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Effect of Plant Identity in Wheat Mixtures on English Grain Aphid (*Sitobion avenae*) Control

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ABSTRACT

Field experiments have demonstrated that wheat mixtures differ in their ability to regulate aphid populations. To further investigate the effectiveness of wheat mixtures (*Triticum aestivum* and *Triticum turgidum*) in controlling aphids, we conducted both laboratory and greenhouse experiments. Specifically, we assessed the associational resistance of two wheat mixtures (Florence-Aurora with Forment, Florence-Aurora with Montcada), and their respective monocultures, in different stages of the aphid host selection process. We analysed aphid acceptance rate, population growth, and load under different wheat treatments. Additionally, we characterised wheat aboveground biomass and nitrogen content as important functional traits for aphid resistant. Aphid acceptance decreased in plants of cv. Forment when exposed to volatiles from undamaged Florence-Aurora plants, whereas the other tested combinations tested had no effect. Aphids performed differently in the two mixtures: Florence-Aurora mixed with Forment significantly reduced aphid population growth and load compared to the monocultures, whereas the combination of Florence-Aurora with Montcada wheat had no effect on aphid performance. The plant–plant interactions also modified the analysed traits. Nitrogen content of Florence-Aurora wheat plants was reduced when mixed with Forment wheat, which may explain the lower aphid load observed in plants of cv. Florence-Aurora when mixed with plants of cv. Forment. However, mixing wheats with similar aboveground biomass resulted in an increase in the average biomass of plants of both cultivars which could have led to a higher aphid population. The data supports the idea of right neighbour, as the benefits of wheat mixtures for aphid control were determined by the identity of the combined plants (or species). Finally, our results suggest that associating wheats with different traits may promote facilitative interactions, which in turn enhances associational resistance, whereas the combination of wheats with similar traits may result in competitive interactions that may hinder aphid control benefits.

1 | Introduction

Wheat diversity (e.g., through cultivation of cultivar mixtures) has become an opportunity to introduce functionality to organic agricultural systems. This approach also ensures easy management for farmers when compared to other diversification strategies such as using a cover crop or some interspecific association (Costanzo and Bàrberi 2014; Wezel et al. 2014). The potential of wheat mixtures to enhance crop functionality is linked to the genetic variability amongst cultivars. This genetic variability gives each plant a unique and distinctive set of functional traits (Barot et al. 2017). In turn, the diversity of functional traits amongst cultivars may lead to complementary relationships that enhance the provision of agroecosystem services in wheat mixtures (Gaba et al. 2015; Tilman, Isbell, and Cowles 2014). For instance, cereal cultivar mixtures reportedly result in aphid population control, amongst others benefits (Costanzo and Bàrberi 2014; Tooker and Frank 2012).

Aphids are commonly used in pest management research as model organisms for plant–herbivore studies because of their direct and indirect economic impacts on cereal crops. In addition, their taxonomy, physiology, and life cycles have been extensively studied and are well understood (Dedryver, Le Ralec, and Fabre 2010; Dixon 1987; Rodriguez-Saona and Stelinski 2009). Control of aphid populations in crop plant mixtures is essentially based on associative resistance, whereby beneficial plant–plant interactions between the combined cultivars can lead to a reduction in aphid population growth (Barbosa et al. 2009; Tahvanainen and Root 1972). Specifically, associational resistance can manifest at different stages of the aphid host selection and population development processes, as a hosts can be accepted or rejected at any point in the sequence (Barbosa et al. 2009; Powell, Tosh, and Hardie 2006). The initial stages of aphid host selection and population development are aphid host location and detection. During these stages, aphids use olfactory and visual cues to locate their hosts (Powell, Tosh, and Hardie 2006). After landing (second stage), aphids used olfactory, gustatory, and tactile cues to decide whether to accept the plant. Wheat mixtures can enhance associational resistance in the first and second stages by reducing the likelihood of aphids locating and accepting host-preferred cultivars through olfactory or visual masking (Grettenberger and Tooker 2016; Tahvanainen and Root 1972). The third stage corresponds to aphid colony development, at which stage, population growth and aphid size depend on host quality (Aqueel and Leather 2011; Nowak and Komor 2010). In the third stages, associational resistance may arise because plant–plant interactions via volatile organic compounds (VOCs) can cause physiological or morphological shifts in the susceptible cultivar, which may ultimately affect herbivores (Kheam et al. 2023; Ninkovic, Markovic, and Dahlin 2016). These modifications may alter host plant quality, or induce herbivore defence mechanisms (Barbosa et al. 2009; Ninkovic, Markovic, and Dahlin 2016).

