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Screening for Resistance to Bird Cherry-Oat Aphid *Rhopalosiphum padi* L. in Barley, *Hordeum vulgare* L.

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Abstract

The bird cherry-oat aphid (BCOA), *Rhopalosiphum padi*, is a major pest of barley worldwide, and few sources of resistance have been identified in this crop. We screened ten barley accessions alongside the resistant line BCOAR001 and the susceptible cultivar Morex for tolerance to BCOA feeding across three independent experiments: a tray screening assay, a single-plant infestation assay, and a physiological assay combining aphid counts with CO_2 response (A/Ci) curves. Morphological parameters were analyzed, and infestation effects within each line were expressed as relative injury (RI; %), calculated from estimated marginal means. Leaf number responded similarly across all lines regardless of genotype. Although shoot height showed no significant line × infestation interaction in the screening tray experiment, a significant interaction was detected in both single-plant and physiological assays. Shoot fresh and dry mass, root length, and chlorophyll content showed significant line × infestation interactions, with line CI 1128 (BCOAR003) consistently sustaining the smallest reductions. Aphid population growth did not differ among genotypes, indicating that the resistance observed reflects tolerance rather than antibiosis. In the physiological assay, aphid infestation significantly altered the A/Ci relationship in Morex and BCOAR002 but not in BCOAR001 or BCOAR003, although the kinetic parameters K_m and V_{max} did not differ between check and infested plants in any genotype. BCOAR003 showed the most consistent reductions in injury across growth traits, while BCOAR001 maintained relatively stable photosynthetic responses, highlighting their potential as parental material for barley breeding programs targeting BCOA resistance.

Keywords

Plant-Insect Interactions, Aphididae, Host Plant Resistance, Germplasm Screening, Antibiosis

1. Introduction

The bird-cherry oat aphid (BCOA), *Rhopalosiphum padi* (Linnaeus, 1758) is a major pest of temperate cereal crops and is widely distributed across cereal-producing regions of the world [1] [2]. BCOA reduces crop productivity both directly, through the extraction of phloem sap, and indirectly, through honeydew accumulation that promotes sooty mold growth and limits photosynthetic capacity and plant growth [3]. Beyond its feeding damage, BCOA is a highly efficient vector of several plant pathogens, notably barley yellow dwarf virus (BYDV-PAV) and cereal yellow dwarf virus (CYDV-RPV), as well as other viruses that affect cereal production worldwide [1] [4].

Chemical insecticides have historically been the primary tool for aphid control [5]-[7]. However, their frequent use can promote the evolution of resistance in target pest populations, thereby reducing the long-term effectiveness of these control measures, and can negatively affect non-target organisms [8] [9]. The limited number of available insecticide modes of action, combined with their intensive and prolonged use, has led to the development of resistance in several aphid species [7] [10]-[13]. Moreover, managing aphids is challenging because of their rapid reproduction through parthenogenesis and their ability to disperse quickly by both flight and passive transport [14].

In contrast to chemical control, host plant resistance can provide effective aphid management while reducing production costs and labor requirements throughout the growing season [5]. Resistance is generally classified into three categories: antibiosis, antixenosis, and tolerance. Antibiosis occurs when plant traits adversely affect insect performance, reducing survival, development, or reproduction, whereas antixenosis involves plant characteristics that deter herbivore colonization, feeding, or oviposition [15] [16]. In contrast, tolerance refers to the ability of a plant to withstand or recover from herbivore damage through compensatory growth and physiological responses [16]. For environmental and economic reasons, there is a growing demand for resistant cultivars as an alternative to insecticides for the management of aphid pests in cereal crops [17].

Despite the economic importance of BCOA, relatively few sources of resistance have been recently identified in barley [18]-[20]. Consequently, continued screening of barley germplasm is needed to identify novel resistance sources and broaden the genetic basis of resistance available for cultivar development. Evaluating a diverse set of barley breeding lines provides an opportunity to identify previously unrecognized resistance sources and quantify variation in plant responses to aphid infestation. Such information is fundamental for selecting pa-

rental material for breeding programs and advancing our understanding of barley-aphid interactions.

2. Materials and Methods

2.1. Insect and Plant Materials

The BCOA colonies were originally collected in August 2017 from barley (*H. vulgare*) fields in Payne County, Oklahoma, USA. At the USDA-ARS laboratory in Stillwater, Oklahoma, the BCOA colony was reared on the susceptible barley cultivar "Aberdeen 812". Although the BCOA colony used in this study was not screened for BYDV or CYDV, no apparent virus-like symptoms, such as leaf yellowing, reddening, or stunting, were observed in the plants used to maintain the colony. Plants were grown in 4.4 L pots fitted with 45 cm tall cylindrical Lexan® sleeve cages (SABIC Polymershapes, OK, USA), each topped with organdy cloth to provide ventilation and prevent aphids from escaping. New seedlings were started every two weeks to maintain the colonies. Plants and BCOA colonies were kept in growth chambers under two T-8 fluorescent lights on a 16:8 h (light:dark) photoperiod at 25˚C ± 2˚C.

Barley seeds used in this study were obtained from the USDA-ARS collection in Stillwater, Oklahoma. Ten barley accessions were selected for evaluation: CI 6493, PI 319867, PI 436149, CI 14305, CI 1128, CI 7260, CI 11162, CI 14089, CI 14285, and PI 69431 alongside Morex, a susceptible six-rowed spring malting barley cultivar [21] [22], and BCOAR001, a BCOA-resistant line derived from the accession CI 1969 [19] [23]. The accession CI 1969, maintained in the National Small Grains Collection, has been previously identified as a source of resistance to Barley yellow dwarf virus (BYDV) [24].

2.2. Screening Tests

Screening Tray experiment

Barley accessions were initially screened using 73-cell flats (Growing Systems, Inc., Milwaukee, WI, USA) filled with Sunshine Redi-earth growing mix (Sun Gro Horticulture Canada Ltd.). For each accession, three seeds were planted per cell, and 10 cells were planted per accession. After seedling emergence, plants were thinned to one plant per cell. Because 12 accessions were evaluated, two trays were required for each treatment (infested and non-infested control). Separate trays were maintained for aphid-infested and control plants to prevent aphid movement between treatments. Each tray/cage (two per infestation level) was treated as a block; accessions were randomized to cells within each tray. Because aphid infestation was applied once per tray/cage rather than per plant, tray was treated as the whole-plot (block) unit for the infestation factor, with barley accession as the sub-plot factor replicated across multiple plants within each tray. Flats were maintained in cages within a greenhouse at 22˚C ± 5˚C under a 16:8 h (light:dark) photoperiod, supplemented with artificial lighting as needed.

