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Physiological and Morphological Responses of Susceptible and Resistant Barleys to Bird Cherry-Oat Aphid Feeding

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Abstract

The bird cherry-oat aphid (*Rhopalosiphum padi* [Linnaeus, 1758]) is considered a key pest of cereal crops worldwide, causing direct damage through sap feeding and by acting as a vector for viral diseases. Managing aphids is challenging because of their biology and potential resistance to insecticides. Developing resistant barley genotypes is a sustainable strategy for managing BCOA. In this study, we assessed responses of susceptible "Morex" and resistant "BCO R001" barley, *Hordeum vulgare* L. genotypes to different initial BCOA densities (0, 50, 100 or 200 aphids.plant^{-1}). Physiological and morphological parameters were measured weekly for four weeks after infestation. Chlorophyll content, photosynthetic rate, plant aerial fresh and dry weight were greater for the resistant cultivar at lower aphid abundances and up to three weeks after infestation. Carbon assimilation curves (A/Ci) of infested "BCO R001" were similar to controls 15 days post infestation, differing from Morex. However, BCOA infestation of 50 aphid.plant^{-1} for two weeks negatively impacted the fitness of both genotypes. Initial resistance by BCO R001 to BCOA infestation can allow growers and natural enemies more time contributing to more effective and sustainable management of BCOA infestations.

Keywords

Plant Tolerance, Hemiptera, Piercing-Sucking Injury, Cereal Crops

1. Introduction

The bird cherry-oat aphid, *Rhopalosiphum padi* (Linnaeus, 1758), BCOA, can feed on numerous species, including all the major cereal crops and pasture

grasses, and is a significant pest of cereal crops worldwide [1] [2]. This aphid causes direct damage to the plant through feeding on the phloem of the plants, which can reduce plant growth, and decrease yield, and quality of the harvested crop [3] [4]. Additionally, BCOA is the most important vector for barley yellow dwarf virus (BYDV) along with other viruses [2] [5].

Managing aphids can be difficult because of their biology that includes parthenogenic reproduction and their ability to quickly spread through active flight passive dispersal [6]. Moreover, aphid populations have developed resistance to insecticides [7]. Consequently, one of the most effective and sustainable strategies for managing BCOA infestations is through the development and deployment of resistant barley genotypes [8].

Mechanisms of plant resistance to herbivores can be generalized as antibiosis, antixenosis, and tolerance [8] [9]. Antibiosis involves the host plant negatively impacting the insect's biology, leading to decreased survival, reproduction, or growth rate, while antixenosis refers to the non-preference of the insect for certain host plants [8].

In contrast, tolerance is defined by a plant's capacity to withstand or recover from injury caused by herbivores, achieved through growth or compensatory physiological mechanisms [10]. While antixenosis and antibiosis can decrease the aphid population and thus, minimize crop loss, these traits also exert selection pressure on the aphid population, potentially leading to a biotype shift towards resistance. In contrast, tolerance is viewed as a more sustainable pest management approach, because it solely involves the plant's response and does not result in resistance development within the target pest population [9] [10].

Previous studies have identified barley genotypes with various levels of resistance to aphids, as well as the underlying genetic mechanisms involved [11] [12]. However, understanding the mechanisms of resistance or susceptibility of barley genotypes to BCOA infestations can aid in developing barley genotypes that are more resistant to aphid attack. This could potentially reduce the use of insecticides, which can have negative impacts on environmental and human health. Thus, in this paper, we investigate the responses of resistant and susceptible barley genotypes under four different BCOA infestation densities.

2. Material and Methods

2.1. Aphid Culture and Plants

In August 2017, a group of BCOA colonies was initially collected from barley, *H. vulgare* fields in Payne County, Oklahoma, United States. These colonies were maintained by cultivating aphids on "Eight-Twelve" barley, a susceptible cultivar [13]. The barley was grown in 4.4 L pots equipped with 45 cm tall cylindrical Lexan® sleeve cages obtained from SABIC Polymershapes, Tulsa OK. These cages were ventilated using organdy cloth coverings positioned at the top. To ensure the continuity of the colony, plants were replaced with fresh seedlings every two weeks. The pots containing the seedling barley were placed on greenhouse

benches illuminated by two T-8 fluorescent lights. These lights were configured to provide supplementary lighting for a photoperiod of 14 hours light to 10 hours darkness, with a temperature range of 21˚C - 31˚C.

The barley seeds utilized in the experimental procedures were sourced from the USDA-ARS located in Stillwater, Oklahoma. Specifically, the material selected for testing was "Morex", recognized as a susceptible [14] [15] six-rowed spring malting barley, and "BCO R001" a resistant selection from "CI-1969". "CI-1969", an accession from the National Small Grains Collection, has been cataloged as resistant to BYDV [16].

2.2. Initial Infestation

Fourteen days after planting, barley seedlings were infested with 0, 50, 100 and 200 BCOA per plant. Nymphs and adults' aphids from the colony were counted and placed onto plant leaves using a camel hair brush. Following the initial infesting of each plant, the aphids were allowed to increase naturally. Control (check, non-infested) plants were caged similarly to infested plants.

2.3. Gas Exchange Response Measurements

Plant physiological responses were recorded at 1, 2, 3 and 4 weeks after aphid introduction for both barley genotypes. The aphids were removed from plants before measuring the physiological responses. There were four replications, with 4 levels of infestations for each barley genotype arranged in a complete randomized design where an experimental unit was a barley plant.