Therefore, it is necessary to investigate the positive (facilitation, complementation), neutral, or negative (competition) plant–plant interactions occurring in different stages of the aphid host selection and population development processes.

In particular, it would be interesting to assess the role of functional traits related with the associational resistance of wheat mixtures (Barot et al. 2017). Previous studies have highlighted the significance of plant odour signals in aphid host identification, location, and acceptance (Pickett et al. 1992; Webster 2012). Once aphids are established on a plant, their development, survival, and reproduction are mainly driven by plant biomass and quality traits such as tissue nitrogen (N) content (Jakobs and Müller 2018; Szpeiner, Martínez-Ghersa, and Ghersa 2009). A larger plant biomass may result in greater herbivore abundance (Barbosa et al. 2009). Furthermore, aboveground plant N content is a limiting factor in the aphids' diet (Taiz and Zeiger 2006). Previous greenhouse studies have indicated a positive relationship between N fertilisation in cereal crop plants and aphid growth rate, individual size, fecundity, and size (Aqueel et al. 2014, Aqueel and Leather 2011; Duffield et al. 1997; Gash 2012; Nowak and Komor 2010). Nevertheless, studies on the heterogeneity of tissue N content across cereal cultivars under identical fertilisation conditions and its effects on aphid control have been widely overlooked (Aqueel and Leather 2011; Gaba et al. 2015).

To this end, we studied the effects of plant–plant interactions on aphid control in different aphid stages, including aphid acceptance and aphid population development, for three wheat monocultures: Florence-Aurora (*Triticum aestivum* L. subsp. *aestivum*), Forment (*Triticum turgidum* L. subsp. *durum* Desf. (Husn.)), and Montcada (*T. aestivum* L. subsp. *aestivum*) and two mixtures: Florence-Aurora with Forment and Florence-Aurora with Montcada. Although *T. aestivum* and *T. turgidum* are taxonomically different, they are closely related (Wang et al. 2013). Hence, for simplicity, we refer to Forment as a cultivar.

Additionally, we investigated the influence of intraspecific plant interactions on wheat aboveground biomass and tissue N content. We hypothesised that (i) aphid acceptance will be negatively affected by VOCs interactions between cultivars with different odour profiles; (ii) the capacity of the wheat mixture to impair aphid populations would depend on the identity of the cultivars combined; and (iii) plant–plant interactions in the wheat mixtures would modify aboveground biomass and N content.

2 | Methodology

2.1 | Plants and Insect Material

We set up a greenhouse experiment with three plants of winter wheat: the modern cultivar Florence-Aurora (released after 1950) (*T. aestivum* L. subsp. *aestivum*) and the traditional cultivars Forment (*T. turgidum* L. subsp. *durum* Desf. (Husn.)) and Montcada (*T. aestivum* L. subsp. *aestivum*) (released before 1950). These cultivars were selected based on agronomic requirements and commercial criteria to assure the viability of the mixtures in a real farming context. When establishing the wheat mixtures, we considered the functional traits related to aphid resistance, including phenology, height and odour profile (Serra-Gironella and Álvaro 2017; Tous-Fandos et al. 2023). We combined two cultivars with similar traits, Florence-Aurora and Montcada, and two cultivars with distinct traits, Florence-Aurora and Forment (Table 1). The combination of a modern wheat with a traditional one was intentional, as modern wheats are more productive but more prone to diseases and herbivore attacks, whereas traditional wheats can complement the modern ones by offering resistance to pests (Serra-Gironella et al. 2019). The combination of Forment and Montcada (FOMO) was not tested because of poor commercial and agronomic interest of mixing these two cultivars. Seeds were supplied by farmers of the Gallecs Agroecological Union (Mollet del Vallès, Barcelona, Spain).

TABLE 1 | List of the functional traits related to aphid resistance considered for the selection of wheat cultivars and the design of wheat mixtures.

Wheat plant	Scientific name	Phenology[a]	Height (cm)[b]	Odour signals[c]	Commercial purposes[a]
Florence-Aurora	*Triticum aestivum* L. *subsp. aestivum*	Early flowering date	70	Similar odour profile	Excellent hard wheat flour for bread-making
Forment	*Triticum turgidum* L. *subsp. durum* Desf. (Husn.)	Late flowering date	180	Distinct odour profile	Great aromatic value
Montcada	*Triticum aestivum* L. *subsp. aestivum*	Early flowering date	70	Similar odour profile	Good wheat flour for bread-making

[a]Consorci de Gallecs website.
[b]Serra-Gironella and Álvaro (2017).
[c]Tous-Fandos et al. (2023).