When plants were two weeks old, each cage was uniformly infested with ap-

proximately 1500 BCOA per tray and maintained under infestation for two weeks. At the end of the infestation period, the number of leaves, shoot height (cm), shoot fresh weight (g), and shoot dry weight (g) were recorded. For dry mass determination, plant shoots were collected, placed in paper bags, and dried in an oven at 65˚C for 5 days until a constant weight was reached. The dried samples were then weighed.

Single-Plant Aphid Infestation Assay

Simultaneously with the tray experiment, individual plants were also evaluated. Three seeds were planted in Cone-tainers™ (model SC10; Stuewe & Sons, Tangent, OR, USA) containing a growth medium composed of three layers: potting soil in the upper layer, followed by clay and sand in the lower layers. After seedling emergence, plants were thinned to one plant per Cone-tainer™. Each Cone-tainer™ was fitted with an 8-cm-diameter Lexan sleeve ventilated with organdy cloth to prevent aphid escape and moisture buildup. For each barley line, 10 Cone-tainers™ per treatment (infested and non-infested control) were used, for a total of 240 plants (12 lines × 2 treatments × 10 replicates). Each plant was considered an experimental unit (replicate). The experiment was arranged in a completely randomized design, and plants were maintained under the same environmental conditions as those used in the screening tray experiment.

Two weeks after sowing, plants were individually infested with 20 BCOA. Twenty-five days after infestation, plants were evaluated for the following parameters: number of leaves, shoot height (cm), shoot fresh and dry mass (g), and root length (cm). Leaf chlorophyll content was estimated using a SPAD-502 Plus chlorophyll meter (Konica Minolta, Tokyo, Japan). Three measurements were taken from the youngest fully expanded leaf of each plant, and the mean value was recorded as SPAD units.

Photosynthetic responses of barley genotypes

After the first two experiments, line CI 1128 (hereafter BCOAR003) was selected for further evaluation of the physiological effects of BCOA in comparison with BCOAR001, line CI 14305 (hereafter BCOAR002), and the susceptible cultivar Morex. Two seeds were sown per Cone-tainer™ on April 27, 2026, and seedlings were thinned to one plant per container after emergence. Cone-tainers™ were arranged in trays in a completely randomized design and maintained under the same environmental conditions described for the previous experiments. Each Cone-tainer™ containing a single plant was considered an experimental replicate, with ten replicates per genotype per treatment (infested and non-infested control), for a total of 80 plants (4 genotypes × 2 treatments × 10 replicates). Two weeks after sowing, plants were individually infested with 10 BCOA.

After two weeks of infestation, CO_2 response curves (A/Ci curves) were determined using a portable photosynthesis system (LI-6400; LI-COR Biosciences, Lincoln, NE, USA). Net CO_2 assimilation rates were measured at CO_2 concentrations ranging from 50 to 1000 ppm following the sequence: 400, 200, 100, 50, 400, 400, 600, 800 and 1000 ppm. Of the 10 replicates per treatment, four plants were randomly selected for A/Ci curve measurements. Measurements were conducted at a photosynthetic photon flux density of 1400 μmol $m^{-2} \cdot s^{-1}$ following the methodol-

ogy described by Paudyal *et al.* [25]. The number of aphids per plant, plant height (cm), and SPAD values were recorded for all 10 replicates per treatment. For each plant, K_m and V_{max} were estimated by fitting a photosynthetic response curve to the nine CO_2 concentration measurements obtained from that plant; these nine measurements were therefore used only to parameterize a single K_m and V_{max} estimate per plant and were not treated as independent replicates.

Statistical analysis

In the screening tray experiment, aphid infestation was applied once per tray/cage rather than to individual plants, so tray (two trays per infestation treatment, nested within aphid infestation) was included as a random intercept to account for the non-independence of plants sharing an infestation tray. Shoot height (cm), shoot fresh mass (g), and shoot dry mass (g) from this experiment were analyzed using generalized linear mixed models (GLMMs) with a Gamma error distribution and a log link function, and the number of leaves was analyzed using a GLMM with a Poisson distribution and a log link function

In the single-plant infestation assay and the physiological assay, each plant was infested individually within its own container, providing independent experimental units. Shoot height (cm), root length (cm), shoot fresh mass (g), and shoot dry mass (g) were analyzed using generalized linear models (GLMs) with a Gamma error distribution and a log link function. Because all check plants had zero aphids by design, the number of aphids could not be modeled with aphid infestation as a factor without producing boundary (zero-variance) estimates; differences in aphid number among barley lines were instead assessed using a negative binomial GLM with a log link, restricted to infested plants, and aphid infestation and its interaction with barley line were not tested for this variable. SPAD values were analyzed using a two-way ANOVA. All models included aphid infestation, barley line, and their interaction (aphid infestation × line) as fixed effects, except for the number of aphids model described above.

The effect of aphid infestation within each barley line was estimated as the ratio of estimated marginal means and expressed as Relative Injury (RI, %) with 95% confidence intervals back-transformed from the log scale. Relative Injury (RI, %) was calculated for each line as the proportional reduction in the mean value of infested plants relative to non-infested (check) plants, multiplied by 100, for each evaluated trait.

$$\text{RI (\%)} = (\text{check} - \text{infested})/\text{check} \times 100$$

Lines were subsequently ranked from least to most injured. Differences in the magnitude of aphid effects among lines were assessed using Tukey-adjusted pairwise comparisons of the aphid infestation × line interaction ($\alpha = 0.05$). All analyses were conducted in RStudio version 2026.07.1 (Posit Software, PBC, 2026) using the car, emmeans, and multcomp packages, and figures were generated using the ggplot2 package [26].

For the A/Ci curves, net CO_2 assimilation rate (A) was modeled as a function of intercellular CO_2 concentration (Ci) for each genotype × treatment combina-

tion. The A/Ci curves were plotted using a three-parameter exponential rise-to-maximum model fitted by nonlinear regression [25]. To test whether infestation altered the A/Ci relationship within each genotype, we used an extra sum-of-squares F-test comparing two nested models. Following generation of the A/Ci curves, the kinetic parameters Michaelis constant K_m and maximum velocity V_{max} were estimated individually for each plant from its own nine-point CO_2 response curve, using the Lineweaver-Burk equation and its linearized form [27] [28]. The nine CO_2 concentration measurements from a given plant were therefore used only to parameterize that plant's own K_m and V_{max} values and were not treated as independent observations. For each genotype, the resulting plant-level K_m and for V_{max} estimates (n = 4 independent plants per treatment) were compared between check and infested treatments using a two-sample t-test (df = 6; $\alpha = 0.05$).

