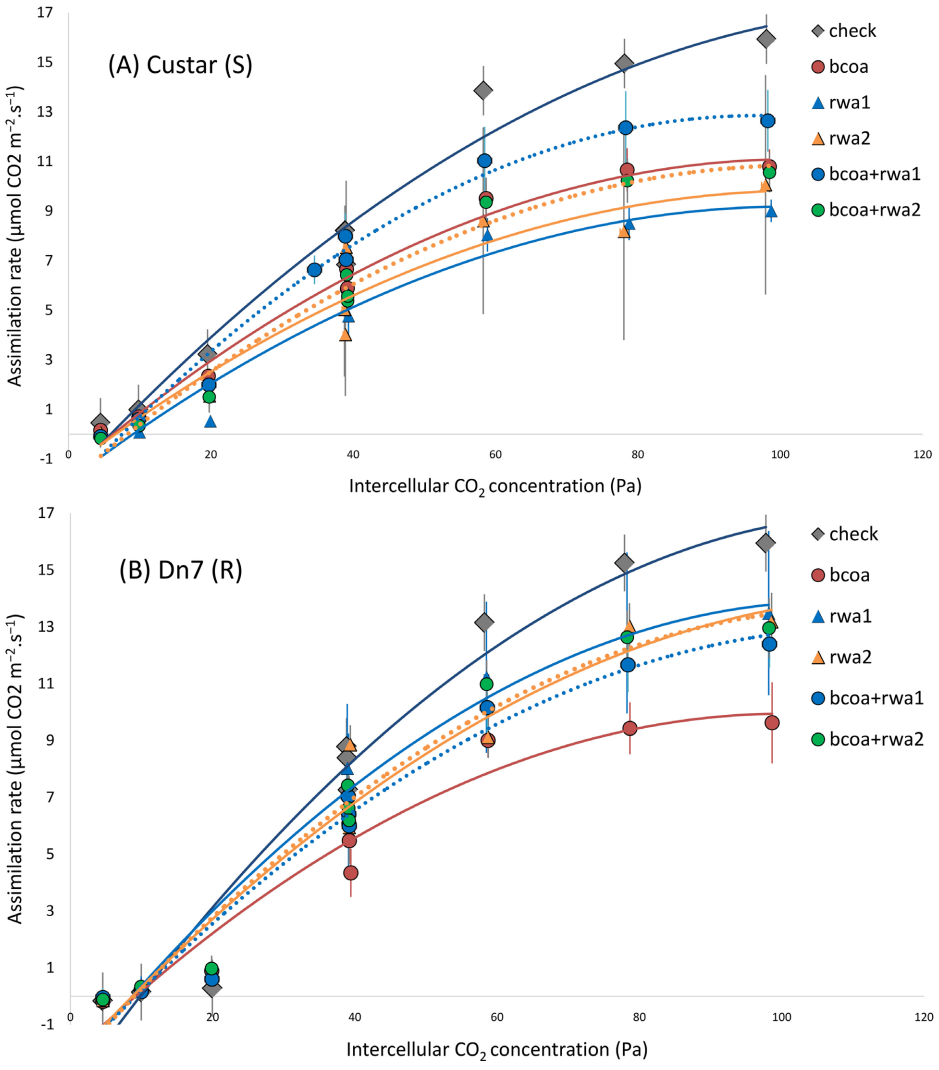

Figure 2. Assimilation rate (µmol CO_2 $m^{-2}{\cdot}s^{-1}$) versus intercellular CO_2 concentration (Ci) in pascals (Pa) susceptible "Custar" and resistant "Dn7" wheat genotypes evaluated 14 days post-infestation with different aphid treatments.