The wheat cultivars were either grown as monocultures, Florence-Aurora (FA), Forment (FO), and Montcada (MO), or in two wheat mixtures in a 1:1 proportion: Florence-Aurora and Forment (FAFO) and Florence-Aurora and Montcada (FAMO), for a total of five treatments. Wheat monoculture treatments served as controls. Temperature inside the greenhouse was 18°C–22°C, with the light regime of 16:8 h light/dark and the relative humidity of 50%–60%. Wheat plants were grown in plastic pots (13.7 × 13.7 × 23 cm) filled with potting soil (Hasselfors P soil, Sweden): four plants were grown in each pot.

English grain aphids, *Sitobion avenae* (Fabricius), were reared on oats (*Avena sativa* L. cv. Belinda) in multi-clonal cultures in a separate rearing chamber under the same conditions.

2.2 | Plant Exposure to VOCs

To analyse aphid acceptance, we exposed the test cultivars (known as receivers) to VOCs emitted by inducer cultivars or the same test cultivar (self-exposure) as a control. Exposure was performed in two-chamber cages (Ninkovic, Olsson, and Pettersson 2002): Air was drawn into the chamber where plants of the inducer cultivar were placed and passed through a hole in the wall into the chamber where plants of the receiver cultivar were placed. Air from the top of the receiving chamber was drawn into the vacuum tank and vented outside the greenhouse. The exposure system had an airflow of 1.3 L min^{-1}. Six wheat plants of the same cultivar were planted together in plastic pots (9 cm × 9 cm × 7 cm) filled with potting soil (Hasselfors P soil, Sweden). The pots were placed in separate petri dishes to prevent plant interactions caused by the root exudates. Plants were watered using an automated drop system (DGT Volmatic). Plants at the one-leaf stage (Z11) were placed in the exposure system and exposed for 5 days. The experimental plants were grown in a separate growing chamber under the same light and temperature conditions as in the chamber with plant exposure cages. To prevent any VOC interactions between the cultivars during the pre-exposure period, pots of different cultivars were placed at least 1 m apart. For the aphid settling tests, five of the six plants per pot from the receiving chambers were randomly chosen. Each combination was replicated four times.

2.3 | Test of Aphid Plant Acceptance

After exposure to VOCs from the emitting cultivar, the plants of the receiving cultivar were tested for aphid acceptance using a no-choice test (Ninkovic, Glinwood, and Dahlin 2009). Ten apterous aphids of mixed ages were released into a polystyrene tube (122 mm × 30 mm) containing the second leaf of a wheat plant. The upper end of the tube was covered with a fine-meshed net, and the lower end was plugged with a piece of plastic foam with a slit for the leaf. A wooden stick was used to support the tube. After 2 h, the aphids that had settled (i.e. not walking and with antennae placed on the body) on the tested leaves were counted.

Aphid acceptance may vary between experiments owing to small differences in testing conditions, therefore, to correct for these variations, the mean number of aphids accepting the VOC treated plants (At) was divided by the mean number of aphids accepting unexposed plants (A0), in a test.

At/A0 < 1 indicates reduced acceptance, At/A0 = 1 indicates no induction relevant to aphids, and At/A0 > 1 indicates an increase in aphid settling.

2.4 | Aphid Population Growth Parameters

We conducted a greenhouse experiment to assess the growth of the aphid population using 150 pots, with 30 replicates for each wheat treatment at the beginning of the experiment. Four weeks after sowing, we inoculated each plant with one apterous aphid and then we recorded the total number of aphids per plant at 10, 15, and 20 days after aphid inoculation. The number of replicates for each treatment decreased over time because of aboveground biomass sampling (see below). Thus, at 10, 15, and 20 days after aphid inoculation, the number of replicates per treatment was 20, 10, and 10, respectively. This reduction in the number of replicates was considered in the data analyses.

Aphid population growth was determined using the mean number of aphids per treatment and sampling time. In addition, we compared aphid load, (i.e., the average number of aphids per unit dry weight), between plants grown in monoculture and those grown in wheat mixtures at 20 days after aphid inoculation to investigate the effects of associational resistance on a specific cultivar. Nevertheless, it is worth discussing that aphid load can be misleading in treatments where aphid growth is identical, as differences in aphid load may simply reflect variations in wheat biomass.

