RESEARCH ARTICLE

Impacts of aphid herbivory on mycorrhizal growth responses across three cultivars of wheat

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Societal Impact Statement

The Earth's population is projected to rise to 9.7 billion by 2050 resulting in mounting pressure to increase agricultural yields in a sustainable manner. Arbuscular mycorrhizal (AM) fungi may be important players in this agricultural transition given their capacity to improve soil and plant health. Benefits gained by crops hosting AM fungi can be cultivar-specific and also affected by insect herbivory, although the combined effect of these factors remains unclear. Here, we show that, in an economically and socially significant tri-partite system, there is interplay between crop cultivar, AM colonisation and aphid herbivory on plant growth and nutritional status.

Summary

- Arbuscular mycorrhizal (AM) fungi are critical components of the rhizosphere across nearly all terrestrial biomes. AM fungi associate with most plants, including major crops, usually increasing plant access to soil nutrients and enhancing defence against pests and pathogens in return for photosynthetic carbon (C). However, plant growth responses to AM fungi vary according to species and genotype, an issue pertinent in agro-ecosystems where crop cultivar can play an important role in AM function. Evidence suggests other biotic interactions, including with sap-feeding aphids, impact the function of AM symbioses in plants. Nonetheless, whether these biotic factors alter genotype (or cultivar)-specific plant growth and nutritional responses to colonisation by AM fungi remains unclear.

- Here, we investigated whether mycorrhizal responsiveness of three cultivars of wheat (*Triticum eastivum* L.) to colonisation by a ubiquitously occurring AM fungus (*Rhizophagus irregularis*) differ in the presence or absence of bird cherry-oat aphids (*Rhopalosiphum padi*), a major pest of cereals.

- Our findings show that although AM fungal colonisation and AM-mediated plant growth responses were not affected by aphid feeding, there was variation between cultivars in the benefits gained by host plants in terms of nutrient acquisition and root growth, while aphid abundances also differed between wheat varieties.

- Understanding what causes cultivar-specific outcomes and how they ultimately impact plant growth promotion, crop yields and food production represent key future research goals in agroecology.

1 | INTRODUCTION

Arbuscular mycorrhizal (AM) fungi are near-ubiquitous components of global agro-ecosystems. The colonisation of plant roots by AM fungi often enhances plant uptake of phosphorus and nitrogen (Leigh et al., 2009; Smith et al., 2003) and may improve protection against insect herbivores by 'priming' phytohormonal pathways (Cameron et al., 2013). In exchange for these benefits, host plants provide AM fungi with 4–20% of their photosynthetically fixed carbon (C) (Cotton, 2018) as hexose sugars or lipids (Jiang et al., 2017). As such, AM colonisation has potential to contribute to increased yield and nutrition in food crops (Pellegrino et al., 2015; Zhang et al., 2018) and in turn reduce agricultural dependence on synthetic agro-chemicals, thereby promoting a more sustainable approach to food production (Thirkell et al., 2017). However, crop-mycorrhizal outcomes are context specific, being driven by plant genotype or cultivar (Ellouze et al., 2016; Hetrick et al., 1992; Thirkell et al., 2021) as well as by which AM fungi are involved (Munkvold et al., 2004), as is also the case in unmanaged ecosystems (Hoeksema et al., 2010; Klironomos, 2003). Abiotic factors, like soil nutrient status (Li et al., 2005), shading (Johnson et al., 2015) and atmospheric CO_2 (Johnson et al., 2005), may intensify this variability. To date, relatively little attention has been paid to how external biotic factors such as interactions between mycorrhizal plants and insect pests could impact plant growth responses to AM fungi.

Most mycorrhizal plants are routinely challenged by insect herbivores (Frew & Price, 2019), of which aphids are common pests (Blackman & Eastop, 2000). Aphids are a diverse group of phytophagous insects (Fereres & Moreno, 2009), which use specialised needle-like mouthparts to imbibe C-rich phloem from plant vascular tissues (Douglas, 2006). Large aggregations of aphids may drain plant C resources and/or result in changes to plant C partitioning (Girousse et al., 2003; Kaplan et al., 2011), perhaps towards plant defence rather than growth or mutualistic symbionts. For instance, plant recognition of aphid infestation increases salicylic acid (SA)-dependent defences (Ali & Agrawal, 2012) and triggers other secondary metabolic changes in cereals (Ahmad et al., 2011), limiting plant C availability. Aphid herbivory may also inhibit photosynthesis (Macedo et al., 2009), although examples exist where aphid feeding stimulates photosynthetic activity (Kucharik et al., 2016).