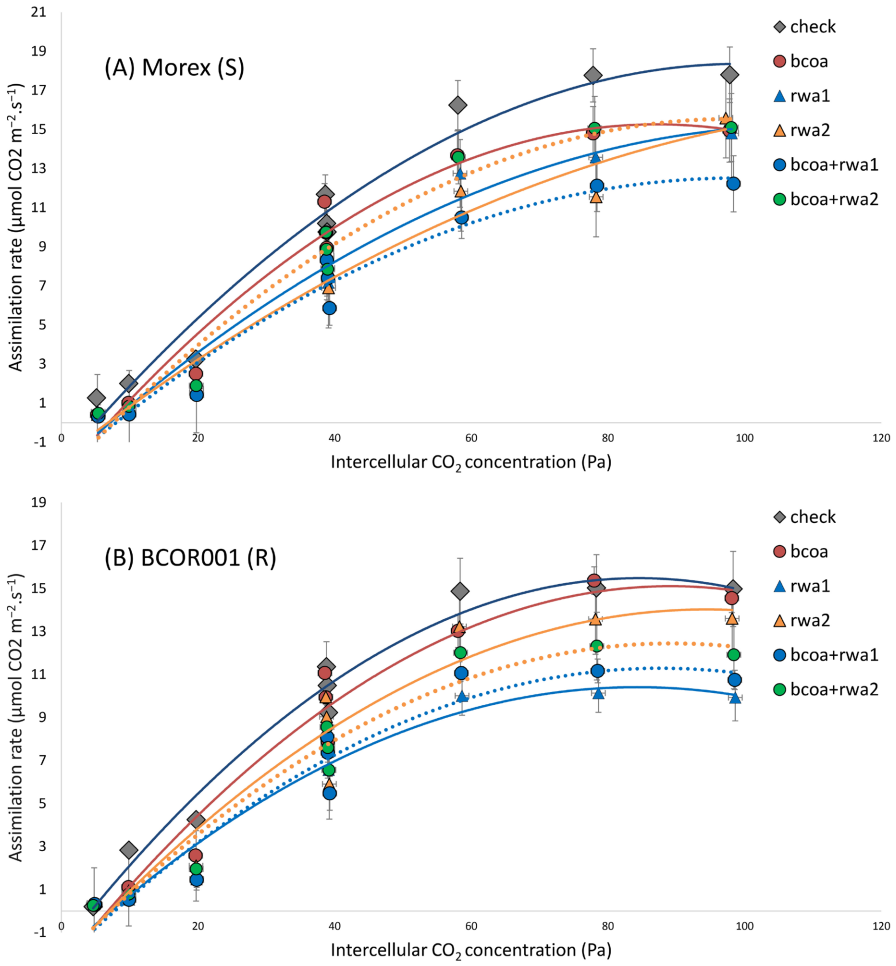

Figure 3. Assimilation rate (µmol CO_2 m^{-2}·s^{-1}) versus intercellular CO_2 concentration (Ci) in pascals (Pa) for susceptible "Morex" and resistant "BCOR001" barley genotypes evaluated 15 days post-infestation with different aphid treatments.

4. Discussion

Although previous studies have identified and reported barley and wheat genotypes resistant to a single aphid species [6] [14] [18] [30]-[32] and multiple aphid species [9] [17], to the best of our knowledge, this is the first study to compare resistant and susceptible barley and wheat genotypes under simultaneous BCOA and RWA1 or RWA2 infestations.

In this study, we observed differences between wheat and barley responses to BCOA, RWA1, and RWA2 within the first week of infestation. However, the differences within aphid treatments (BCOA, RWA1, RWA2, BCOA + RWA1, and RWA2 + BCOA) were noted for either susceptible or resistant genotypes of barley and wheat.

As anticipated, differential responses between resistant and susceptible cultivars were observed within one week of infestation by the aphid species for which resistance was selected. Cross-resistance was also noted in resistant cultivars. The resistant barley cultivar BCOR001 showed no impact on the number of leaves or

aerial dry weight (g) regardless of aphid species or combination, in contrast to the susceptible cultivar Morex, which experienced a reduction in the number of leaves across all aphid-infested treatments. In contrast, during the first week, no impact from aphid feeding was observed on the number of leaves, and aerial and root dry weight was noted for either wheat genotype. Number of leaves, as well the plant dry weight are important parameters in cereal crops for early growth stages because they directly corelate with grain yield [33].