A portable photosynthesis system (model LI-6400, LI-COR, Lincoln, NE) was used to measure the gas exchange responses, following methods described by Paudyal [17] and Carey [18]. A stomatal ratio of 1 was adopted, as abaxial and adaxial stomatal densities exhibit similarity [19]. Measurements were conducted within greenhouse conditions (maintained at 26˚C ± 5˚C and relative humidity 75% ± 10%). For each trial, two leaves were placed into the Li-Cor's 6 cm^2 measurement chamber. Net photosynthetic rate (μmol CO_2 $m^{-2}{\cdot}s^{-1}$), stomatal conductance (mol H_2O $m^{-2}{\cdot}s^{-1}$) and CO_2 assimilation (μmol CO_2 $m^{-2}{\cdot}s^{-1}$) were recorded at 1200 μmol photons $m^{-2}{\cdot}s^{-1}$ light intensity with a reference carbon dioxide of 400 ppm generated from a 12 g CO_2 cylinder connected to the meter. Measurements were taken at days 10, 15, and 20 post-infestations.

2.4. Chlorophyll Concentration

Chlorophyll content was determined using a SPAD model 502 chlorophyll meter (Minolta, Tokyo, Japan). To ensure accuracy, three readings were taken from each seedling, and the mean SPAD value was computed. This handheld meter functions by absorbing light within the range of 430 to 750 nm as it moved across a leaf surface [20].

2.5. Plant Growth

Weekly, after the collection of physiological data, morphological paraments were

measured. Plant height (cm) was determined by measuring from the soil line to the tip of the plant. The number of true leaves was counted, the plant was harvested and the aerial fresh weight (g) and the root fresh weight (g) were determined. The upper portion of the plant from the soil line was clipped and weighed, as was the root system after dislodging soil. Tissues were labeled and dried in a drying oven at 50˚C for 72 h, before being reweighed following methods described by Limaje *et al.* [21]. Subsequently, aerial dry weight (g), root dry weight (g), and total dry weight (g) were calculated from the reweighed tissues.

2.6. CO_2 Response

Using 14 d-old plants, we evaluated the CO_2 response (A/Ci curves) of the susceptible "Morex" and the resistant "BCO R001" genotypes under two different densities (0 = check and 50 aphids.plant^{-1}), with 5 replications, 10, 15 and 20 d after infestation. CO_2 assimilation rates were measured adopting CO_2 concentrations ranging from 50 to 1000 ppm (sequence of 400, 200, 100, 50, 400, 400, 600, 800, 1000, 2000 and 400 ppm) and at 1400 µmol photon $m^{-2}{\cdot}s^{-1}$ light intensity following methods described by Paudyal *et al.* [17].

2.7. Statistical Analysis

The statistical analyses were conducted in the R computing environment, utilizing the "AgroR" package [22] and "ggplot2" package [23] for the graphs. Before proceeding with the ANOVA, we performed exploratory data analysis to assess the assumptions of normality of residuals [24] and homogeneity of variances [25]. When necessary, the data were transformed by sin(x) or using Box-Cox transformation [26]. The data were analyzed using a two-way ANOVA, considering the factors genotypes and aphid densities levels, with a significance level of $\alpha = 0.05$. The A/Ci curves were plotted with a polynomial regression model of order 2 in Excel software (Microsoft Office; Version 2019) for each genotype at 10, 15 and 20 d after infestation.

3. Results

Based on our analysis, the chlorophyll content (%) was affected by BCOA density from the first evaluation (one week after infestation) to the last one (four weeks after infestation; **Table 1**). Except for the initial assessment, all subsequent evaluations indicated a significant interaction between barley genotype and aphid density (**Table 1**). At the first evaluation, plants with less than 100 aphids.plant^{-1} had a reduction of 5% of chlorophyll content compared to the check (0 aphid.plant^{-1}; $p = 0.04$; $F = 3.29$, $DF = 24$).

Following the second week, distinct responses in chlorophyll content were observed in both susceptible and resistant barley genotypes and were affected by densities of BCOA. The resistant genotype "BCO R001" only had a reduction in chlorophyll content with 200 aphids.plant^{-1}, whereas the susceptible "Morex"

Table 1. Chlorophyll content (%) and gas-exchange responses of "BCO R001" and "Morex" barley genotypes under different levels of bird cherry-oat aphid infestation (aphids.plant^{-1}). Means ± 1SE followed by the same letter, lowercase in column (within aphid density) and uppercase in the line (within barley cultivars), do not differ from each other ($\alpha = 0.05$).