According to the carbon-limitation hypothesis, the removal or redirection of plant C by herbivores away from mycorrhizal roots may compromise the ability of AM fungi to maintain root-internal and root-external structures (Barto & Rillig, 2010; Gehring & Whitham, 1994). Reduced fungal growth because of competition for resources with insects (Larson & Whitham, 1997) could explain lower AM colonisation in roots of aphid exposed plants (Babikova et al., 2014; Meier & Hunter, 2018). In turn, this could limit the capacity of AM fungi to supply plants with nutrients, driving a more one-sided symbiosis. However, neutral (Cabral et al., 2018; Wilkinson et al., 2019) and even positive (Meier & Hunter, 2018) effects of aphids have been reported on AM colonisation, potentially due to changes in root exudation (Hoysted et al., 2018) or honeydew deposits (Milcu et al., 2015). The identity of the organisms at each trophic level appears to determine these outcomes; aphid feeding rates vary (Puterka et al., 2017), AM fungi differ in their C requirements (Lendenmann et al., 2011) and plant genotype—particularly in crops—can influence mycorrhizal function (Thirkell et al., 2019, 2021). Recent evidence suggests aphids (*Rhopalosiphum padi*), which cost UK wheat growers £120 million annually in reduced yield (Loxdale et al., 2017) through direct feeding and plant virus transmission (Leather et al., 1989), can impact the functioning of wheat-mycorrhizal symbioses (Charters et al., 2020), but how this translates into wheat growth responses remains unknown.

Colonisation of wheat by AM fungi may also, indirectly, affect herbivore fitness. The survival of chewing herbivores tends to be adversely affected by AM colonisation (Koricheva et al., 2009), while sap-feeding pests like aphids may achieve greater abundance (Ueda et al., 2013). This is perhaps a consequence of altered leaf physiology (Simon et al., 2017) or the enhanced nutritional quality of host plants which can gain improved nutrient access and supply from the fungus (Hartley & Gange, 2009). By increasing resource availability, AM fungi may also improve the ability of plants to maintain or increase their biomass when challenged by herbivores (Bennett et al., 2005; Kula et al., 2005). However, despite the near ubiquity of AM fungi in agroecosystems, their effect on crop responses to cereal aphids and their impact on aphid abundance across cultivars is unclear.

To address these important knowledge gaps, we investigated how virus-free aphids (*R. padi*) impact the responses of three wheat cultivars commonly grown by farmers in the UK to colonisation by a generalist AM fungus (*Rhizophagus irregularis*) (van der Heijden et al., 2015) and, in turn, how colonisation of host plants by the fungus impacts aphid abundance. Two of the cultivars, cv. Avalon and cv. Cadenza, represent parental lines of the UK bread wheat reference population (Ma et al., 2015), with known differences in canopy traits (Piñera-Chavez et al., 2021), resistance to biotic stressors (Bass et al., 2006; Gardiner et al., 2020) and function of mutualists (Thirkell et al., 2019, 2022). The third, cv. Skyfall, is the most widely sown variety in the UK (RAGT, 2018).

2 | MATERIALS AND METHODS

2.1 | Wheat pre-germination and AM fungal inoculation

Twenty seeds of each of the wheat cultivars Avalon, Cadenza, and Skyfall (RAGT Seeds Ltd) were sterilised, germinated and transplanted into 12 cm diameter pots containing substrate consisting of three parts sand to one part perlite (Methods S1; 60 plants, $n = 5$). cvs. Avalon and Cadenza were first described in 1991 and 1993, respectively, and were crossed by the Wheat Genetic Improvement Network in 2003, with their progeny used to study the genetic basis of a wide variety of wheat characteristics (Ma et al., 2015). cv. Skyfall was developed in 2012 and has strong agronomic traits, producing high yield and exhibiting disease resistance (RAGT, 2018).

