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Article

Effects of Confinement and Wheat Variety on the Performance of Two Aphid Species

Maria Elisa D. A. Leandro [1], Joe M. Roberts [1], Ed T. Dickin [1] and Tom W. Pope [1,*]

Centre for Crop and Environmental Science, Agriculture and Environment Department, Harper Adams University, Newport, Shropshire TF10 8NB, UK; mdamascena@live.harper.ac.uk (M.E.D.A.L), jroberts@harper-adams.ac.uk (J.M.R.); edicken@harper-adams.ac.uk (E.T.D), tpope@harper-adams.ac.uk (T.W.P.)

* Correspondence: tpope@harper-adams.ac.uk

Simple Summary: This study investigates how two different experimental methods (specifically confining the insect to a leaf section on a plant or leaving it to move freely on a plant) impact the performance of two aphid species on different wheat varieties, including old and modern varieties. Aphid performance was found to be affected by both confinement method, and wheat variety, highlighting the complexity of these interactions. Possible reasons for this include species-specific responses to feeding site and differences in microclimate. Results presented here show the importance of carefully selecting the correct method based on the aphid species being studied. The fact that wheat variety affected aphid performance highlighted the fact that while none of the varieties tested were resistant, useful traits may already be present in commercial wheat varieties.

Abstract: Bird cherry-oat aphid (*Rhopalosiphum padi* L.; Hemiptera: Aphididae) and English grain aphid (*Sitobion avenae* Fabricius; Hemiptera: Aphididae) are important vectors of barley yellow dwarf virus (BYDV) in cereal crops traditionally managed using synthetic chemical insecticides. Exploiting the genetic diversity within different wheat varieties offers an alternative to current controls for managing both aphid pests and virus transmission. Confining individual aphids onto specific plant parts using a clip cages is often used to screen for resistance traits in host plants. However, clip cages can damage plants and influence aphid performance. In this study, the performance of bird cherry-oat aphid and English grain aphid was recorded when aphids were 'confined' or left 'free' on different wheat varieties. Aphid performance was found to be affected by both confinement method and cereal variety, highlighting the complexity of these interactions. Performance of English grain aphid was increased when 'confined' while performance of bird cherry-oat aphid was increased when left 'free'. These differences reflect species-specific differences to feeding sites and responses to microclimate. Differences in aphid performance were also recorded in response to cereal variety, with the lowest performance recorded on the BYDV resistant variety Wolverine. While none of the varieties tested were 'truly' resistant, useful traits for aphid management may already be present in commercial wheat varieties.

Keywords: bird cherry-oat aphid; English grain aphid; intrinsic rate of increase; mean relative growth rate; aphid performance; clip cage; integrated pest management; varietal mixtures

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1. Introduction

Aphids are among the most economically important pests in cereal crops due to their role in vectoring plant viruses [1]. Bird cherry-oat aphid (*Rhopalosiphum padi* L.; Hemiptera: Aphididae) and English grain aphid (*Sitobion avenae* Fabricius; Hemiptera: Aphididae) vector barley yellow dwarf virus (BYDV) in cereal crops. Estimates suggest that BYDV may be responsible for yield losses of up to 84% in wheat and 64% in barley. The impact on yield is mainly as a result of reduced grain number [2]. Current BYDV management strategies include foliar pyrethroid insecticide applications and

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delayed sowing to avoid peak aphid migration events [3]. However, insecticide use is associated with negative impacts on non-target organisms and the evolution of resistance in the target organism to active ingredients that reduces the efficacy of key plant protection products [e.g. 4,5]. Meanwhile, delayed sowing can result in reduced yields [6]. Reduced yields are due to low temperatures during crop vegetative growth and shortened duration of various phases of crop development.

Exploiting the genetic diversity found within different wheat varieties offers an alternative to current controls for managing both aphid pests and virus transmission [7,8]. Host plant genetics influence insect performance parameters, which may reduce aphid infestations through increased resistance [9]. Aphid performance typically focuses on mean relative growth rate (MRGR) and intrinsic rate of increase (r_m) for individual aphids to assess relative resistance/susceptibility across host plants [10–13]. Intrinsic rate of increase describes the rate at which a population changes size per unit of time in the absence of limiting factors such as predation or resource competition [14]. The r_m value integrates both reproductive output and survival probabilities to provide a comprehensive measure of population growth, which is strongly influenced by the nutritional quality of the host plant [15,16]. On the other hand, MRGR focuses on biomass changes in individual aphids over a specific period by calculating the increase in an individual's weight relative to its initial size over time [17]. Thus, MRGR is a measure of how efficiently an individual aphid grows, which can influence developmental speed and, consequently, reproductive timing, indirectly affecting population growth.