2.5 | Wheat Biomass Analysis

Four weeks after sowing (inoculation time), the aboveground plant biomass was assessed. Additionally, biomass was collected at 10- and 20-days post-inoculation from 10 pots for each treatment and dried at 60°C for 72 h. In the mixture treatments, plants were tagged, and the two cultivars were weighed separately.

2.6 | Wheat N and Carbon Content Analysis

Before aphid inoculation, we collected the aboveground biomass of four plants from each of the pots in each wheat treatment and dried them at 60°C for 72 h for N content analysis. Dried samples were ground using an agate-mortar grinding mill. Homogeneous subsamples (2.5–3 μg) of ground plant tissue were collected for analysis of total N and C contents using the Dumas dry-combustion method.

2.7 | Statistical Analysis

All statistical analyses were conducted using the R software version 4.1.1 (R Development Core Team 2021). Differences in aphid acceptance between cultivars exposed to clean air and those exposed to VOCs from other or the same cultivars were analysed using generalised linear mixed models (GLM) with a binomial error distribution in *lme4* (Bates et al. 2015). The results were expressed as the proportion of settled aphids out of the 10 introduced aphids as replicates. Tukey's test was used for post hoc comparisons.

The measured variables of aphid population growth, aphid load, wheat biomass and wheat N content were analysed using linear mixed effects models (LMM) or generalised linear mixed effects models (GLMM) using the glmmTMB function from the *glmmTMB* package (Brooks et al. 2017). The best model for each response variable was determined by using the Akaike information criterion. The significance of the fixed effect factors and their interactions was determined using an F test with the Kenward-Roger approximation for LMMs or a likelihood ratio test for GLMMs. Pairwise comparisons were performed using Tukey-adjusted estimated marginal means (EMMs; a.k.a. least-squares means) using the *emmeans* package (Lenth 2019). Aphid population growth, aphid load, and wheat biomass models were fitted by considering two fixed covariables: wheat treatment (categorical variable with five levels: FA, FO, MO, FAFO, and FAMO), sampling time (categorical variable with three levels: 10, 15, and 20 days) and their interactions. Aphid population growth fitted a negative binomial error distribution without any interactions. The number of pot replicates per treatment (categorical variable with 10 levels) was added as a random factor to fit the GLMM with repeated measurements. Aphid load was independently analysed for each cultivar. Hence, we categorised the wheat treatment factors into two distinct levels: monoculture (FA, MO, or FO) and mixture (FAMO or FAFO). To equalise the sowing ratios between the monocultures and mixtures, the total number of aphids per plant in the mixture plots was doubled. Aphid load was fitted to a negative binomial GLMM. The wheat biomass model was fitted to a Gaussian LMM with an interaction between treatment and sampling time. For statistical analysis of N and carbon content, the dataset was separated by mixture. Hence, we assessed Florence-Aurora monoculture, Forment monoculture, and Florence-Aurora with Forment mixture data on the one hand, and Florence-Aurora monoculture, Montcada monoculture, and Florence-Aurora with Montcada mixture data on the other. The wheat N and carbon model included two fixed covariables: wheat treatment (categorical variable with two levels: monoculture or mixture) and cultivar treatment (categorical variable with two levels: Florence-Aurora or Forment, Florence-Aurora or Montcada). The wheat N content and carbon model was fitted to a Gaussian LMM with an interaction between treatment and cultivar factors. The correlation between aphid abundance and wheat N and carbon content was analysed via the Pearson correlation method.

3 | Results

3.1 | Aphid Plant Acceptance

Analysis of aphid plant acceptance indicated no difference for the settlement of *S. avenae* between self-exposed cultivars. However, plants exposed to Florence-Aurora VOCs had a significantly lower aphid acceptance ($F_{4,89} = 5.74$, $p < 0.01$). Specifically, aphid acceptance of plants of the Forment individuals exposed to VOCs emitted by plants of the Florence-Aurora cultivar was reduced by 20% relative to that of unexposed plants (Table 2). No other significant effects were observed.

3.2 | Aphid Population Development

The total number of aphids per pot was used to calculate the size of the aphid populations in the monocultures and mixtures. The aphid population size increased over time in all wheat treatments ($\chi_{2,90} = 2538.91$, $p < 0.001$), differing between wheat treatments ($\chi_{4,88} = 24.20$, $p < 0.001$). From the first count, the FA cultivar supported the greatest aphid population, which was significantly higher than that of FAFO ($p < 0.001$) at 20 days after inoculation (Figure 1).