3. Results

Screening Tray experiment

The parameters plant shoot height (cm) and number of leaves showed no significant interaction between barley lines and BCOA infestation, indicating that the magnitude of the aphid effect on these parameters did not differ among barley lines ($\chi^2(11) = 17.03$, P = 0.11 and $\chi^2(11) = 3.98$, P = 0.97, respectively; Table 1). Overall, the barley lines exhibited an RI ranging from 59.18% to 72.98% for plant height and from 54.35% to 76.92% for number of leaves (Table 2).

Table 1. Statistical test results for the effects of aphid infestation, barley line, and their interaction on growth, physiological, and aphid population parameters across three experiments.

Experiment	Parameter	Model	Aphid infestation (χ^2 or F)	P-value	Barley lines (χ^2 or F)	P-value	Infestation × line (χ^2 or F)	P-value
Screening Tray Experiment	Number of leaves	Poisson GLMM	$\chi^2(1) = 18.13$	<0.001	$\chi^2(11) = 4.54$	0.951	$\chi^2(11) = 3.98$	0.971
	Shoot height (cm)	Gamma GLMM	$\chi^2(1) = 17.09$	<0.001	$\chi^2(11) = 60.07$	<0.001	$\chi^2(11) = 17.03$	0.107
	Fresh mass (g)	Gamma GLMM	$\chi^2(1) = 17.51$	<0.001	$\chi^2(11) = 60.77$	<0.001	$\chi^2(11) = 53.00$	<0.001
	Dry mass (g)	Gamma GLMM	$\chi^2(1) = 19.17$	<0.001	$\chi^2(11) = 57.32$	<0.001	$\chi^2(11) = 26.24$	0.006
Single-Plant Aphid Infestation Experiment	Number of leaves	Poisson GLM	$\chi^2(1) = 148.32$	<0.001	$\chi^2(11) = 7.76$	0.734	$\chi^2(11) = 5.13$	0.925
	Shoot height (cm)	Gamma GLM	$\chi^2(1) = 1805.48$	<0.001	$\chi^2(11) = 109.46$	<0.001	$\chi^2(11) = 63.26$	<0.001
	Root length (cm)	Gamma GLM	$\chi^2(1) = 1481.48$	<0.001	$\chi^2(11) = 134.93$	<0.001	$\chi^2(11) = 89.76$	<0.001
	Fresh mass (g)	Gamma GLM	$\chi^2(1) = 1339.64$	<0.001	$\chi^2(11) = 67.88$	<0.001	$\chi^2(11) = 56.34$	<0.001
	Dry mass (g)	Gamma GLM	$\chi^2(1) = 3291.20$	<0.001	$\chi^2(11) = 138.20$	<0.001	$\chi^2(11) = 63.10$	<0.001
	SPAD	Two-way ANOVA	F(1, 205) = 473.63	<0.001	F(11, 205) = 6.80	<0.001	F(11, 205) = 2.39	0.008
Aphid population growth and A/Ci curves experiment	Number of aphids	Negative binomial GLM (infested plants only)[1]	—	—	$\chi^2(3) = 0.88$	0.831	—	—
	Plant height (cm)	Two-way ANOVA	F(1, 67) = 0.64	0.426	F(3, 67) = 2.17	0.099	F(3, 67) = 0.24	0.865
	SPAD	Two-way ANOVA	F(1, 65) = 0.52	0.472	F(3, 65) = 4.54	0.0059	F(3, 65) = 1.38	0.255

[1]Genotype differences in aphid number were assessed using a negative binomial generalized linear model restricted to infested plants, since all control plants had zero aphids; the infestation and infestation × line terms are not applicable under this model (see Methods).

Table 2. Response of barley lines to Bird cherry-oat aphid infestation based on number of leaves, shoot height (cm), shoot fresh mass (g), and shoot dry mass (g) of plants under check (aphid-free) and aphid-infested conditions in the screening tray experiment, with Relative Injury (RI, %) for each line.