Confining individual aphids onto specific plant parts using a clip-cages is a common practice in aphid performance studies to facilitate data collection and avoid losing individuals during experiments [7,18,19]. Although effective at containing aphids, clip cages have unintended consequences on their biology that may impact any conclusions drawn from performance studies [20]. For instance, as clip cages are designed to restrict aphid movement it prevents an individual from choosing a feeding site. This is important as each aphid species may preferentially feed on specific plant parts [21]. Attaching and detaching clip cages during data collection may also damage plant tissue and upregulate defence responses in the host plant, indirectly affecting aphid development or limit plant physiology in other ways [22,23]. To address the effects of confinement and wheat variety on aphid performance, the present study measured the intrinsic rate of increase and mean relative growth rate of the bird cherry-oat aphid and English grain aphid on five different wheat varieties and one barley variety using two different confinement methods. Through this study, we aim to determine the importance of methods used for aphid research investigating host plant resistance.

2. Materials and Methods

2.1. Plant Material

Plants were grown under glasshouse conditions at Harper Adams University (52.777385, -2.427895) (mean temperature: 20 ± 5 °C/10 ± 5 °C day/night; 16h:8h light:dark photoperiod). Wheat (*Triticum aestivum* L.) and spring barley (*Hordeum vulgare* L.) seeds from each tested variety were sown 1 cm deep into 9 x 9 cm pots (Teku, Poeppelmann GmbH, Lohne, Germany) filled with peat-free John Innes No. 2 compost (Sylva Grow®, Melcourt, Tetbury, UK). Plants were grown in 60 x 60 x 60 cm fine nylon mesh (160 µm) cages (BugDorm-6E Insect Rearing Cage, Taichung, Taiwan) with a tray (58 x 58 cm) placed underneath the pots for watering until the plants had reached BBCH Growth Stage 12 (GS12) [24] before being used for experiments. The wheat varieties used in this study were selected to include a range of end uses and parental lineages (Table 1). Spring barley (var. Planet) was used to rear all aphid populations and included in experiments as a control to account for possible influence of previous generations feeding experience [25].

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Table 1. Wheat varieties chosen for experiments.

Breeder	Wheat variety	End use group	Parents
Desprez	Flanders	Old (1976-1983)	Champlein x FD 2816-348
Syngenta	Gleam	Hard group 4	Kielder x Hereford
Plant Breeding Institute	Maris Huntsman	Old (1972-1983)	[(CI 12633 x Cappellle Desprez*5) x Hybrid 46] x Professeur Marchal
LG	Skyscraper	Soft group 4	(Cassius x NAWW29) x KWS Santiago
RGT	Wolverine	Hard group 4	(09TC2654 x Panorama) x Coronation

2.2. Aphid Populations and Age-Synchronised Cohorts

English grain aphid (*Sitobion avenae*) and bird cherry oat aphid (*Rhopalosiphum padi*) were established by collecting 10 individuals of each species from cereal fields located at Harper Adams University and transferring them to potted spring barley (var. Planet) seedlings. Aphid infested barley plants were then placed in insect cages (47.5 x 47.5 x 47.5 cm) (Bugdorm-4 Insect Rearing Cage, Taichung, Taiwan) separated by species and housed in a plant growth room (Fitotron® Weiss Technik, Loughborough, UK) maintained at 18 °C and 60 % relative humidity under a 16:8 light:dark photoperiod. Population maintenance was carried out on a weekly basis by replacing heavily infested barley plants with clean plants. Each population of aphids was maintained in this way for over 10 generations before being used in experiments (i.e., approximately 10 to 12 weeks).

Age-synchronised aphid cohorts were produced prior to use in experiments in order to standardise the fitness of aphids at the start of each experiment. Aphid cohorts were established by transferring 20 adult aphids from the stock populations to clean barley plants (var. Planet) at BBCH GS12 in a new cage (47.5 x 47.5 x 47.5 cm) and left to larviposit for 24 hours. Adult aphids were then removed and only first instar nymphs were left to develop under the same conditions as stock populations until they had become adults.