		Chlorophyll Content (%)		Photosynthetic Rate (μmol CO_2 m^{-2}·s^{-1})		Stomatal Conductance (μmol CO_2 m^{-2}·s^{-1})	
		1 week after infestation					
		BCO R001[1]	Morex[1]	BCO R001	Morex	BCO R001	Morex
Aphid.plant^{-1}	0	32.70 ± 0.90 a	32.50 ± 1.15 a	10.87 ± 0.94	11.23 ± 0.92	0.04 ± 0.01	0.03 ± 0.00
	50	31.08 ± 2.70 ab	31.33 ± 0.61 ab	12.05 ± 1.47	14.02 ± 1.26	0.06 ± 0.02	0.05 ± 0.02
	100	26.63 ± 1.00 b	29.13 ± 1.39 b	11.11 ± 1.27	11.77 ± 1.82	0.05 ± 0.01	0.05 ± 0.01
	200	29.00 ± 1.68 ab	33.13 ± 1.96 ab	11.46 ± 0.95	12.83 ± 1.01	0.06 ± 0.01	0.05 ± 0.01
Statistics	p; $F_{genotype}$	0.14; 2.30		0.23; 1.53		0.34; 0.95	
	p; $F_{density}$	0.04; 3.29		0.42; 0.98		0.42; 0.97	
	p; $F_{genotypexdensity}$	0.48; 0.84		0.91; 0.17		0.94; 0.14	
	$DF_{residuals}$	24		24		24	
		2 weeks after infestation					
		BCO R001	Morex	BCO R001[2]	Morex[2]	BCO R001[2]	Morex[2]
Aphid.plant^{-1}	0	31.90 ± 2.60 aA	38.65 ± 0.85 aA	12.29 ± 1.05 aA	13.63 ± 1.51 aB	0.12 ± 0.01 aA	0.09 ± 0.02 aB
	50	25.15 ± 2.78 abA	27.33 ± 2.32 aA	7.18 ± 0.69 bA	6.11 ± 0.41 bB	0.11 ± 0.01 aA	0.09 ± 0.01 aB
	100	24.50 ± 1.64 abA	16.30 ± 4.79 bB	6.77 ± 0.34 bA	4.42 ± 0.71 bB	0.11 ± 0.00 aA	0.08 ± 0.01 aB
	200	21.68 ± 2.02 4bA	10.33 ± 1.5 2bB	4.64 ± 0.44 bA	1.50 ± 0.64 bB	0.10 ± 0.01 bA	0.04 ± 0.01 aB
Statistics	p; $F_{genotype}$	0.02; 6.24		0.03; 5.16		<0.001; 20.15	
	P; $F_{density}$	<0.001; 13.87		<0.001; 53.88		0.03; 3.64	
	P; $F_{genotypexdensity}$	0.01; 4.37		0.06; 2.91		0.36; 1.11	
	$DF_{residuals}$	22		22		22	
		3 weeks after infestation					
		BCO R001	Morex	BCO R001[3]	Morex[3]	BCO R001	Morex
Aphid.plant^{-1}	0	30.38 ± 2.05 aA	33.47 ± 2.48 aA	4.41 ± 1.05 aA	5.12 ± 2.05 aA	0.013 ± 0.003	0.021 ± 0.009
	50	10.88 ± 4.31 bB	24.47 ± 3.91 bA	2.62 ± 1.36 bB	3.96 ± 1.25 aA	0.029 ± 0.011	0.004 ± 0.018
	100	8.70 ± 1.50 bA	5.30 ± 0.96 cA	1.19 ± 0.44 bA	0.033 ± 0.045 bA	0.021 ± 0.004	0.004 ± 0.001
	200	5.25 ± 0.83 bA	7.30 ± 1.57 cA	0.07 ± 0.27 bA	0.29 ± 0.12 bA	0.008 ± 0.003	0.013 ± 0.005
Statistics	p; $F_{genotype}$	0.02; 6.24		0.29; 1.18		0.18; 1.96	
	P; $F_{density}$	<0.001; 13.87		<0.001; 37.37		0.12; 2.20	
	P; $F_{genotypexdensity}$	0.01; 4.37		0.02; 4.21		0.07; 2.76	
	$DF_{residuals}$	22		22		22	
		4 weeks after infestation					
		BCO R001	Morex	BCO R001[1]	Morex[1]	BCO R001	Morex
Aphid.plant^{-1}	0	30.38 ± 2.05 aA	33.47 ± 2.48 aA	4.41 ± 1.05 aA	5.12 ± 2.05 aA	0.01 ± 0.00 abA	0.02 ± 0.01 aA
	50	10.88 ± 4.32 bB	24.47 ± 3.91 bA	2.62 ± 1.36 bB	3.96 ± 1.25 aA	0.03 ± 0.01 aA	0.04 ± 0.02 aA
	100	8.70 ± 1.50 bA	5.30 ± 1.36 cA	1.19 ± 0.44 bA	0.03 ± 0.06 bA	0.02 ± 0.00 abA	0.00 ± 0.00 bB
	200	5.25 ± 0.83 bA	7.30 ± 2.21 cA	0.07 ± 0.27 bA	0.33 ± 0.17 bA	0.01 ± 0.00 bA	0.02 ± 0.00 aA

Continued

Statistics	p; $F_{genotype}$	0.05; 4.22	0.11; 2.84	0.91; 0.01
	P; $F_{density}$	<0.001; 41.00	<0.001; 29.57	0.06; 2.99
	P; $F_{genotypexdensity}$	0.03; 3.62	0.03; 3.60	0.03; 3.72
	$DF_{residuals}$	20	20	20

[1]No significant interaction, test performed for density. [2]No significant interaction, test performed for density and genotype separately. [3]Data were transformed using the function Box-Cox for analysis.

was impacted with densities of 100 and 200 aphids.plant^{-1} (p = 0.01; F = 4.37; DF = 22). By the third and fourth weeks post-infestation, both barley genotypes had the chlorophyll content reduced with densities staring from 50 aphids.plant^{-1} in comparison to the control (p = 0.01, 4.37; DF = 22; and p = 0.03; F = 3.62; DF = 20, respectively). Compared with "BCO R001", "Morex" showed an even more substantial reduction in chlorophyl when exposed to 100 and 200 aphids.plant^{-1} (Table 1).