Plants in the mycorrhizal treatment ('+AMF') were inoculated with the AM fungus *Rhizophagus irregularis*, a widely distributed isolate (Savary et al., 2018) and the most frequently used strain in commercial inocula (Rosikiewicz et al., 2017). A total of 15 ml of inoculum (Methods S2) containing 23,500 spores was mixed throughout the substrate of each +AMF replicate. Remaining plants ('−AMF') were inoculated with the same volume of inoculum that was autoclaved at 121°C for 30 min.

2.2 | Growth conditions

Plants were grown for 8 weeks inside nylon-lined insect rearing cages in semi-controlled glasshouse conditions at Leeds University. Temperature was kept at a constant 20°C during a 16:8 h light:dark cycle. Natural daylight was supplemented with LED lighting and automatic blinds, providing an average light intensity of 190 μmol m^{-2} s^{-1} at canopy level. Plants were fed once each week with 30 ml of 40% nitrate-type Long Ashton Solution (Table S1) and watered with tap water when necessary.

2.3 | Insect culture and exposure

Bird cherry-oat aphids (*R. padi*) were provided by Dr. Tom Pope at Harper Adams University. Virus-free cultures of this specialist cereal-feeding insect were maintained on plants of cvs. Avalon, Cadenza and Skyfall grown in composted soil inside insect rearing cages. Growth conditions for aphid cultures were the same as those for experimental plants. After experimental plants had grown for 8 weeks, 30 wingless aphids of mixed life-cycle stages were added to half of all −AMF and +AMF plants (30 plants, $n = 5$ per treatment), hereafter termed the '+aphids' treatment (Methods S3). Remaining plants of each cultivar were not exposed to aphids ('−aphids' treatment). All plants were grown for a further 14 days, a duration of aphid exposure shown previously to elicit changes in AM fungal colonisation (Meier & Hunter, 2018) and mycorrhizal function (Charters et al., 2020), after

which final aphid abundance per plant was recorded and insects were removed.

2.4 | Wheat, AMF and aphid growth measures

At the end of the 2-week aphid exposure period, plants were extracted from their pots, and substrate was dislodged from the roots. A known volume of substrate was collected from each pot and stored at 5°C. This was used to calculate extra-radical hyphal lengths of the AM fungus (Methods S4). Roots were washed with water to remove remaining substrate. Shoots and roots were separated, and roots were split equally in two with half being stored in 50% EtOH (v/v) at 5°C. These roots were cleared and stained, and % AMF root length colonisation, % arbuscules and % vesicles were determined using the gridline intersection methodology (Methods S5). Remaining roots were freeze-dried with shoot material for 72 h and biomass measured using a digital scale. Aphid load, measured as the number of aphids per gramme of dry shoot weight, on each aphid exposed plant, was calculated in accordance with Petermann et al. (2010).

2.5 | Plant P determination

Freeze-dried shoot material was homogenised separately in a mill. A known amount of sample (30–40 mg) was weighed in triplicate into acid-washed tubes (1% HCl). One millilitre concentrated sulphuric acid (H_2SO_4) was added to each tube, and samples were digested at 365°C for 15 min using a digest block. Samples were allowed to cool and then returned to the block with 100 μl hydrogen peroxide (H_2O_2) until clear. Digests were diluted to 10 ml with distilled water and total P within resulting solutions quantified using colourimetry, following an adapted method from Murphy and Riley (1962) and John (1970) (Methods S6; Figure S1a).