Aphid load was lower in plants grown in for Florence-Aurora and Forment mixture than those grown in monocultures (FA: $\chi_{2,60} = 7.14$, $p < 0.05$, FO: $\chi_{1,72} = 10.83$, $p < 0.01$). However, the aphid load on plants of the Florence-Aurora and Montcada cultivars grown in the FAMO mixture was comparable to those on plants grown in monoculture (FA: $\chi_{2,60} = 3.04$, $p = 0.09$, MO: $\chi_{1,72} = 5.41$, $p = 0.11$) (Figure 2).

TABLE 2 | Aphid cultivar acceptance expressed as the proportion between the number of aphids settled on receiver wheat cultivars (Florence-Aurora, Forment and Montcada) after exposure to VOCs from inducer cultivars and unexposed plants. Asterisks indicate a significant shift in aphid cultivar acceptance according to Tukey's-adjusted pairwise test ($p < 0.05$).

	Inducers		
Receivers	**Florence-Aurora**	**Forment**	**Montcada**
Florence-Aurora	0.98	0.98	0.99
Forment	0.80	0.89	0.94
Montcada	1.06	1.02	0.99

3.3 | Wheat Biomass

At 30 and 50 days after sowing, the aboveground biomass of plants grown in monocultures differed significantly (30 days: $F_{1,3} = 3.34$, $p < 0.05$, 50 days: $F_{1,3} = 2.21$, $p < 0.05$). Plants of the Florence-Aurora and Montcada cultivars had larger biomass than those of the Forment cultivars at 30 days after sowing ($p < 0.05$) whereas at 50 days Forment plants showed larger wheat biomass than those of the cultivar Florence-Aurora ($p < 0.05$). Aboveground plant biomass was affected by plant–plant interactions in cultivar mixtures ($F_{1,3} = 5.48$, $p < 0.05$). Particularly, plants grown in the FAMO mixture showed significantly ($p > 0.05$) greater aboveground biomass at 30 and 50 days after sowing than those grown in monocultures (Table 3).

3.4 | Nitrogen and Carbon Content

Nitrogen content in the aboveground biomass of 1-month-old wheat plants grown in monoculture varied between cultivars. Florence-Aurora had a higher N content than the Forment monocultures ($F_{1,3} = 4.94$, $p < 0.05$). The N content of Florence-Aurora plants decreased significantly when grown together with Forment ($F_{1,3} = 5.91$, $p < 0.05$), resulting in a similar N content in the two mixed cultivars. In contrast, plants of the Florence-Aurora and Montcada cultivars had similar N contents whether grown in monocultures ($F_{1,3} = 0.14$, $p = 0.71$), or in a mixture ($F_{1,3} = 0.24$, $p = 0.62$). Neither Forment nor Montcada plant N content varied when grown in a mixture (Table 3). The results showed a low positive correlation between the number of aphids per plant and aboveground N content ($r = 0.30$, $p = 0.02$). Carbon