2.3. Experimental Design

Each aphid species These aphid cohorts was studied separately in consecutive experiments to avoid cross contamination and both experiments were completed in a plant growth room (Fitotron® Weiss Technik, Loughborough, UK) maintained at 20°C and 60 % relative humidity under a 16:8 light:dark photoperiod. Two confinement methods, ('confined' and 'free') were simultaneously tested on each experimental plant to facilitate direct comparison. Adults from the age-synchronised cohorts were individually placed on each experimental plant, confined to a leaf section inside an open 1.5 ml microcentrifuge tube (Eppendorf AG, Hamburg, Germany) sealed with ¼ of a cotton pad ('confined') or placed on whole plants covered with a clear plastic cylinder (13 cm in height, 7 cm in diameter at the top, and 5.5 cm in diameter at the bottom) mounted with a fine mesh organza bag at the top (18 x 13 cm) ('free') (Figure 1). The leaf used for aphid confinement was randomly selected for each plant using a random number generator. Adults were left to larviposit for a 24-hour period on experimental plant and after 24-hours the adult aphids and all but one first instar nymph was removed from each plant. Nymphs removed from these plants were weighed (XPR10/M Microbalance, Mettler Toledo, Columbus, USA) in groups of ten to obtain a mean first-instar nymph weight. Each experimental nymph was carefully removed from the plant using a fine paintbrush (size 000) on day five, individually weighed and then returned to the same plant and position from which it was taken. These aphids were monitored every day to track their development using exuviae. After reaching adulthood, each aphid was monitored every one to two days for a period equal to its development time, and the number of offspring was recorded and removed periodically. To evaluate aphid performance in the different host plants and confinement conditions, multiple biological parameters were measured and are described in Table 2, adapted from [25,26]. Five blocks with six replicates per treatment (variety) and two methods was carried out using a complete randomised block design for each aphid species.

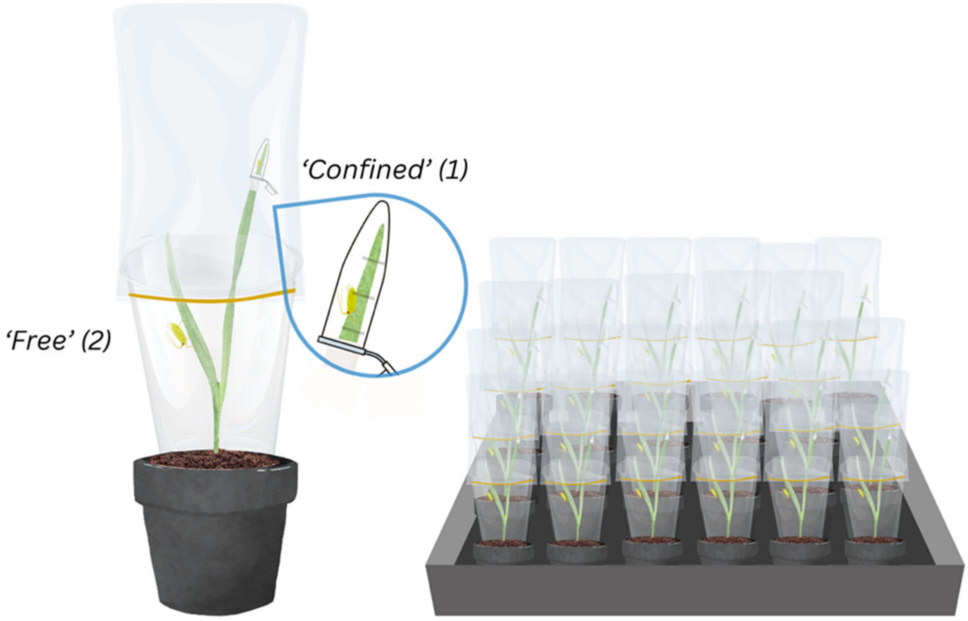

Figure 1. Host plant (wheat or barley) as in the aphid performance experiment (left) and experimental block consisting of 36 plants (right). Each aphid corresponds to one experimental unit in each of the methods tested simultaneously: 'Free' (1) and 'Confined' (2).

Table 2. Biological parameters measured to evaluate aphid performance on different wheat varieties.

Biological Parameter	Measurement
Development Time (DT)	Duration from birth to adult emergence +0.5 d [27]
Weight Gain (Wg)	Wa-Wn[1] [27]
Mean Relative Growth Rate (MRGR)	(lnWa - lnWn)/DT [17,27]
Intrinsic Rate of Natural Increase (r_m)	0.738 ln(fecundity)/DT [17,28]

[1]Wa is the nymph weight 5 days after birth, Wn is the first-instar nymph weight (newborn within 24h).