2.6 | Plant N determination

A total of 20–30 mg of freeze-dried homogenized shoot material was weighed into acid-washed tubes with 1.1 ml 'mixed digestion reagent' containing 0.21 g selenium powder and 7 g lithium sulphate dissolved in a solution of sulphuric acid (210 ml) and hydrogen peroxide (175 ml). Samples were digested at 365°C for up to 60 min until clear. Digest solutions were diluted to 6.25 ml with distilled water, and total N was determined as described by Thirkell et al. (2016) (Methods S7; Figure S1b).
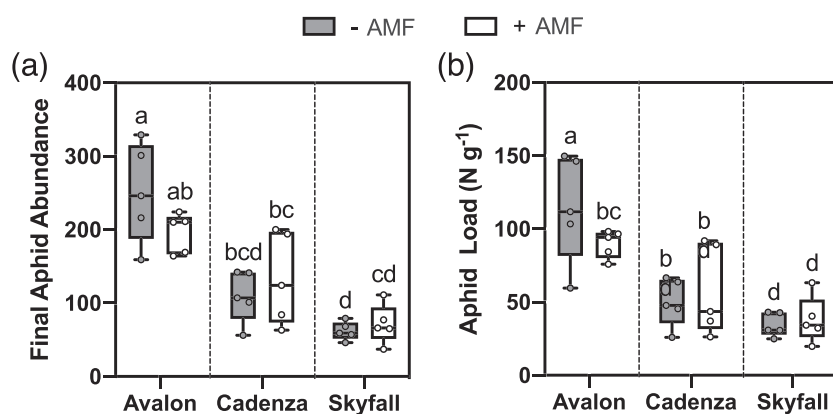
2.7 | Data analyses

All data analyses were conducted in R Studio v1.1.453. Data were tested to ensure assumptions of normality, and homogeneity of

variances was met using residuals vs. fitted and normal Q-Q plots. Shoot biomass was found to differ between cultivars (see results) so herbivore pressure was measured as aphid load (number of aphids per gramme of dry shoot weight), which standardised herbivore pressure per cultivar, and was treated as a continuous covariate to account for the effect of aphids on AMF and cultivar treatments. AM colonisation datasets were tested using one-way ANCOVAs followed by Tukey HSD post hoc tests, with percentage data being arcsine square root transformed. For this, cultivar was the categorical, independent variable, and aphid treatment (represented by aphid load) was a continuous covariate. Plant biomass, plant P and plant N data sets were tested using two-way ANCOVAs, followed by Tukey HSD post hoc tests. For this, cultivar and AMF treatment were the categorical, independent variables, and aphid treatment (represented by aphid load) was a continuous covariate. AM colonisation affecting aphid performance data sets was tested using two-way ANOVAs, followed by Tukey HSD post hoc tests. A generalised linear model (GLM) was used to test the effect of AMF, cultivar and their interaction on aphid performance on wheat. Spearman rank-test correlation analyses were conducted between final aphid abundance or aphid load and shoot P/[P] and N/[N], in order to investigate the relationship between plant nutrient status and aphid performance. All figures were produced using GraphPad Prism v8.2.0.

3 | RESULTS

3.1 | AM colonisation does not impact aphid performance

There was no difference in aphid abundance on wheat grown with or without the AM fungus across the cultivars tested (Figure 1a; Table S2). However, aphid abundance differed significantly between cultivar, being greatest on cv. Avalon (223 ± 18, mean ± SE) followed by cv. Cadenza (121 ± 16) and cv. Skyfall (67 ± 6) ($p \leq 0.001$, GLM). Similarly, aphid loads were unaffected by mycorrhizal treatment but varied between cultivar (Figure 1b; Table S2), again being highest on cv. Avalon (102 ± 9 N g^{-1}), followed by cv. Cadenza (54 ± 7 N g^{-1}) and cv. Skyfall (36 ± 4 N g^{-1}) ($p \leq 0.001$, GLM).

### 3.2 | AM colonisation of wheat is unaffected by aphid feeding

The roots and soils of all plants that were inoculated were colonised by the AM fungus (hereafter AMF). % root length colonisation (RLC) was not affected by aphid herbivory in any of the cultivars (Figure 2a; Table S3), but the extent of RLC varied significantly between cultivars. cv. Avalon had higher mean (± SE) RLC (45.2 ± 5.2%) than cv. Cadenza (39.6 ± 4.7%), with cv. Skyfall having lowest mean RLC (24.1 ± 3.1%). cv. Avalon was significantly different from cv. Skyfall ($p \leq 0.01$, one-way ANCOVA), but there was no difference between cv. Cadenza and the other two cultivars. The % of arbuscules and vesicles in roots was unaffected by aphid treatment, cultivar or their interaction (Figure 2b,c; Table S3). Hyphal length densities (HLDs) were also unchanged by aphid exposure, but varied according to cultivar (Figure 2d; Table S3), with cv. Cadenza supporting higher HLDs (2.07 ± 0.11 m g^{-1}, mean ± SE) than cvs. Avalon (1.29 ± 0.14 m g^{-1}) and Skyfall (1.43 ± 0.09 m g^{-1}). cv. Cadenza was significantly different from the other cultivars ($p \leq 0.01$, one-way ANCOVA).