Photosynthetic rate (µmol CO_2 $m^{-2}{\cdot}s^{-1}$) was not impacted by any of the tested aphid densities in one week after infestation for either variety (p = 0.91; F = 0.17; DF = 24). After two weeks of infestation, there was a significant difference between genotypes and among aphids' densities, where "BCO R001" demonstrated a notably higher photosynthetic rate compared to "Morex" (p = 0.03; F = 5.16; DF = 24). By week 2, plants infested with 50 and 100 aphids.plant^{-1} had lower photosynthetic rates compared with the control (p < 0.001; F = 53.88; DF = 24). After three and four weeks of infestation, there was a significant interaction between genotype and aphid density (p = 0.02; F = 4.17; $DF_{residuals}$ = 22; and p < 0.001; F = 29.57; $DF_{residuals}$ = 20). Interestingly, "BCO R001" experienced a reduction in photosynthetic rate (µmol CO_2 $m^{-2}{\cdot}s^{-1}$) with all aphid densities, while "Morex" was impacted by densities starting with 100 aphid.plant^{-1} (Table 1).

A significant difference in stomatal conductance (mol H_2O $m^{-2}{\cdot}s^{-1}$) was only observed after two and four weeks of infestation. After two weeks of infestation, there was significant reduction conductance for both genotypes with density of 200 aphids.plant^{-1} (p = 0.03; F = 3.64; DF = 24). Four weeks after infestation, there was a significant interaction between aphid density and genotypes tested (p = 0.03; F = 3.72; DF = 20). Specifically, "Morex" exhibited a reduction in response when subjected to an aphid density of 100 aphids.plant^{-1}, whereas "BCO R001" had a reduction in response at the higher density of 200 aphids.plant^{-1} (Table 1).

Plant height (cm) was only impacted by aphid density after two weeks of infestation (Table 2). Two weeks after infestation, there was a significant difference between the check and plants with aphid densities of 100 and 200 aphids.plant^{-1}. Three weeks and four after infestation, plants with 50 or more aphids.plant^{-1} had reduced height compared to the check, and plants with 200 aphids.plant^{-1} were the shortest (Table 2).

The number of leaves was impacted by aphid density in all the evaluation periods

Table 2. Plant growth parameters of barley genotypes "BCO R001" and "Morex" under different levels of bird cherry-oat aphid infestation (aphids.plant^{-1}). Means ± 1SE followed by the same letter, lowercase in column (within aphid density) and uppercase in the line (within barley cultivars), do not differ from each other ($\alpha = 0.05$).

		Plant height (cm)		Number of leaves		Aerial fresh weight (g)		Root fresh weight (g)	
		1 week after infestation							
		BCO R001	Morex	BCO R001[1]	Morex[1]	BCO R001[1]	Morex[1]	BCO R001[1]	Morex[1]
Aphid.plant^{-1}	0	49.50 ± 2.72	41.25 ± 3.84	3.75 ± 0.25 a	3.25 ± 0.25 a	1.49 ± 0.15 a	1.20 ± 0.17 a	2.72 ± 0.35 a	4.02 ± 0.86 a
	50	45.75 ± 1.38	38.75 ± 2.59	3.25 ± 0.25 a	3.75 ± 0.25 a	1.21 ± 0.09 ab	1.19 ± 0.11 ab	2.75 ± 0.41 a	3.30 ± 0.68 a
	100	39.25 ± 1.93	40.25 ± 2.84	3.00 ± 0.00 b	3.00 ± 0.00 b	0.98 ± 0.14 b	0.88 ± 0.20 b	2.33 ± 0.42 ab	1.99 ± 0.40 ab
	200	42.25 ± 2.32	38.25 ± 3.17	3.25 ± 0.25 ab	3.00 ± 0.00 ab	1.11 ± 0.08 ab	0.88 ± 0.06 ab	1.30 ± 0.26 b	1.97 ± 0.38 b
Statistics	p; $F_{genotype}$	0.02; 5.74		0.66; 0.20		0.09; 3.04		0.14; 2.31	
	P; $F_{density}$	0.17; 1.80		0.03; 3.40		0.02; 4.14		0.01; 4.94	
	P; $F_{genotypexdensity}$	0.34; 1.17		0.10; 2.33		0.73; 0.43		0.46; 0.89	
	$DF_{residuals}$	22		22		22		22	
		2 weeks after infestation							
		BCO R001[1]	Morex[1]	BCO R001[2]	Morex[2]	BCO R001[2]	Morex[2]	BCO R001[1]	Morex[1]
Aphid.plant^{-1}	0	54.50 ± 2.10 a	50.00 ± 1.00 a	4.75 ± 0.25 aA	4.50 ± 0.50 aB	2.48 ± 0.11 aA	1.65 ± 0.20 aB	6.00 ± 1.21 a	6.10 ± 1.48 a
	50	46.75 ± 3.73 ab	45.75 ± 2.32 ab	4.00 ± 0.41 bA	3.75 ± 0.25 bB	0.88 ± 0.20 bA	0.64 ± 0.24 bB	2.64 ± 0.73 b	2.65 ± 0.82 b
	100	39.50 ± 4.87 b	43.50 ± 3.40 b	3.25 ± 0.25 cA	3.25 ± 0.25 cB	0.68 ± 0.18 bA	0.41 ± 0.09 bB	1.76 ± 0.33 b	2.51 ± 0.16 b
	200	44.50 ± 3.23 b	34.75 ± 1.75 b	3.75 ± 0.25 bcA	3.00 ± 0.00 bcB	0.54 ± 0.04 bA	0.35 ± 0.14 bB	1.71 ± 0.42 b	2.79 ± 0.28 b
Statistics	p; $F_{genotype}$	0.12; 2.64		0.04; 5.03		0.00; 20.13		0.82; 0.06	
	P; $F_{density}$	0.01; 5.61		<0.001; 8.91		<0.001; 41.49		<0.001; 12.25	
	P; $F_{genotypexdensity}$	0.20; 1.68		0.57; 0.69		0.27; 22.00		0.84; 22.00	
	$DF_{residuals}$	22		22		22		22	
		3 weeks after infestation							
		BCO R001[1]	Morex[1]	BCO R001[1]	Morex[1]	BCO R001[1]	Morex[1]	BCO R001[1]	Morex[1]
Aphid.plant^{-1}	0	58.50 ± 1.85 a	54.00 ± 1.53 a	5.00 ± 0.00 a	5.33 ± 0.33 a	3.04 ± 0.25 a	2.82 ± 0.41 a	3.59 ± 0.88 a	4.63 ± 1.03 a
	50	45.25 ± 2.96 b	41.67 ± 0.67 b	4.00 ± 0.41 b	4.00 ± 0.00 b	0.79 ± 0.20 b	0.60 ± 0.08 b	0.83 ± 0.23 b	1.67 ± 0.11 b
	100	43.25 ± 2.66 bc	33.75 ± 2.53 bc	3.50 ± 0.29 c	3.50 ± 0.29 c	0.71 ± 0.11 bc	0.12 ± 0.03 bc	0.83 ± 0.22 b	0.69 ± 0.12 b
	200	37.00 ± 0.82 c	34.50 ± 1.76 c	3.25 ± 0.25 bc	3.50 ± 0.29 bc	0.17 ± 0.05 c	0.27 ± 0.05 c	0.46 ± 0.06 b	0.93 ± 0.22 b
Statistics	p; $F_{genotype}$	0.12; 2.64		0.75; 0.10		0.19; 1.81		0.82; 0.06	
	P; $F_{density}$	<0.01; 5.61		<0.001; 16.41		<0.001; 2.59		<0.001; 12.25	
	P; $F_{genotypexdensity}$	0.20; 1.68		0.91; 0.18		0.09; 2.42		0.84; 0.27	
	$DF_{residuals}$	22		22		22		22	
		4 weeks after infestation							
		BCO R001[2]	Morex[2]	BCO R001[1]	Morex[1]	BCO R001[4]	Morex[4]	BCO R001[2,3]	Morex[2,3]
Aphid.plant^{-1}	0	58.50 ± 1.85 aA	54.00 ± 1.53 aB	5.00 ± 0.00 a	5.33 ± 0.33 a	3.04 ± 0.25aA	2.82 ± 0.41aA	3.59 ± 0.88 aB	4.63 ± 1.03 aA
	50	45.25 ± 2.96 bA	41.67 ± 0.67 bB	4.00 ± 0.41 b	4.00 ± 0.00 b	0.79 ± 0.20bA	0.60 ± 0.08bA	0.83 ± 0.23 bB	1.67 ± 0.11 bA
	100	43.25 ± 2.66 bcA	35.67 ± 2.33 bcB	3.50 ± 0.29 bc	3.67 ± 0.33 bc	0.71 ± 0.11bA	0.14 ± 0.03dB	0.83 ± 0.22 bcB	0.81 ± 0.05 bcA
	200	37.00 ± 0.82 cA	33.67 ± 2.19 cB	3.25 ± 0.25 c	3.33 ± 0.33 c	0.17 ± 0.05cB	0.30 ± 0.04cA	0.46 ± 0.06 cB	0.93 ± 0.31 cA