2.4. Statistical Analysis

Data were first checked for normality (Shapiro–Wilk) and homoscedasticity (Levene's). Because all datasets satisfied these assumptions, or were sufficiently close, linear mixed-effects models (LMMs) were fitted using the lme4 package [29], with 'Replicate' as a random effect and 'Variety' and 'Method' as fixed effects. Separate models were fitted for each response variable (MRGR and r_m), initially including the Variety × Method interaction. Likelihood-ratio tests compared the full (interaction) model against the reduced (no-interaction) model, which was non-significant in all cases and so final inferences were based on the reduced models. Post-hoc pairwise comparisons were carried out using the emmeans package with Sidak adjustment [30]. All analyses were carried out using R (version 4.4.1).

3. Results

3.1. English Grain Aphid

3.1.1. Mean Relative Growth Rate (MRGR)

No differences were found in MRGR between cereal varieties (Figure 2A). Confinement method, however, had a significant effect, with a higher MRGR recorded for 'confined' aphids MRGR (0.227 ± 0.008) compared to 'free' aphids (0.185 ± 0.008) (Figure 2B).

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3.1.2. Intrinsic Rate of Increase (r_m)

Significant differences were observed between cereal varieties. The highest r_m (0.305 ± 0.004) was recorded for aphids feeding on barley, whilst the lowest r_m (0.266 ± 0.006) was recorded on Wolverine (Figure 2C). Confinement also had a significant effect on r_m, with higher values recorded for 'confined' aphids (0.288 ± 0.003) compared to 'free' aphids (0.268 ± 0.003).

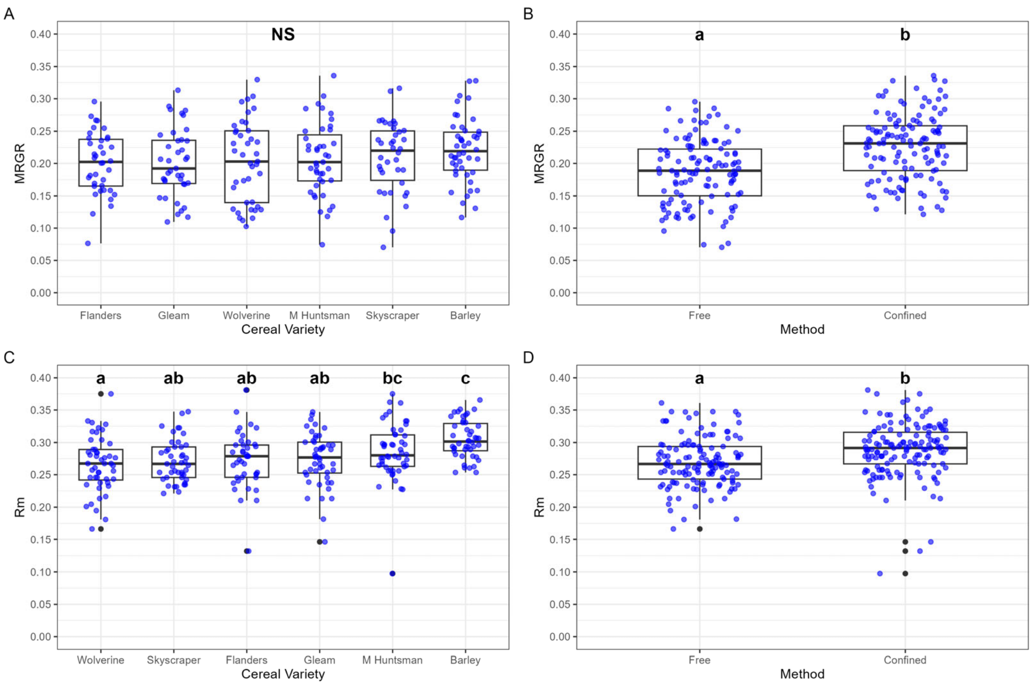

Figure 2. Effects of cereal variety and confinement method on the mean relative growth rate (MRGR) and intrinsic rate of increase (r_m) of English grain aphid (*Sitobion avenae*). (A) MRGR across six cereal varieties, showing no significant difference; (B) MRGR under two confinement methods, with "Confined" resulting in significantly higher values than "Free"; (C) r_m across six cereal varieties, with significant differences among varieties; (D) r_m under two confinement methods, with "Confined" leading to significantly lower values compared to "Free." Letters indicate significant differences using post-hoc comparisons with Sidak correction ($p < 0.05$). Boxplots show median, interquartile range, and individual data points.