Number of leaves					Shoot height (cm)				
Line	Check[1]	Infested[1]	RI %[2]		Line	Check[1]	Infested[1]	RI %[2]	
CI 1128	4.50 ± 0.22	1.70 ± 0.15	62.22 (34.00 - 78.38)	a	CI 1128	30.71 ± 2.09	10.96 ± 0.67	64.31 (57.28 - 70.18)	a
PI 319867	5.00 ± 0.00	2.10 ± 0.10	58.00 (29.50 - 74.98)	a	PI 319867	35.51 ± 0.84	14.28 ± 1.16	59.79 (51.63 - 66.57)	a
CI 14305	5.20 ± 0.13	2.10 ± 0.10	59.62 (32.97 - 75.67)	a	CI 14305	39.44 ± 0.87	14.04 ± 1.29	64.40 (57.39 - 70.26)	a
CI 14089	4.60 ± 0.43	2.10 ± 0.10	54.35 (23.50 - 72.76)	a	CI 14089	34.11 ± 3.32	13.65 ± 0.92	59.98 (52.10 - 66.57)	a
PI 436149	5.00 ± 0.00	2.10 ± 0.18	58.00 (30.08 - 74.77)	a	PI 436149	40.43 ± 1.07	15.79 ± 1.49	60.94 (53.25 - 67.37)	a
CI 6493	4.80 ± 0.20	2.00 ± 0.15	58.33 (29.80 - 75.27)	a	CI 6493	34.30 ± 1.99	14.00 ± 0.94	59.18 (51.14 - 65.90)	a
CI 11162	4.80 ± 0.13	2.00 ± 0.00	58.33 (29.80 - 75.27)	a	CI 11162	32.21 ± 0.81	11.74 ± 0.68	63.55 (56.37 - 69.55)	a
CI 14285	5.40 ± 0.16	2.20 ± 0.13	59.26 (33.12 - 75.18)	a	CI 14285	39.60 ± 0.37	14.05 ± 1.43	64.52 (57.53 - 70.36)	a
PI 69431	6.10 ± 0.10	2.10 ± 0.10	65.57 (43.47 - 79.03)	a	PI 69431	36.52 ± 1.21	12.56 ± 1.03	65.61 (58.83 - 71.27)	a
BCOAR001	5.40 ± 0.16	2.00 ± 0.00	62.96 (38.13 - 77.83)	a	BCOAR001	39.03 ± 1.29	13.20 ± 0.75	66.18 (59.52 - 71.75)	a
CI 7260	4.90 ± 0.10	2.00 ± 0.00	59.18 (31.34 - 75.74)	a	CI 7260	33.03 ± 1.41	11.97 ± 1.01	63.76 (56.62 - 69.72)	a
Morex	5.20 ± 0.20	1.20 ± 0.13	76.92 (56.77 - 87.68)	a	Morex	32.64 ± 1.48	8.82 ± 0.74	72.98 (67.66 - 77.42)	a
Shoot fresh mass (g)					**Shoot dry mass (g)**				
Line	Check[1]	Infested[1]	RI %[2]		Line	Check[1]	Infested[1]	RI %[2]	
CI 1128	1.69 ± 0.18	0.11 ± 0.03	93.60 (89.96 - 95.92)	a	CI 1128	0.237 ± 0.022	0.027 ± 0.005	88.50 (84.31 - 91.57)	a
PI 319867	2.23 ± 0.09	0.13 ± 0.02	93.98 (90.44 - 96.21)	a	PI 319867	0.347 ± 0.008	0.036 ± 0.003	89.72 (85.85 - 92.53)	ab
CI 14305	2.83 ± 0.22	0.14 ± 0.04	95.19 (92.46 - 96.94)	ab	CI 14305	0.376 ± 0.031	0.028 ± 0.005	92.58 (89.88 - 94.56)	ab
CI 14089	2.33 ± 0.38	0.10 ± 0.02	95.66 (93.19 - 97.23)	ab	CI 14089	0.346 ± 0.040	0.031 ± 0.003	91.12 (87.78 - 93.55)	ab
PI 436149	2.97 ± 0.26	0.12 ± 0.04	95.93 (93.62 - 97.41)	ab	PI 436149	0.419 ± 0.027	0.036 ± 0.006	91.39 (88.25 - 93.69)	ab
CI 6493	2.31 ± 0.26	0.09 ± 0.02	96.14 (93.95 - 97.54)	ab	CI 6493	0.372 ± 0.041	0.025 ± 0.003	93.25 (90.79 - 95.05)	ab
CI 11162	2.06 ± 0.09	0.07 ± 0.01	96.46 (94.45 - 97.75)	ab	CI 11162	0.281 ± 0.011	0.033 ± 0.002	88.31 (84.06 - 91.43)	a
CI 14285	3.82 ± 0.16	0.12 ± 0.01	96.75 (94.90 - 97.93)	ab	CI 14285	0.482 ± 0.017	0.046 ± 0.005	90.55 (87.10 - 93.07)	ab
PI 69431	4.13 ± 0.33	0.11 ± 0.01	97.41 (95.94 - 98.35)	abc	PI 69431	0.476 ± 0.033	0.028 ± 0.003	94.08 (91.92 - 95.66)	ab
BCOAR001	2.60 ± 0.27	0.05 ± 0.01	98.03 (96.92 - 98.75)	bc	BCOAR001	0.363 ± 0.031	0.026 ± 0.003	92.78 (90.15 - 94.71)	ab
CI 7260	1.96 ± 0.16	0.03 ± 0.01	98.27 (97.28 - 98.90)	bc	CI 7260	0.266 ± 0.020	0.024 ± 0.004	91.10 (87.86 - 93.48)	ab
Morex	2.11 ± 0.24	0.02 ± 0.01	99.05 (98.51 - 99.40)	c	Morex	0.312 ± 0.031	0.016 ± 0.003	94.84 (92.97 - 96.22)	b

[1]Mean ± SE. [2]RI (%) = (check − infested)/check × 100, with 95% confidence intervals in parentheses; values followed by the same letter do not differ significantly among lines ($\alpha = 0.05$).

For shoot fresh mass (g), a significant interaction between barley lines and BCOA infestation was observed ($\chi^2(11) = 53.00$, $P < 0.001$; Table 1). Line CI 1128 exhibited the lowest RI, with a 93.60% reduction in fresh mass, whereas Morex showed the highest RI, corresponding to a 99.05% reduction (Table 2). Similarly, plant dry mass (g) shower a genotype-dependent response to infestation [$\chi^2(11) = 26.24$, $P = 0.006$]. Lines CI 11162 and CI 1128 showed the lowest reductions in dry

mass (RI values of 88.31% and 88.31%, respectively), whereas Morex exhibited the highest reduction, with an RI of 94.84% (Table 2).

Single-Plant Aphid Infestation Assay

Similar to the screening tray experiment, no significant interaction between barley lines and BCOA infestation was observed for number of leaves in the single-plant aphid infestation assay [$\chi^2(11) = 5.13$, P = 0.92; Table 1]. All lines exhibited similar reductions under BCOA infestation, with RI values ranging from 36.73% to 63.79%. However, the response for shoot height (cm) differed among barley lines, resulting in a significant interaction between barley lines and BCOA infestation [$\chi^2(11) = 63.26$, P < 0.001; Table 1]. Line CI 1128 exhibited the lowest reduction (RI = 34.83%), whereas line CI 14285 showed an intermediate RI (51.73%; Table 3). No significant differences were observed among the remaining lines, whose RI values ranged from 54.06% to 64.78% (Table 3).

Table 3. Number of leaves, shoot height (cm), shoot fresh mass (g), shoot dry mass (g), root length (cm), and SPAD of barley lines under check (non-infested) and aphid-infested conditions in the single-plant aphid infestation assay, with Relative Injury (RI, %) for each line.