Continued

Statistics	p; $F_{genotype}$	0.01; 9.78	0.48; 0.51	0.03; 5.18	0.01; 8.07
	P; $F_{density}$	<0.001; 36.15	<0.001; 16.23	<0.001; 72.43	<0.001; 18.36
	P; $F_{genotypexdensity}$	0.74; 0.41	0.95; 0.12	<0.001; 14.04	0.27; 1.42
	$DF_{residuals}$	20	20	20	20

[1]No significant interaction, test performed for density. [2]No significant interaction, test performed for density and genotype separately. [3]Data were transformed using the function sin(x) for analysis. [4]Data were transformed using the function Box-cox for analysis.

(Table 2). Plants under density of 100 aphids.plants^{-1} after one week of infestation had a mean reduction of 0.5 leaf/plant compared to controls ($p = 0.03$; $F = 3.40$; $F = 22$). Plants under 50 aphids.plant^{-1}, exhibited average reductions of 0.79 and 1.14 leaf per plant after two and three weeks of infestation, respectively. Notably, this reduction became more pronounced at a density of 100 aphids.plant^{-1} ($p < 0.001$; $F = 8.91$; $DF = 22$; and $p < 0.001$; $F = 16.41$; $DF = 22$). At four-weeks after infestation, plants continued to be influenced by a density of 50 aphids.plant^{-1}, resulting in a reduction of 1.14 leaves per plant when compared to the control group. Under the more substantial infestation of 200 aphids.plant^{-1}, a more pronounced impact was observed, with a mean reduction of 1.86 leaves per plant ($p = 0.00$; $F = 16.23$; $DF = 20$).

Similar to the number of leaves, aerial fresh weight (g) was impacted by aphid density in all of the evaluation periods, and especially in the fourth week after infestation, where there was a significant interaction between aphid density and genotype ($p < 0.001$; $F = 14.04$; $DF = 20$; Table 2). Plants under density of 100 aphids.plant^{-1} had a significant mean reduction of about 30% of aerial fresh weight (g) after one week of infestation. Densities of 50 aphids.plant^{-1} were responsible for reductions of about 65% and 22% after two and three weeks of infestation, respectively. At the conclusion of four weeks following infestation, the tested genotypes exhibited similar responses with "BCO R001" showing reductions of up to 94% in aerial fresh weight, while "Morex" showing an approximate 90% decrease with an initial density of 200 aphids.plant^{-1}.