3.3 | Cultivar-specific root growth responses to AM colonisation but not aphid exposure

Wheat cultivar had a strong significant effect on shoot biomass (Figure 3a; Table S4), with cv. Cadenza plants (2.27 ± 0.03 g, mean ± SE) being larger than cvs. Avalon (2.11 ± 0.04 g) and Skyfall (1.79 ± 0.03 g), and cv. Skyfall plants being significantly smaller than the other two cultivars ($p \leq 0.0001$, Two-way ANCOVA), regardless of AM colonisation and aphid feeding.

Root biomass was significantly affected by the interaction between AMF and cultivar (Figure 3b; Table S4). AM colonisation reduced the root biomass of cv. Avalon, but not cv. Cadenza or Skyfall, regardless of aphid treatment ($p \leq 0.0001$, two-way ANCOVA). The root biomass of cv. Avalon and Skyfall were significantly different from each other ($p \leq 0.05$, two-way ANCOVA), but not from cv. Cadenza, regardless of AMF colonisation or aphid treatment.

FIGURE 1 Aphid abundance (a) and aphid load (number of aphids per gramme of dry shoot weight) (b) on three wheat cultivars inoculated with the arbuscular mycorrhizal fungus (AMF) *Rhizophagus irregularis* (+AMF) or a sterile control (−AMF). Boxes extend from the 25th to the 75th percentile. Middle lines represent median values, and whiskers extend to minimum and maximum data points (closed or open markers, $n = 5$). Different letters indicate significant differences between treatment means (where $p < 0.05$, Tukey HSD tests).

FIGURE 2 Arbuscular mycorrhizal colonisation of roots and soils of three wheat cultivars grown in the presence (+Aphid) and absence (−Aphid) of cultivar-specific loads of the bird cherry-oat aphid (*Rhopalosiphum padi*). (a) % root length colonised; (b) % arbuscules; (c) % vesicles; (d) hyphal length densities. Boxes extend from the 25th to the 75th percentile. Middle lines represent median values, and whiskers extend to minimum and maximum data points (closed or open markers, *n* = 5). Different letters indicate significant differences between treatment means (where *p* < 0.05, Tukey HSD tests). 'ns' indicates no significant differences. Aphid effects were treated as continuous co-variates to account for differences in aphid load between cultivars, which differed in size.
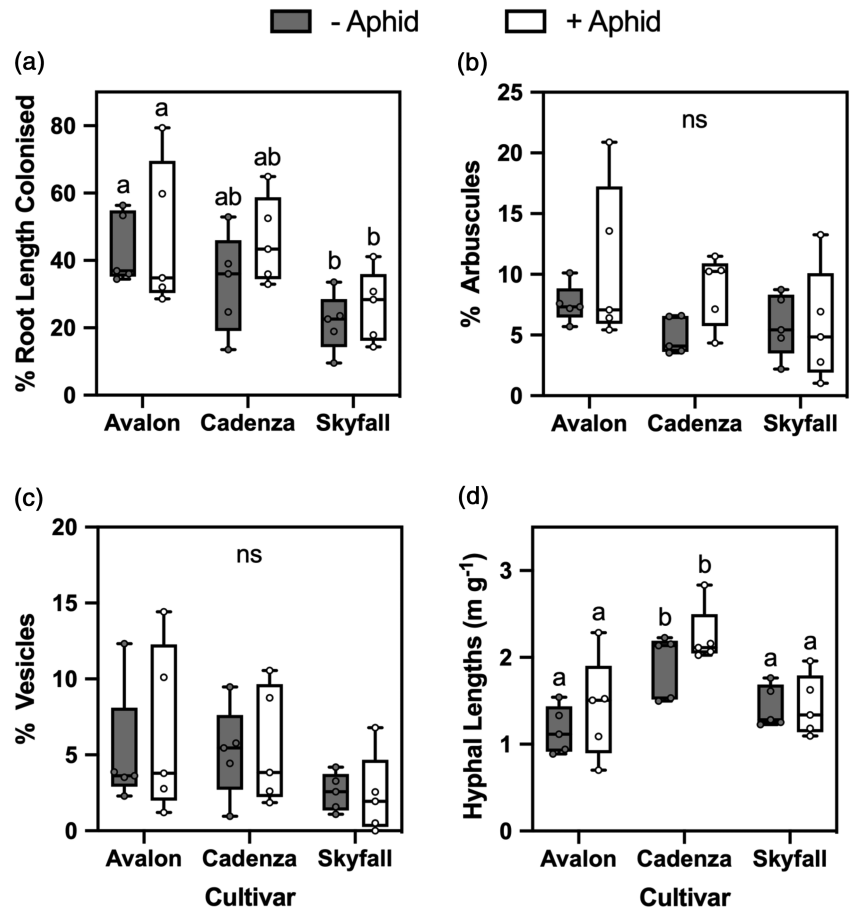
3.4 | Variable effects of AM colonisation and aphids on wheat P, but not N, status