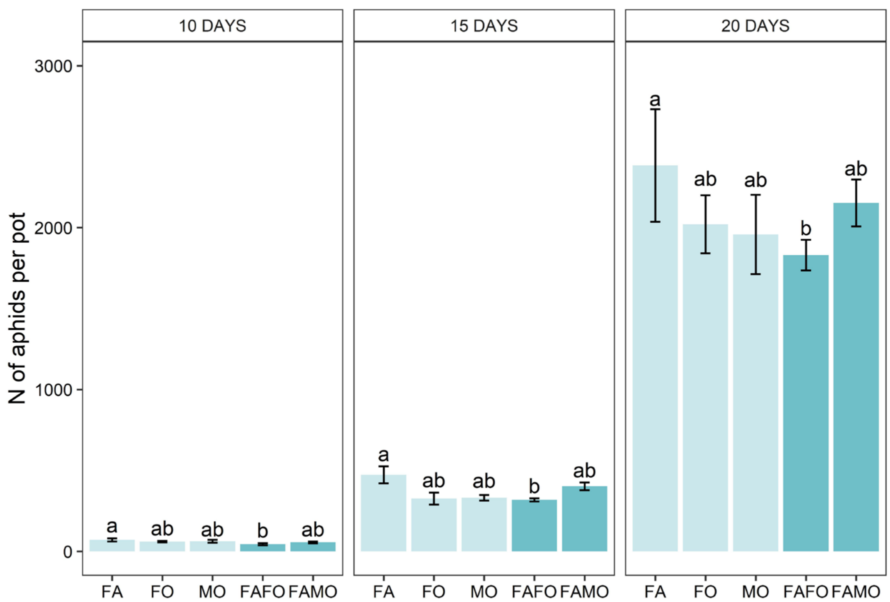

FIGURE 1 | Aphid population growth over time (mean ± SE) at 10, 15, and 20 days after aphid inoculation on five wheat treatments: Three monocultures, Florence-Aurora (FA), Forment (FO), and Montcada and two wheat mixtures, Florence-Aurora with Forment (FAFO) and Florence-Aurora with Montcada (FAMO). Different letters indicate significant differences between wheat cultivars and mixtures within each sampling time according to Tukey-adjusted pairwise EMMS comparisons ($p < 0.05$). [Colour figure can be viewed at wileyonlinelibrary.com]

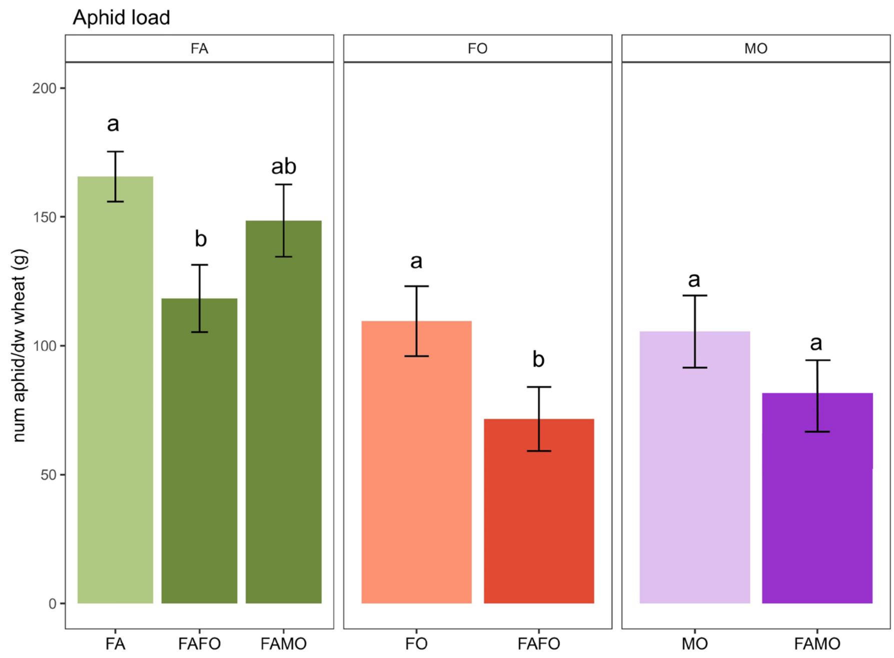

FIGURE 2 | Aphid load (mean number of aphids per unitdry weight ± SE) at 20 days after inoculation; (a) Florence-Aurora cultivar grown in monoculture (FA), mixed with Forment (FAFO or Montcada FAMO); (b) Forment cultivar grown in monoculture (FO), or mixed with Florence-Aurora (FAFO); and (c) Montcada cultivar grown in monoculture (MO), or mixed with Florence-Aurora (FAMO). Different letters indicate significant differences according to Tukey's-adjusted pairwise EMMS comparisons ($p < 0.05$). [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 3 | Aboveground dry weight of wheat plants (mean ± SE) at 30, 40, and 50 days after sowing grown in monoculture (FA, FO, and MO) or in wheat mixtures Florence-Aurora with Forment (FA in FAFO or FO in FAFO) and Florence-Aurora with Montcada (FA in FAMO or MO in FAMO). Asterisks indicate significant differences within the FAFO (FA, FA in FAFO, FO in FAFO, and FO) and FAMO (FA, FA in FAMO, MO in FAMO, and MO) categories according to Tukey's-adjusted pairwise EMMS comparisons ($p < 0.05$).