The root fresh weights were exclusively influenced by the densities of aphids, with no significant differences between genotypes and no notable interaction of the two factors tested (Table 2). One-week post-infestation, plants subjected to a density of 200 aphids.plant^{-1} exhibited a substantial reduction in root weight of approximately 50% compared to the control ($p = 0.01$; $F = 4.94$; $DF = 22$). Subsequent evaluations conducted two weeks post-infestation indicated significant reductions in root weight, with discernible impacts from the lowest initial density of 50 aphids.plant^{-1}. Plants assessed two and three weeks after infestation demonstrated root weight reductions exceeding 50%. By the fourth week of infestation, reductions in infested plants compared with controls were even greater. Specifically, plants exposed to 50 aphids.plant^{-1} experienced reductions of about 70%, while those subjected to 200 aphids.plant^{-1} had approximately 85%

reduction.

The aerial dry weight (g) displayed sensitivity to aphid density across all evaluation periods, with a significant interaction during the second and third weeks after infestation ($p < 0.001$; $F = 54.99$; $DF = 22$ and $p < 0.001$; $F = 48.80$; $DF = 20$; Table 3). In the initial week following infestation, a significant reduction of over 35% in aerial dry weight was observed in plants subjected to 100 aphids.plant^{-1}. During the second and third evaluation periods, both "Morex" and "BCO R001" exhibited impacts from infestations beginning at 50 aphids.plant^{-1}. Notably, "Morex" was more profoundly affected by infestations of 200 aphids.plant^{-1} during the second evaluation and by densities of 100 and 200 aphids.plant^{-1} during the third evaluation (Table 3).

The root dry weight (g) and total dry weight (g) were impacted by aphid density of 100 or more aphids.plant^{-1} beginning from the second week of infestation (Table 3). The only exception was observed in plants subjected to 50 aphids.plant^{-1} at the three-week interval after infestation. During this period, these plants exhibited an intermediate value that did not significantly differ from the control and those under 100 aphids.plant^{-1}.

In the initial assessment conducted at 10 days post-infestation, the CO_2 response (A/Ci curves) exhibited similar patterns for both infested and control barley plants across the tested genotypes (Figure 1). At 15-days post-infestation, a noticeable reduction in assimilation rate (μmol CO_2 $m^{-2}{\cdot}s^{-1}$) was observed in infested plants when compared to the control, which was especially evident in the susceptible genotype "Morex". At 20 days post infestation both genotypes showed a reduction in assimilation rates (Figure 1).

4. Discussions

The present study compared physiological and morphological responses of a known susceptible barley genotype "Morex" with a resistant genotype "BCO R001" to infestation by bird cherry-oat aphid (BCOA). Although the level of "BCO R001" resistance is not yet classified, our results indicate a degree of tolerance to BCOA. Distinct responses in terms of chlorophyll content (%), aerial weight (g), and aerial dry weight (g) were observed among infestation levels between the two genotypes, with "BCO R001" showing a lesser impact from aphid-induced injury.

Chlorophyll content is commonly employed as a parameter to investigate the physiological effects of hemipteran pests on plants, and it can also serve as a basis for calculating economic injury levels and economic thresholds [27]. The reduction in chlorophyll content is typically correlated with the population density of aphids and serves as an indicator of damage. The differential response in chlorophyll loss of "BCO R001" highlights that even when subjected to high BCOA infestations for two weeks, there was less impact in comparison to the susceptible "Morex" genotype.

The analysis of A/Ci curves further supports that "BCO R001" exhibited a degree

Table 3. Dry weight (g) of barley genotypes "BCO R001" and "Morex" under different levels of bird cherry-oat aphid infestation (aphids.plant^{-1}). Means ± 1SE followed by the same letter, lowercase in column (within aphid density) and uppercase in the line (within barley cultivars), do not differ from each other ($\alpha = 0.05$).