Number of leaves					Shoot height				
Line[1]	Check[2]	Infested[2]	RI %[3]		Line	Check[2]	Infested[2]	RI %[3]	
CI 1128	4.90 ± 0.10	3.10 ± 0.23	36.73 (0.80 - 59.65)	a	**CI 1128**	61.72 ± 1.29	40.22 ± 2.61	34.83 (25.63 - 42.90)	a
PI 69431	6.00 ± 0.00	3.20 ± 0.20	46.67 (18.09 - 65.27)	a	**CI 14285**	63.85 ± 1.62	30.82 ± 1.43	51.73 (44.91 - 57.70)	ab
CI 14285	5.90 ± 0.10	2.70 ± 0.21	54.24 (27.84 - 70.98)	a	**CI 14305**	62.12 ± 1.28	28.54 ± 0.97	54.06 (47.57 - 59.74)	b
CI 6493	5.00 ± 0.00	2.20 ± 0.13	56.00 (27.35 - 73.35)	a	**PI 69431**	61.64 ± 1.19	27.06 ± 2.97	56.10 (49.90 - 61.53)	b
CI 7260	5.10 ± 0.10	2.10 ± 0.10	58.82 (31.55 - 75.23)	a	**BCOAR001**	56.68 ± 1.56	24.76 ± 1.60	56.32 (50.15 - 61.72)	b
CI 14089	5.00 ± 0.00	2.00 ± 0.00	60.00 (30.71 - 76.91)	a	**CI 6493**	56.55 ± 1.23	24.25 ± 1.84	57.12 (51.06 - 62.42)	b
BCOAR001	5.80 ± 0.13	2.30 ± 0.15	60.34 (35.72 - 75.53)	a	**PI 319867**	57.25 ± 1.04	23.92 ± 1.57	58.21 (52.31 - 63.38)	b
PI 319867	5.30 ± 0.15	2.10 ± 0.10	60.38 (34.32 - 76.10)	a	**PI 436149**	63.24 ± 0.73	26.41 ± 1.62	58.24 (52.34 - 63.41)	b
Morex	5.60 ± 0.16	2.20 ± 0.13	60.71 (35.67 - 76.01)	a	**CI 7260**	62.15 ± 1.53	24.78 ± 1.24	60.13 (54.50 - 65.06)	b
PI 436149	5.00 ± 0.00	1.90 ± 0.10	62.00 (35.56 - 77.59)	a	**CI 11162**	56.54 ± 0.78	20.81 ± 0.88	63.19 (58.00 - 67.75)	b
CI 11162	5.30 ± 0.15	2.00 ± 0.00	62.26 (36.88 - 77.44)	a	**Morex**	61.40 ± 1.35	22.03 ± 1.23	64.12 (59.05 - 68.56)	b
CI 14305	5.80 ± 0.13	2.10 ± 0.10	63.79 (40.35 - 78.02)	a	**CI 14089**	64.56 ± 2.78	22.74 ± 1.26	64.78 (59.26 - 69.55)	b
Shoot fresh mass (g)					**Shoot dry mass (g)**				
Line	Check[2]	Infested[2]	RI %[3]		Line	Check[2]	Infested[2]	RI %[3]	
CI 1128	3.47 ± 0.24	0.64 ± 0.14	81.69 (70.80 - 88.52)	a	**CI 1128**	0.325 ± 0.021	0.085 ± 0.009	73.94 (66.89 - 79.48)	a
CI 6493	3.50 ± 0.16	0.31 ± 0.06	91.05 (85.72 - 94.39)	ab	**CI 14285**	0.437 ± 0.014	0.056 ± 0.008	87.24 (83.79 - 89.95)	b
PI 319867	3.59 ± 0.18	0.23 ± 0.05	93.57 (89.75 - 95.97)	abc	**PI 69431**	0.427 ± 0.022	0.048 ± 0.006	88.70 (85.64 - 91.10)	b
BCOAR001	4.76 ± 0.17	0.29 ± 0.06	93.84 (90.18 - 96.14)	abc	**CI 11162**	0.313 ± 0.012	0.033 ± 0.003	89.48 (86.64 - 91.72)	b
PI 69431	6.29 ± 0.35	0.37 ± 0.10	94.05 (90.51 - 96.27)	bc	**CI 14089**	0.336 ± 0.015	0.035 ± 0.002	89.54 (86.39 - 91.96)	b
PI 436149	4.40 ± 0.17	0.24 ± 0.03	94.50 (91.23 - 96.55)	bc	**CI 7260**	0.314 ± 0.016	0.032 ± 0.004	89.79 (87.03 - 91.96)	b
CI 14285	6.05 ± 0.27	0.32 ± 0.10	94.65 (91.46 - 96.64)	bc	**CI 6493**	0.312 ± 0.015	0.032 ± 0.005	89.89 (87.16 - 92.04)	b

Continued

CI 7260	3.64 ± 0.19	0.18 ± 0.05	95.00 (92.02 - 96.86)	bc	**BCOAR001**	0.329 ± 0.034	0.033 ± 0.004	90.10 (87.43 - 92.21)	b
CI 11162	3.67 ± 0.14	0.17 ± 0.03	95.34 (92.57 - 97.08)	bc	**PI 436149**	0.353 ± 0.015	0.032 ± 0.003	90.96 (88.52 - 92.89)	b
CI 14305	4.45 ± 0.15	0.19 ± 0.05	95.76 (93.23 - 97.34)	bc	**PI 319867**	0.329 ± 0.016	0.030 ± 0.003	90.98 (88.54 - 92.90)	b
CI 14089	3.79 ± 0.19	0.09 ± 0.01	97.60 (95.99 - 98.57)	c	**CI 14305**	0.345 ± 0.012	0.031 ± 0.002	91.03 (88.61 - 92.94)	b
Morex	5.97 ± 0.40	0.13 ± 0.03	97.87 (96.61 - 98.67)	c	**Morex**	0.462 ± 0.028	0.041 ± 0.003	91.23 (88.86 - 93.10)	b