	FAFO			
	FA	FA in FAFO	FO in FAFO	FO
30 days dw (g)	0.64 ± 0.02	0.65 ± 0.02	0.55 ± 0.01	0.54 ± 0.01
40 days dw (g)	2.34 ± 0.06	2.18 ± 0.10	2.11 ± 0.06	2.18 ± 0.04
50 days dw (g)	4.92 ± 0.05	4.76 ± 0.11	5.07 ± 0.28	5.24 ± 0.16

	FAMO			
	FA	FA in FAMO	MO in FAMO	MO
30 days dw (g)	0.64 ± 0.02	0.80 ± 0.01	0.73 ± 0.01	0.62 ± 0.02
40 days dw (g)	2.34 ± 0.06	2.39 ± 0.06	2.72 ± 0.11	2.56 ± 0.04
50 days dw (g)	4.92 ± 0.05	5.08 ± 0.09	6.08 ± 0.14	5.63 ± 0.29

content of the aboveground biomass was not affected by cultivation as monocultures or in a mixture (Table 4).

4 | Discussion

4.1 | Wheat Mixtures Benefit in Aphid Control Are Mixture Specific

In the present study, we investigated the effectiveness of two wheat mixtures in controlling aphid populations. We conducted the experiment in a greenhouse, as this allowed us to control major environmental factors and focus solely on the influence of plant–plant interactions on aphids.

In the early stages of aphid host detection, plant interactions through VOCs affects aphid responses. Thus, aphid acceptance decreased when inducer and receiver plants had different odour profiles. This effect was only observed in Forment plants exposed to Florence-Aurora VOCs. Interestingly, the effect was not reciprocal, hinting at the specificity of plant communication.

It has been hypothesised that the reduced acceptance of aphids may be due to a physiological modification of the receiving

TABLE 4 | Total percentage of biomass nitrogen (N %) and carbon (C %) (mean ± SE) of Florence-Aurora, Forment and Montcada cultivars grown in monocultures (FA, FO, and MO) or in wheat mixtures Florence-Aurora with Forment (FA in FAFO or FO in FAFO) and Florence-Aurora with Montcada (FA in FAMO or MO in FAMO). Sampling for tissue nitrogen and carbon was conducted before aphid inoculation. Asterisks indicate significant differences within cultivar mixtures according to Tukey's-adjusted pairwise EMMS comparisons ($p < 0.05$).

	FAFO			
	FA	**FA in FAFO**	**FO in FAFO**	**FO**
N (%)	5.28 ± 0.07	5.08 ± 0.05	5.06 ± 0.02	5.09 ± 0.05
C (%)	36.31 ± 0.15	36.88 ± 0.12	36.54 ± 0.18	36.70 ± 0.39
	FAMO			
	FA	**FA in FAMO**	**MO in FAMO**	**MO**
N (%)	5.28 ± 0.07	5.16 ± 0.08	5.29 ± 0.08	5.22 ± 0.05
C (%)	36.31 ± 0.15	36.61 ± 0.22	36.70 ± 0.39	36.51 ± 0.20

plants, making them less attractive to aphids (Kheam et al. 2023). Alternatively, it has been proposed that changes in VOCs of the exposed plants might interfere with the location and acceptance of the host by the aphid upon perceiving such changes (Kheam, Gallinger, and Ninkovic 2024).

In terms of aphid population development, our results showed that the effectiveness of a wheat mixture for aphid control depended on the identity of the combined plants, supporting the right neighbour concept (Dahlin et al. 2018; Kheam et al. 2023). In this study, the mixture of Florence-Aurora and Forment effectively reduced aphid populations compared to Florence-Aurora monoculture. In addition, both cultivars showed significantly lower aphid loads when mixed, despite no changes in their above-ground biomass when grown together. In contrast, the mixture of Florence-Aurora and Montcada wheats had no effect on aphid population development. The contrasting performance of the two mixtures studied here might be due to an enhancement of facilitation interactions, and consequently, associational resistance that results from combining plants with different functional traits, such as tissue N content and aboveground biomass, as observed when cultivars Florence-Aurora and Forment grew together in a mixture. However, the association between similar wheats may lead to functional redundancy.

4.2 | Effect of Quality Traits on Aphid Populations Control in Wheat Stands

Our results indicate that wheat cultivars grown in monoculture regime, particularly Forment, differed in both aboveground biomass and N content under the same soil fertility conditions. These findings revealed the heterogeneity of functional traits related to aphid susceptibility in wheat plants. It is important to highlight that the benefits of wheat mixture in aphid population control rely on complementary functions between associated cultivars (Barot et al. 2017).