		Aerial dry weight (g)		Root dry weight (g)		Total dry weight (g)	
		1 week after infestation					
		BCO R001[1]	Morex[1]	BCO R001	Morex	BCO R001	Morex
Aphid.plant^{-1}	0	0.15 ± 0.01 a	0.14 ± 0.02 a	1.09 ± 0.38	0.88 ± 0.08	1.23 ± 0.38	1.01 ± 0.09
	50	0.12 ± 0.02 ab	0.12 ± 0.00 ab	1.18 ± 0.15	1.04 ± 0.23	1.29 ± 0.16	1.16 ± 0.24
	100	0.09 ± 0.02 b	0.09 ± 0.02 b	0.73 ± 0.14	0.86 ± 0.22	0.82 ± 0.15	0.94 ± 0.23
	200	0.10 ± 0.01 b	0.09 ± 0.00 b	0.84 ± 0.17	0.52 ± 0.14	0.93 ± 0.18	0.61 ± 0.14
Statistics	p; $F_{genotype}$	0.70; 0.15		0.36; 0.85		0.37; 0.84	
	P; $F_{density}$	<0.001; 5.93		0.18; 1.75		0.15; 1.94	
	P; $F_{genotypexdensity}$	0.95; 0.12		0.74; 0.42		0.75; 0.40	
	$DF_{residuals}$	24		24		24	
		2 weeks after infestation					
		BCO R001	Morex	BCO R001[1]	Morex[1]	BCO R001[1]	Morex[1]
Aphid.plant^{-1}	0	0.28 ± 0.01 aA	0.22 ± 0.03 bA	1.65 ± 0.45 a	1.41 ± 0.40 a	1.94 ± 0.44 a	1.63 ± 0.37 a
	50	0.10 ± 0.02 aB	0.14 ± 0.02 aB	0.78 ± 0.34 b	0.74 ± 0.22 b	0.87 ± 0.35 b	0.88 ± 0.23 b
	100	0.07 ± 0.02 aB	0.11 ± 0.01 aBC	0.33 ± 0.06 b	0.74 ± 0.10 b	0.40 ± 0.08 b	0.85 ± 0.11 b
	200	0.09 ± 0.01 aB	0.06 ± 0.01 aC	0.35 ± 0.06 b	0.58 ± 0.15 b	0.44 ± 0.06 b	0.64 ± 0.15 b
Statistics	p; $F_{genotype}$	0.12; 2.63		0.94; 0.01		0.43; 0.63	
	P; $F_{density}$	<0.001; 56.00		0.001; 7.43		<0.001; 8.29	
	P; $F_{genotypexdensity}$	0.01; 5.00		0.62; 0.61		0.52; 0.78	
	$DF_{residuals}$	22		22		22	
		3 weeks after infestation					
		BCO R001[2]	Morex[2]	BCO R0012[1,2]	Morex[1,2]	BCO R001[1,3]	Morex[1,3]
Aphid.plant^{-1}	0	0.43 ± 0.02 aA	0.38 ± 0.05 aB	1.13 ± 0.36 a	1.40 ± 0.38 a	1.55 ± 0.37 a	1.78 ± 0.44 a
	50	0.13 ± 0.02 bA	0.13 ± 0.02 bA	0.26 ± 0.08 b	0.52 ± 0.12 b	0.39 ± 0.10 b	0.64 ± 0.12 b
	100	0.10 ± 0.01 bA	0.07 ± 0.01 bcA	0.29 ± 0.09 b	0.21 ± 0.01 b	0.39 ± 0.10 b	0.28 ± 0.01 b
	200	0.07 ± 0.01 bA	0.08 ± 0.01 cA	0.20 ± 0.06 b	0.27 ± 0.06 b	0.26 ± 0.07 b	0.35 ± 0.06 b
Statistics	p; $F_{genotype}$	0.13; 2.50		0.08; 3.28		0.43;	
	P; $F_{density}$	<0.001; 54.99		0.04; 3.33		<0.001; 8.29	
	P; $F_{genotypexdensity}$	0.01; 4.91		0.69; 0.49		0.52; 0.78	
	$DF_{residuals}$	22		22		22	
		4 weeks after infestation					
		BCO R001[1,3]	Morex[1,3]	BCO R001[1]	Morex[1]	BCO R001[1,3]	Morex[1,3]
Aphid.plant^{-1}	0	0.43 ± 0.02 a	0.38 ± 0.05 a	1.13 ± 0.36 a	1.40 ± 0.38 a	1.55 ± 0.37 a	1.78 ± 0.44 a
	50	0.13 ± 0.02 b	0.13 ± 0.02 b	0.26 ± 0.08 b	0.52 ± 0.12 b	0.39 ± 0.10 b	0.64 ± 0.12 b
	100	0.10 ± 0.01 c	0.07 ± 0.01 c	0.29 ± 0.09 b	0.21 ± 0.01 b	0.39 ± 0.10 bc	0.28 ± 0.01 bc
	200	0.07 ± 0.01 c	0.07 ± 0.01 c	0.20 ± 0.06 b	0.28 ± 0.08 b	0.26 ± 0.07 c	0.35 ± 0.08 c

Continued

Statistics	p; $F_{genotype}$	0.40; 0.73	0.13; 2.47	0.25; 1.38
	P; $F_{density}$	<0.001; 48.80	<0.001; 9.29	<0.001; 13.11
	P; $F_{genotypexdensity}$	0.24; 1.53	0.32; 1.26	0.28; 1.36
	$DF_{residuals}$	20	20	20

[1]No significant interaction, test performed for density. [2]Data were transformed using the function sin(x) for analysis. [3]Data were transformed using the function Box-cox for analysis.