Root length (cm)					SPAD				
Line	**Check[2]**	**Infested[2]**	**RI %[3]**		**Line**	**Check[2]**	**Infested[2]**	**RI %[3]**	
CI 1128	35.98 ± 0.99	25.98 ± 1.03	27.79 (18.64 - 35.92)	a	**BCOAR001**	35.47 ± 1.74	21.08 ± 2.01	40.58 (19.19 - 61.96)	a
CI 7260	30.64 ± 1.01	18.08 ± 0.72	40.99 (33.51 - 47.63)	ab	**CI 1128**	44.69 ± 1.14	24.85 ± 2.22	44.40 (27.88 - 60.92)	ab
CI 6493	39.08 ± 0.75	21.48 ± 1.42	45.04 (37.87 - 51.38)	abc	**CI 6493**	41.85 ± 1.05	22.80 ± 2.23	45.53 (27.41 - 63.66)	ab
PI 319867	37.97 ± 0.67	20.57 ± 0.96	45.83 (38.96 - 51.92)	bcd	**CI 11162**	44.82 ± 1.85	21.84 ± 2.40	51.28 (34.80 - 67.75)	ab
PI 69431	43.16 ± 1.34	21.91 ± 1.62	49.23 (42.38 - 55.27)	bcde	**PI 436149**	44.34 ± 0.85	19.23 ± 1.36	56.62 (39.97 - 73.28)	ab
PI 436149	39.99 ± 0.71	19.12 ± 0.74	52.18 (45.94 - 57.70)	bcde	**CI 14285**	33.54 ± 1.26	13.92 ± 2.60	58.50 (35.88 - 81.12)	ab
CI 14089	37.63 ± 1.45	17.72 ± 0.39	52.91 (46.29 - 58.71)	bcde	**PI 319867**	52.05 ± 9.97	18.62 ± 2.22	64.22 (50.04 - 78.41)	b
BCOAR001	53.38 ± 1.07	24.40 ± 0.96	54.29 (48.33 - 59.57)	bcde	**PI 69431**	31.95 ± 0.74	11.15 ± 2.24	65.10 (41.98 - 88.21)	ab
CI 14285	48.09 ± 1.13	21.77 ± 1.97	54.74 (48.83 - 59.96)	bcde	**CI 14305**	36.57 ± 0.95	11.49 ± 2.02	68.57 (45.25 - 91.88)	ab
CI 11162	43.57 ± 0.83	18.08 ± 0.88	58.51 (53.09 - 63.30)	cde	**CI 7260**	46.12 ± 1.17	14.14 ± 1.93	69.35 (53.33 - 85.36)	ab
CI 14305	44.66 ± 0.72	18.15 ± 1.14	59.36 (53.87 - 64.19)	de	**CI 14089**	40.50 ± 0.97	9.57 ± 2.47	76.37 (55.83 - 96.91)	ab
Morex	47.75 ± 1.58	18.45 ± 1.73	61.36 (56.46 - 65.71)	e	**Morex**	36.95 ± 1.14	7.96 ± 1.23	78.46 (58.48 - 98.45)	ab

[1]n = 10 per line per treatment, except CI 14089 check (n = 7). [2]Mean ± SE. [3]RI (%) = (check − infested)/check × 100; (95% confidence intervals); values followed by the same letter do not differ significantly among lines ($\alpha = 0.05$).

For root length (cm) and shoot fresh mass (g), line CI 1128 also exhibited the lowest reductions, with RI values of 27.79% and 81.69%, respectively, whereas Morex showed the highest reductions, with RI values of 61.36% and 97.87%, respectively ($\chi^2(11) = 89.76$, $P < 0.001$ and $\chi^2(11) = 56.34$, $P < 0.001$; Table 1). Shoot dry mass (g) was also significantly affected by the interaction between barley line and BCOA infestation ($\chi^2(11) = 63.14$, $P < 0.001$; Table 1). Once again, line CI 1128 exhibited the lowest reduction (RI = 73.94%); however, no significant differences were observed among the other lines (Table 3).

Chlorophyll content was also significantly affected by the interaction between barley lines and BCOA infestation ($F(11, 205) = 2.39$, $P = 0.008$; Table 1). Line BCOAR001 exhibited the lowest reduction in SPAD values, with an RI of 40.58%, whereas line PI 319867 showed the highest reduction, with an RI of 64.22% (Table 3).

Aphid population growth and A/Ci curves

As expected by design, aphid-free check plants had zero aphids, while infested plants developed established populations within each genotype by the end of the infestation period (Table 1). Among infested plants, aphid populations ranged from 186.9 ± 57.8 aphids on BCOAR001 to 221.8 ± 136.0 aphids on Morex, with

no significant difference among barley lines ($\chi^2(3) = 0.88$, P = 0.831; Table 1 and Figure 1).

Plant height (cm) was similar among barley lines and between infested and check plants, and no barley line × aphid infestation interaction was observed (F(3, 67) = 0.24, P = 0.865; Table 1, Figure 2). Plant height ranged from 39.05 ± 3.81 cm to 48.05 ± 1.28 cm (Figure 2). SPAD values differed only among barley lines; however, no barley line × aphid infestation interaction was observed (F(3, 67) = 1.38, P = 0.255; Table 1, Figure 1(B)).

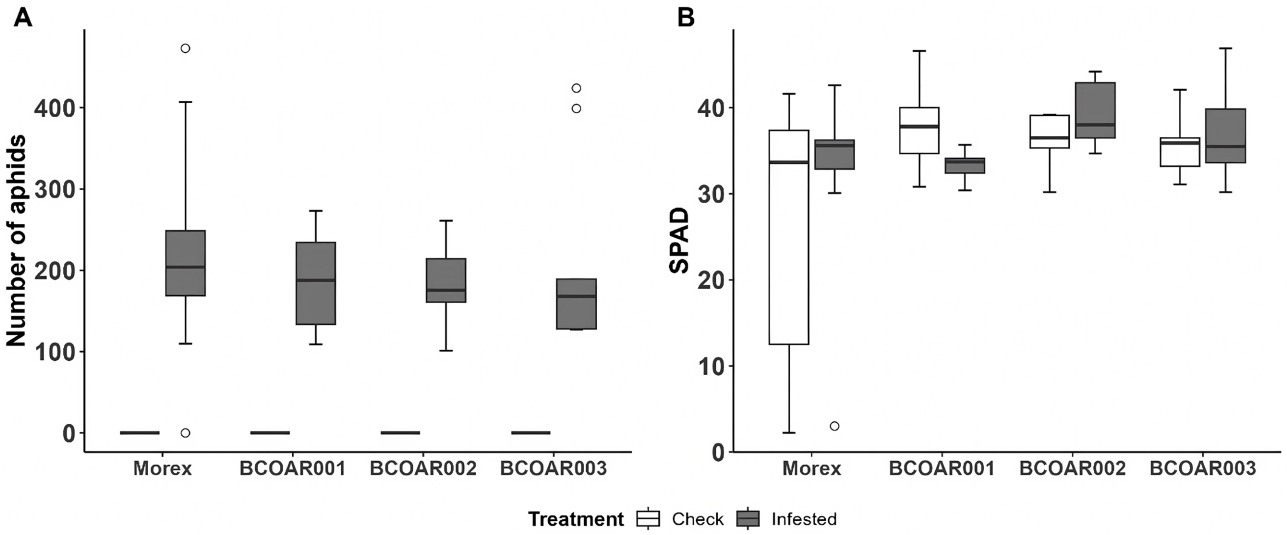


Figure 1. Aphid population size (A) and SPAD index (B) across a barley susceptible line Morex and BCOAR001, BCOAR002, and BCOAR003 lines two weeks after infestation with 10 BCOA per plant, under check (aphid-free) or infested conditions. Boxes represent the median and interquartile range (IQR); whiskers extend to the most extreme values within 2 × IQR; open circles denote values beyond that range (outliers).