There is a considerable body of literature related to plant nutritional parameters, such as plant biomass, tissue N content, and aphid performance (Dixon 1987; Duffield et al. 1997; Gash 2012; Jakobs and Müller 2018). The evidence consistently indicates that wheat cultivars with greater biomass can sustain larger aphid populations (Szpeiner, Martínez-Ghersa, and Ghersa 2009), whereas high N fertilisation inputs boost aphid populations, leading to important grain yield losses (Duffield et al. 1997; Gash 2012). Cereal aphids, such as *S. avenae* and *Metopolophium dirhodum* (Walker), have been found to increase in size, longevity and fecundity under high N fertilisation rates, which enhance their performance (Aqueel and Leather 2011; Duffield et al. 1997; Nowak and Komor 2010).

Furthermore, our findings support the hypothesis that intraspecific interactions lead to physiological modifications in plants. Indeed, previous studies have shown that the interactions between plants through VOCs can cause shifts in both plant physiological and morphological characteristics (Barbosa et al. 2009; Dahlin et al. 2018; Ninkovic, Markovic, and Dahlin 2016). For instance, Tous-Fandos et al. (2023) showed that VOC interactions in wheat cultivar mixtures can alter the odour profile of the mixture. Additionally, Dahlin et al. (2020) showed that cultivar mixtures can affect the plasticity of specific leaf area, plant height, and phenological traits.

Our own observations revealed that mixing cultivars with similar aboveground biomass, such as Florence-Aurora and Montcada, increased the mean plant biomass possibly owing to competitive interactions. This greater aboveground biomass, and hence the possibility of sustaining larger aphid populations, may be one of the reasons why the Florence-Aurora with Montcada mixture was not effective for aphid control (Szpeiner, Martínez-Ghersa, and Ghersa 2009).

Nitrogen content varied depending on the identity of the neighbouring cultivar. In this case, N content in plants of cv. Florence-Aurora decreased significantly when mixed with Forment plants but remained constant when mixed with Montcada plants. This reduction in N content may explain the lower aphid load in plants of cv. Florence-Aurora individuals growing together in a mixture with plants of cv. Forment.

This study revealed that plant–plant interactions in wheat mixtures results in physiological modifications of functional traits. Regarding the limitations of assessing only two of the multiple attributes related to aphid control, our findings suggest that combining cultivars with different traits, such as Florence-Aurora and Forment, may enhance positive interactions, such as facilitation, which boosts the associational resistance of the mixture. Conversely, mixing cultivars with similar attributes can result in neutral or even negative interactions that can minimise the beneficial effects of some wheat cultivar mixtures on pest control, as described by the “right neighbour” hypothesis.

Finally, the correlation between N content and aphid abundance was low, suggesting that other factors might also have contributed significantly to the development of aphid populations in the mixtures. It is important to discuss that this study only tested two functional traits, N content and aboveground biomass. However, other attributes closely related to aphid performance, such as plant height, odour signals, and secondary metabolite production, were not considered here (Finch and Collier 2000; Tous-Fandos et al. 2023). For instance, previous studies have highlighted the importance of specific phloem sap compositions for host acceptance and the performance of phloem-feeding insects (Gallinger and Gross 2020; Jakobs, Schweiger, and Müller 2019; Karley, Douglas, and Parker 2002; Nowak and Komor 2010). Jakobs and Müller (2018) found that high aminoacid concentrations, and hence N do not necessarily benefit aphid performance and development. Future studies should include these additional traits to gain a more comprehensive understanding of the factors influencing aphid resistance.

5 | Conclusions

This study highlights the importance of the neighbour identity and trait approach in assessing the potential of wheat mixture for aphid control. In this regard, further investigation of the concept of the "right neighbour", identification of intraspecific interactions in wheat mixtures and the underlying mechanisms leading to associational resistance, are crucial. Future research on the heterogeneity of phloem sap quality, including amino acids, sucrose, and organic acids amongst wheat cultivars may prove important in identifying functional traits for pest control.

Author Contributions

Alba Tous-Fandos: conceptualization, data curation, formal analysis, software, visualization, writing – original draft. **Jannicke Gallinger:** conceptualization, formal analysis, visualization, investigation, methodology, software, supervision, writing – review and editing. **Arnoud Enting:** data curation, investigation. **Lourdes Chamorro-Lorenzo:** conceptualization, writing – review and editing. **F. Xavier Sans:** conceptualization, funding acquisition, writing – review and editing. **Velemir Ninkovic:** conceptualization, funding acquisition, project administration, supervision, writing – review and editing.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are openly available in CORA Repositori de Dades de Recerca at https://doi.org/10.34810/data1637.

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