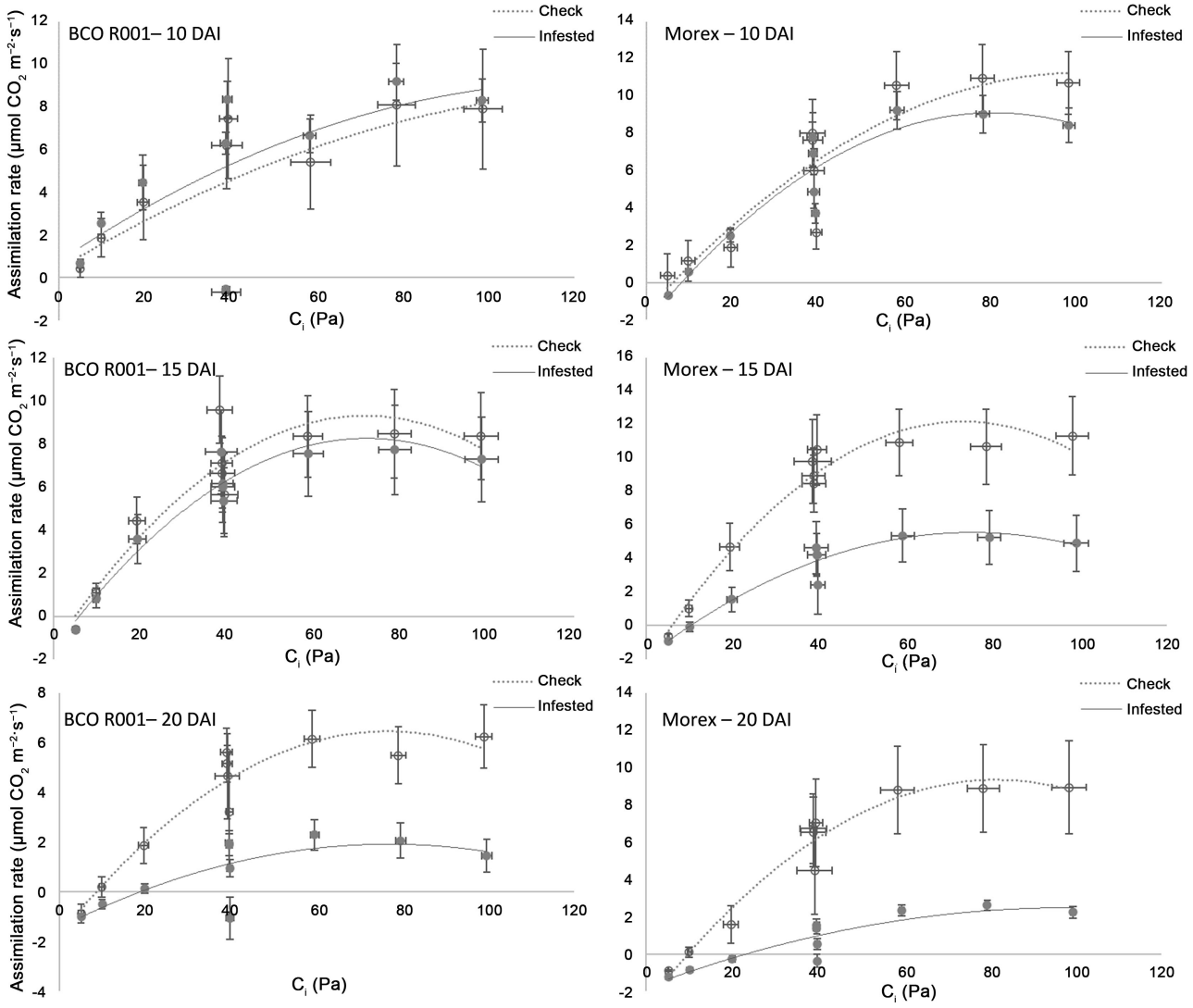

Figure 1. Assimilation rate (µmol CO_2 m^{-2}·s^{-1}) versus intercellular CO_2 concentration (Ci) in pascals (Pa) for susceptible "Morex" and resistant "BCO R001" barley genotypes evaluated at 10, 15 and 20 days post-infestation with bird cherry-oat aphids (DAI).

of tolerance to BCOA. This tolerance was observed for up to 15 days post-infestation. The A/Ci slope is related to the ribulose-1-5 bisphosphate (RuBP) regeneration and CO_2 fixation which are directly related to photosynthetic performance and crop yield [28]. During photosynthesis, carbon dioxide (CO_2) is absorbed through stomata, and the CO_2 concentration significantly influences

the amount of carbohydrate products [29]. Thus, the A/Ci curves observed in "BCO R001" indicated similar efficiency of the RuBP in the plants under 15d of infestation compared to the check.

Although many attempts have been made to find resistance sources in barley for BCOA, the genes involved in barley resistance to the BCOA remain unknown [8]. This knowledge gap is also responsible for the scarcity of barley genotypes exhibiting resistance to BCOA [12]. Adopting a genotype that exhibits resistance traits can prove beneficial for barley growers, serving as a valuable tool not only for managing Barley Yellow Dwarf Virus (BYDV) [16], but also for integrated BCOA management [4].

It is worth noting that even the lowest tested starting aphid density (50 aphids.plant^{-1}) over a period of 2 weeks proved detrimental to barley fitness for both genotypes. This level of aphid pressure far surpasses any natural infestation in the field. According to Peterson [9], among the variables that confer plant tolerance to insect injury, the magnitude and duration of the injury substantially contribute to the differential tolerance capacity between genotypes. Allowing aphids to feed and reproduce for a continuous 2-week period appears to have surpassed the tolerance capacity of "BCO R001".

Previous studies have shown that uncontrolled BCOA infestations in barley reduce growth and leaf area, decrease dry weight, and result in fewer leaves and tillers [30]. In our experiment, the reduction in the number of leaves and photosynthetic rate resulted in a decrease in plant height and reduced total dry mass for both genotypes. Although the assessment of tillering was not conducted during the experimental period, it is plausible that aphid infestation led to a reduction in tillers as well, potentially impacting yield [31].

Overall, the results obtained in this study reveal that BCOA densities of 50 aphids per plant for more than two weeks or a density of 100 aphids per plant for one week can have a detrimental impact on barley plant fitness. Further studies are necessary to fine-tune the economic thresholds and determine the role of resistance in Integrated Pest Management. Future investigations should take into account the relationship between BCOA density and barley yields, aphid reproduction rate on resistant and susceptible genotypes, the presence and effect of natural enemies, agronomic conditions like insecticide efficacy, as well as economic factors such as market values of barley and control costs [32].

Further exploration of "BCO R001" to determine the mechanisms of resistance is warranted. In addition, field trials that address the timing of infestation with plant growth stage could be informative to understanding the ability of barley to compensate for aphid damage. Additional physiological measures that compare aphid density to carbon assimilation will also likely be useful in refining economic thresholds in the absence of disease pressure.

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Conflicts of Interest

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

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