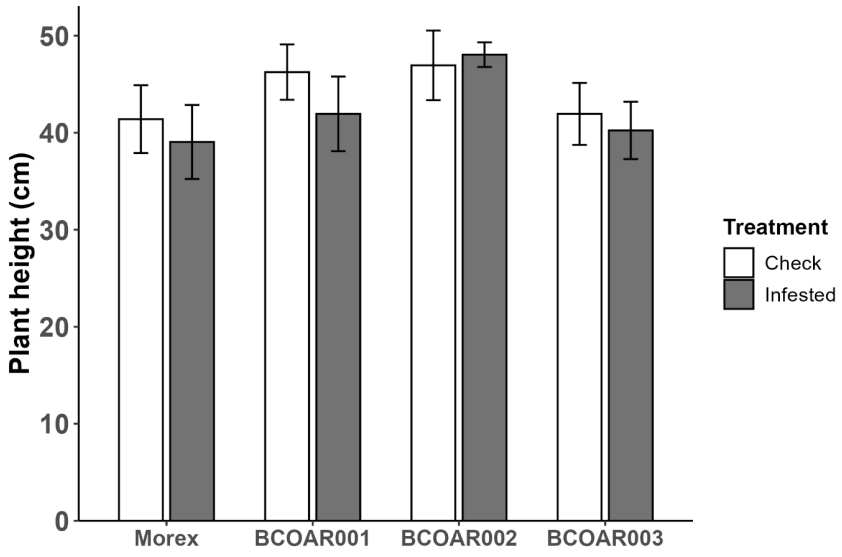


Figure 2. Mean plant height (±SE) of the susceptible cultivar Morex and BCOAR001, BCOAR002, and BCOAR003 lines two weeks after infestation with 10 BCOA per plant, under check (aphid-free) or infested conditions.

Comparison of the A/Ci curve parameters between check and BCOA-infested plants indicated that aphid infestation significantly altered the A/Ci relationship in Morex [F(3, 66) = 3.66, P = 0.017] and BCOAR002 [F(3, 66) = 3.32, P = 0.025], whereas no significant differences were detected in BCOAR001 [F(3, 66) = 0.51, P = 0.677] or BCOAR003 [F(3, 66) = 0.02, P = 0.997; Figure 3]. However, no significant differences in the kinetic parameters K_m (Michaelis constant) and V_{max} (maximum reaction rate) were observed between check and BCOA-infested plants across the barley lines evaluated (Table 4).

Table 4. Michaelis constant (K_m) and maximum velocity (V_{max}) estimated from A/Ci curves in check and infested treatments across four barley lines after 14 days of infestation with Bird cherry-oat aphid.

	K_m (μmol·mol^{-1})					V_{max} (μmol CO$_2$ m^{-2}·s^{-1})				
Barley line	Check[1]	Infested[1]	t	Df[2]	P-value	Check[1]	Infested[1]	t	Df[2]	P-value
Morex	1291.08 ± 517.87	3371.13 ± 1517.80	−1.30	6	0.24	67.76 ± 13.94	125.78 ± 44.90	−1.23	6	0.26
BCOAR001	627.84 ± 79.38	623.27 ± 123.60	0.03	6	0.98	35.58 ± 2.43	35.4 ± 7.88	0.02	6	0.98
BCOAR002	863.14 ± 226.51	728.47 ± 186.90	0.46	6	0.66	32.82 ± 7.62	23.72 ± 4.710	1.02	6	0.35
BCOAR003	924.36 ± 178.44	631.98 ± 46.71	1.59	6	0.16	66.00 ± 10.27	50.9 ± 5.32	1.31	6	0.24

[1]Mean ± SE. [2]n = 4 plants per treatment (check and infested) per line.