In our experiment, we also observed differential responses to aphid infestation between crops. Although previous work has reported that wheat infested with aphids tends to experience a more rapid reduction in fitness compared to barley [9], our results showed that aphid-infested wheat genotypes exhibited reductions in the number of leaves, root weight (g), and aerial dry weight (g) only after two weeks. In contrast, barley showed reductions in number of leaves and aerial fresh weight after just one week of infestation. Previous studies have observed reductions in barley aerial fresh and dry matter accumulation in aphid-infested plants within two days of infestation [30]. The differential responses between crops might be explained by the speed of the plant's response to stress.

Although Dn7 is reported to be resistant to RWA1 and RWA2 [13] [14], morphological responses in our treatments did not demonstrate clear resistance. In general, all aphid treatment equivalently reduced some parameters in Dn7, especially at three and four weeks after infestation.

Both susceptible and resistant genotypes of barley and wheat exhibited higher photosynthetic assimilation rates under non-infested (check) conditions, suggesting that aphid feeding negatively impacted photosynthetic efficiency. Additionally, the A/Ci curves revealed distinct physiological responses between susceptible and resistant genotypes.

The susceptible wheat genotype Custer exhibited a relatively uniform reduction in assimilation rate across different CO_2 concentrations. In contrast, the resistant genotype Dn7 showed a partial reduction in assimilation rate under treatments with RWA1, RWA2, and their combinations with BCOA, when compared to the BCOA treatment alone, which resulted in the lowest assimilation rate across all CO_2 concentrations. Interestingly for barley, the resistant genotype BCOR001 under BCOA infestation maintained assimilation rates comparable to the check under high CO_2 concentrations. This suggests a potential tolerance mechanism that allows compensation or recovery of photosynthetic capacity in response to BCOA feeding at elevated intercellular Ci levels [34]. The resistant barley's ability to maintain high assimilation under BCOA infestation could be linked to efficient ribulose-1,5-bisphosphate (RuBP) regeneration and CO_2 fixation, processes that are directly linked to photosynthetic performance and, consequently, crop yield [35].

The findings of this study confirm that aphid infestation, whether by BCOA, RWA1, RWA2, or their combinations, results in significant reductions in morphological and physiological parameters in both barley and wheat. However, the

magnitude and timing of the impact varied considerably between resistant and susceptible genotypes, supporting the hypothesis that plant genotype plays a critical role in controlling host responses to aphid stress.

Contrary to our expectations, combinations of aphids on susceptible varieties did not cause more damage than single aphid species, suggesting that Economic Injury Levels (EILs) do not need to distinguish aphid species. Resistant cultivars generally responded similarly to single or combination treatments suggesting the existence of cross-resistance. The observation suggests that EILs can be standardized across different aphid species, which simplifies pest management protocols and reduces the complexity of monitoring and intervention strategies. Farmers can implement a unified economic threshold, streamlining decision-making processes and potentially lowering management costs.

It is important to note that aphid density was not controlled during the four-week evaluation period of our experiments, resulting in variability in the number of aphids per plant. It is likely that each treatment experienced different population growth rates of the species used. This variability may have impacted wheat's tolerance capacity, as both aphid density and feeding duration are known to influence plant tolerance [34]. To better understand these interactions, future studies should include species-specific life table analyses to assess the impact of single and combined aphid infestations on different barley and wheat genotypes [8]. Additionally, the variability in aphid density highlights the dynamic nature of pest populations. IPM strategies should incorporate regular monitoring and adaptive management practices to respond to fluctuating pest pressures, considering the differences in aphid population growth in different genotypes. This could involve periodic sampling and adjusting control measures based on real-time data, ensuring that interventions are both effective and economical.

Additionally, our results highlight the importance of evaluating plant resistance not only through visual symptoms but also through physiological parameters, including CO_2 assimilation and dry weight retention. Finally, the variable response of Dn7 under uncontrolled aphid densities indicate that resistance can be overcome under complex infestation dynamics, reinforcing the need for integrated pest management (IPM) approaches alongside the adoption of resistant genotypes.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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