3.2. Bird Cherry-Oat Aphid

3.2.1. Mean Relative Growth Rate (MRGR)

A significant effect of cereal variety was found on bird cherry-oat aphid MRGR, but no significant interaction with confinement method was identified. The highest MRGR was recorded for aphids feeding on barley (0.250 ± 0.007), while the lowest was on Wolverine (0.191 ± 0.009) (Figure 3A). Wolverine-fed aphids exhibited significantly lower MRGR than those on the older wheat varieties Flanders (0.214 ± 0.008) and Maris Huntsman (0.219 ± 0.007) (Figure 3A). Confinement significantly reduced MRGR, with confined aphids exhibiting a lower growth rate (0.189 ± 0.005) compared to free aphids (0.242 ± 0.003, n = 122), indicating that the restriction negatively impacted aphid growth (Figure 3B).

3.2.2. Intrinsic Rate of Increase (r_m)

There were significant differences in bird cherry-oat aphid r_m across cereal varieties and confinement methods. Among varieties, aphids feeding on barley exhibited the highest r_m (0.422 ± 0.007), while those on Wolverine had the lowest (0.315 ± 0.014) (Figure 3C). Confinement had a

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significant negative effect on r_m, with confined aphids having a lower intrinsic rate of increase (0.317 ± 0.007) compared to free aphids (0.394 ± 0.005), demonstrating a strong negative impact of restricted movement on aphid reproductive potential (Figure 3D).

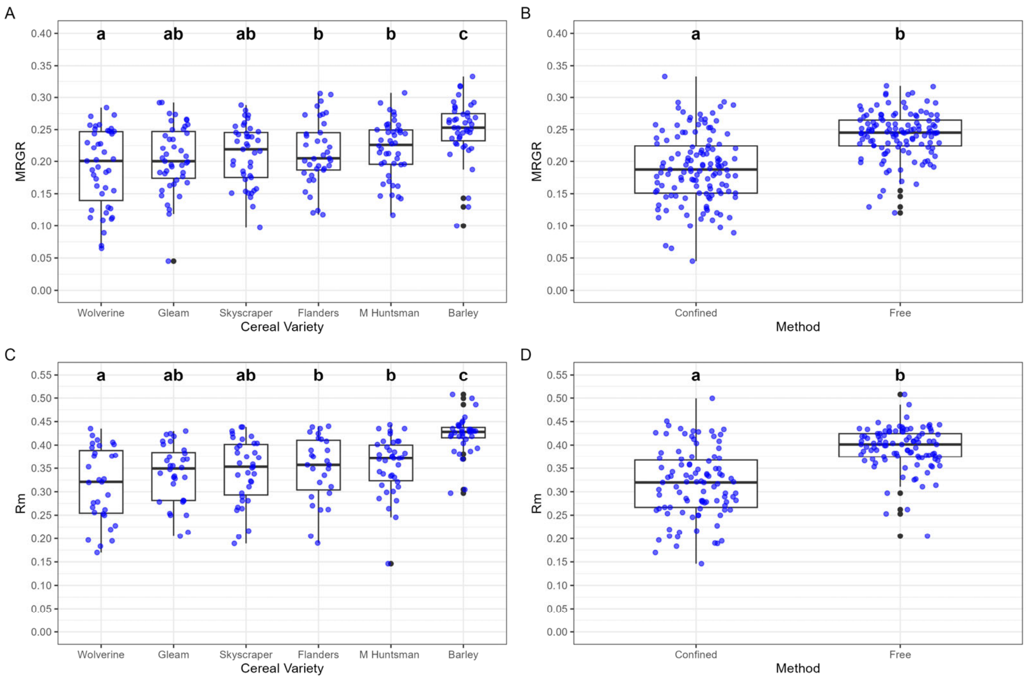

Figure 3. Effects of cereal variety and confinement method on the mean relative growth rate (MRGR) and intrinsic rate of increase (*r_m*) of *R. padi*. (A) MRGR across six cereal varieties, showing significant differences among varieties; (B) MRGR under two confinement methods, with "Confined" showing significantly higher values than "Free"; (C) *r_m* across six cereal varieties, with significant differences among varieties; (D) *r_m* under two confinement methods, with "Confined" resulting in significantly lower values compared to "Free". Letters indicate significant differences ($p < 0.05$), based on statistical analysis.