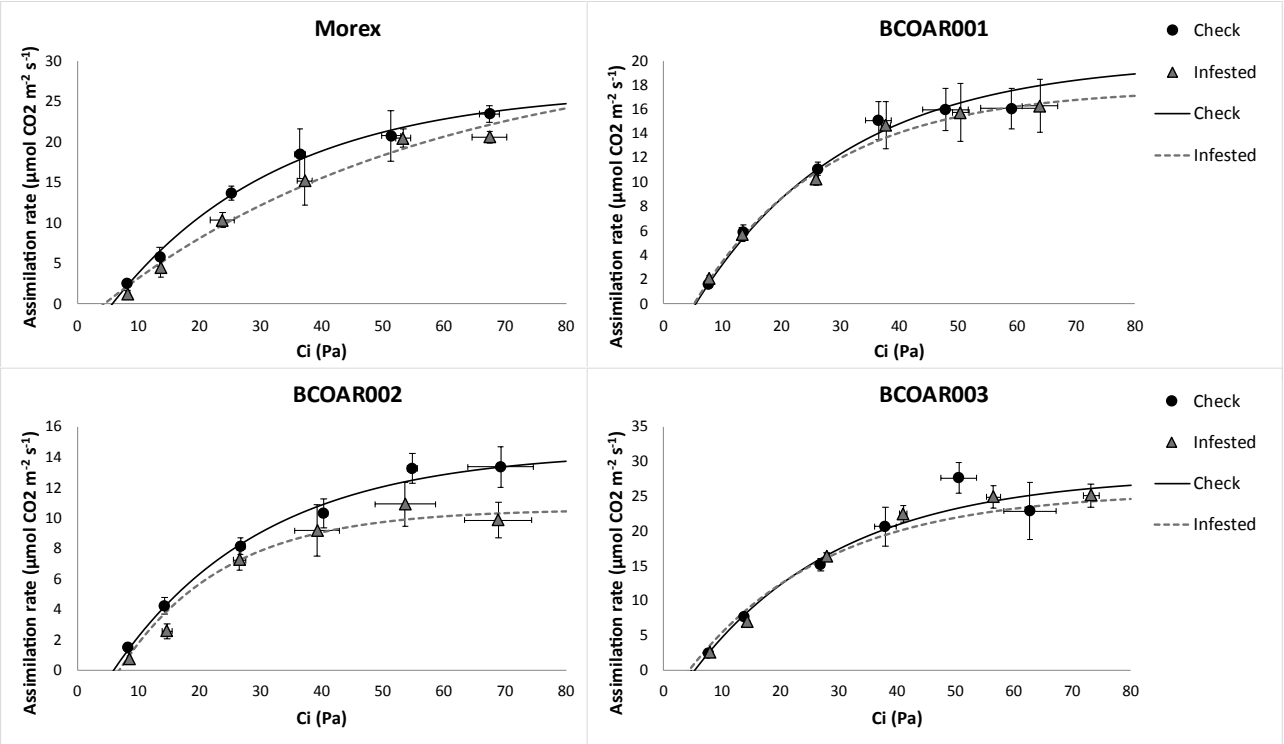


Figure 3. Assimilation rate (μmol CO_2 m^{-2}·s^{-1}) versus intercellular CO_2 concentration (Ci, in pascals) for Morex, BCOAR001 and BCOAR002 under check (aphids-free) and aphid-infested conditions, evaluated 14 days postinfestation with 10 BCOA aphids.

4. Discussion

The plant response to BCOA feeding is generally considered asymptomatic, par-

ticularly at low infestation densities, although physiological alterations may occur in the absence of obvious external symptoms [29] [30]. Across these three experiments, we observed that higher infestation levels were associated with measurable effects on plant morphology. In the first experiment (screening tray experiment), in which each tray received approximately 1500 aphids, and in the second experiment (single-plant aphid infestation experiment), in which each individual plant was infested with 20 aphids, we observed reductions in all morphological parameters evaluated in infested plants compared with non-infested checks. In contrast, in the third experiment (aphid population growth and A/Ci curve experiment), in which plants were initially infested with 10 aphids, plant height and SPAD index values were similar between infested and non-infested plants; however, differences were detected in the physiological responses characterized by the A/Ci curves.

Previous studies demonstrated that BCOA densities of 50 aphids per plant sustained for more than two weeks or 100 aphids per plant sustained for one week can negatively affect barley plant morphology [19]. Additionally, the authors found that BCOA feeding had little effect on photosynthetic performance at 10 days, but prolonged infestation progressively reduced CO_2 assimilation, with clear effects emerging after 15 days [19]. Together with the results of the present study, these findings suggest that the effects of BCOA feeding may depend on infestation intensity and duration, with physiological alterations becoming detectable even when external morphological symptoms are limited or absent.

Line CI 1128 (BCOAR003) exhibited the most consistent tolerance across the two independent screening experiments. It had the lowest RI (%) for shoot fresh and dry mass in the screening tray experiment and, in the single-plant assay, the lowest RI (%) for four parameters that showed a significant genotype × infestation interaction: shoot height, shoot fresh and dry mass and root length. This consistency justified carrying BCOAR003 forward into the physiological comparison. Although the kinetic parameters Michaelis constant (K_m) and maximum velocity (V_{max}) were not affected after 14 days of BCOA infestation in any of the four barley genotypes tested, the overall A/Ci response varied among genotypes.

Franzen *et al.* [29] found no significant differences in gas-exchange responses between BCOA-infested and control Morex barley plants after 6 days of infestation. However, after 9 days of infestation, BCOA-infested plants exhibited lower A_{max} and J_{max} values than check plants. In our study, BCOAR003 exhibited similar A/Ci curves between aphid-infested and check plants after 14 days of infestation, a pattern also observed for BCOAR001 in both the present experiment and previous studies [19] [23]. In contrast, Morex and BCOAR002 exhibited significant changes in their A/Ci curves in response to aphid infestation.

The shape of an A/Ci response curve reflects multiple biochemical and diffusional limitations, including Rubisco activity and ribulose-1,5 bisphosphate (RuBP) regeneration. Attributing a change in the curve to one process requires a mechanistic photosynthesis model [31] [32]. During photosynthesis, CO_2 enters the leaf through the stomata and is assimilated into carbohydrates, making CO_2

availability an important factor influencing photosynthetic carbon assimilation [33]. The similarity between the A/Ci curves of aphid-infested and non-infested BCOAR001 and BCOAR003 plants suggests that RuBP regeneration was relatively unaffected after 14 days of aphid infestation.

Because final aphid counts did not differ among genotypes after 14 days, the smaller plant responses in selected lines are consistent with relative tolerance. However, a non-significant aphid count difference does not establish equivalent aphid survival or reproduction, suggesting that the resistance identified in this screening is more closely associated with tolerance than with antibiosis [16]. However, similar aphid population growth among genotypes does not exclude the possibility that additional resistance mechanisms affect aphid feeding behavior. Using the electrical penetration graph (EPG) technique, Leybourne *et al.* [34] demonstrated that partial resistance in the wild barley *Hordeum spontaneum* to BCOA involves mesophyll- and phloem-based mechanisms that restrict aphid feeding and reduce phloem ingestion. Further investigation of aphid feeding behavior and plant responses may help determine whether similar mechanisms contribute to the resistance observed in BCOAR001 and BCOAR003. Additionally, transcriptional profiling has been used to identify differentially expressed genes associated with BCOA resistance in barley [17] [35]. EPG and transcriptional profiling could therefore serve as complementary approaches for identifying the mechanisms underlying BCOA resistance and supporting future barley resistance-screening programs.

5. Conclusion

In conclusion, the present screening trials identified BCOAR001 and line CI 1128 (BCOAR003) as the most tolerant barley genotypes to BCOA feeding, showing the smallest reductions in plant morphology and photosynthetic performance across three independent experiments despite supporting aphid populations comparable to those on the susceptible cultivar Morex. These findings align with those of Mornhinweg and Armstrong [20] who identified CI 1969 (the source of BCOAR001) and ascension CI 1128 (BCOAR003) as potential sources of resistance for breeding programs.

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Author Contributions

Conceptualization, R.H. and D.M.; methodology, R.H. and D.M.; software, R.H.; validation, R.H. and D.M.; formal analysis, R.H.; investigation, R.H. and D.M.; resources, D.M., W.W.H. and N.E.; data curation, R.H.; writing—original draft preparation, R.H. and D.M.; writing—review and editing, R.H., W.W.H. and N.E.; visualization, R.H. and D.M.; supervision, D.M.; project administration, D.M. and N.E.; funding acquisition, N.E. and W.W.H. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

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

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