4. Discussion

Results from this study show that use of clip cages decreased bird cherry-oat aphid performance in terms of both MRGR and *r_m*, regardless of host plant or variety. As this aphid species typically feeds at the base of the plant stem [31], confinement to a leaf section is likely to compromise nutrient availability for this species. Similar findings have been reported in other aphid species, where confinement to non-preferred feeding sites alters growth and reproduction [32]. Other factors involving the microclimate formed in a confined space, such as increased humidity, may also play a role in aphid life-history traits [32,33]. In contrast, the use of confinement increased the performance of English grain aphid. An earlier study investigating the potential of a modified lightweight clip cage reported that the performance of this species was similar when confined to this clip cage or left free on the plant [34]. While it is not possible to directly compare between the two studies, the contrasting confinement methods used further supports the conclusion that experimental method influences aphid performance recorded. However, the fact that these studies indicate a similar or increase in aphid performance when confined is likely to be in part at least due to the fact that this species typically feeds on leaves and so the position of the confinement apparatus would have had a reduced effect on feeding behaviour.

Several studies have investigated resistance to both English grain aphid and bird cherry-oat aphid in wheat lines [e.g. 7,34,35]. However, none of these studies have considered the impact that confinement may have on aphid performance of both aphid species. This is likely to be most important where wheat collections, such as the Watkins and Gediflux collections, are screened [35]

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and presence of partial plant resistance is masked by the impact of the experimental technique. Missing the presence of partial plant resistance will have the impact of delaying and possibly preventing useful traits from being introgressed into elite breeding lines. The impact on aphid performance across cereal varieties highlights the role of plant genotype in shaping aphid population dynamics. Resistance mechanisms may include antixenosis, where aphids avoid specific varieties, and antibiosis, where host plant properties negatively impact aphid development and reproduction [36]. Previous work has reported significant variation in aphid performance when feeding on different lines within wheat collections [35]. The fact that here we report reduced aphid performance on commercial varieties, such as Wolverine, suggests that selective breeding, such as the inclusion of the Bdv2 gene to confer resistance to BYDV-PAV, may have unintentionally introduced traits that confer partial resistance to these pests [35]. This is important because recent work has shown that mixtures of wheat varieties have the potential to reduce the performance of English grain aphid [37]. However, the combination of varieties appears to be important in achieving this effect, therefore, an understanding of aphid performance on and behavioural response to each wheat variety included in a mixture are likely to be important factors in determining the success of this approach. Further considerations could include the use of wheat varieties known to reduce the transmission of BYDV by aphids [26,38]. Indeed, on this final point, it is interesting to note that the lowest performance of both species of aphid in this study was recorded when aphids were feeding on the BYDV resistant variety Wolverine.

The higher performance of both aphid species on barley is consistent with findings from previous studies, where host plant suitability was linked to aphid fecundity and growth rates [18,39]. This may be attributed to nutritional factors, such as variations in plant secondary metabolites and amino acid profiles, which have been shown to influence aphid development [40]. The suitability of barley to both aphid species is likely to also reflect the rearing history of aphid populations on this host and is likely due to maternal effects. Maternal effects represent the impact of environmental variation in previous generations on phenotypic variations in the offspring generation. When reproducing parthenogenetically, asexual mother aphids develop telescoping generations (embryos within embryos) so that granddaughters are present inside the bodies of their grandmothers. As a result, aphids have strong maternal and transgenerational effects that can extend for three or more generations [41,42].

5. Conclusions

Intrinsic crop resistance is a fundamental basis for functional IPM [42]. Differences in aphid performance among cereal varieties highlight the potential to identify resistance traits but the experimental methods used to identify such resistance must be given full consideration as these may impact aphid fitness. This knowledge could accelerate resistance breeding programs aimed at developing aphid-resistant lines by enabling the development of molecular markers for aphid susceptibility, thereby aiding plant breeders in selecting for resistant traits more efficiently and reducing reliance on chemical control. Furthermore, an understanding of how varieties vary in susceptibility to *S. avenae* and *R. padi* may enable development of varietal mixtures that reduce the performance and spread of aphid vectors of BYDV [37].

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