Cultivar significantly affected plant-acquired shoot P and [P] (Figure 4a,b; Table S5; *p* ≤ 0.0001 and *p* ≤ 0.05, respectively, two-way ANCOVA), regardless of AMF and aphid treatments. cvs. Avalon and Cadenza had a greater amount of shoot P and a greater shoot [P] than cv. Skyfall. AM colonisation also played a significant role in affecting shoot P and [P] across cultivars, regardless of aphid treatment (Figure 4a,b; Table S5; *p* ≤ 0.01 and *p* ≤ 0.05, respectively, two-way ANCOVA).

For both shoot P and [P], there was a significant interaction between aphid exposure and cultivar, no matter the status of AMF colonisation (Figure 4a,b; Table S5; *p* ≤ 0.01 and *p* ≤ 0.01, respectively, two-way ANCOVA). For shoot P, cv. Avalon and cv. Skyfall were affected by this interaction (Figure 4a; Table S5; *p* ≤ 0.01, two-way ANCOVA). Exposure to aphids increased shoot P of cv. Avalon (−AMF: +10%; +AMF: +6%), did not affect shoot P of cv. Cadenza but reduced shoot P of cv. Skyfall (−AMF: −9%; +AMF: −8%). For shoot [P], aphid exposure significantly reduced shoot [P] of cv. Skyfall (− AMF: −9%; + AMF: −19%) but had no effect on the shoot [P] of cv. Avalon or Cadenza (Figure 4b; Table S5; *p* ≤ 0.01, two-way ANCOVA).

There was no effect of AM colonisation or aphid exposure on plant- and AM fungal-acquired shoot N (Figure 5a; Table S6).

However, shoot N differed between cultivars, being greatest in cvs. Cadenza (13.68 ± 0.41 mg) and Avalon (13.13 ± 0.17 mg) and lowest in cv. Skyfall (11.07 ± 0.19 mg) (Figure 5a; Table S6; *p* ≤ 0.0001, two-way ANCOVA). N concentrations ([N]) in wheat shoots were also unaffected by AMF, aphids, cultivar or any interactive term (Figure 5b; Table S6).

3.5 | Impact of nutritional status of wheat on aphid performance

There were strong positive correlations between the number of aphids after 2 weeks and shoot P (Figure 6a; *r* = 0.604, *p* < 0.001, Pearson's) and N (Figure 6b; *r* = 0.512, *p* < 0.004, Pearson's). This was also the case in terms of aphid load, with plants with greater shoot P (Figure 6c; *r* = 0.534, *p* = 0.02, Pearson's) and N (Figure 6d; *r* = 0.406, *p* < 0.026, Pearson's) supporting a greater aphid load. Shoot [P] was likewise positively associated with aphid performance, but shoot [N] was not (Figure S2).
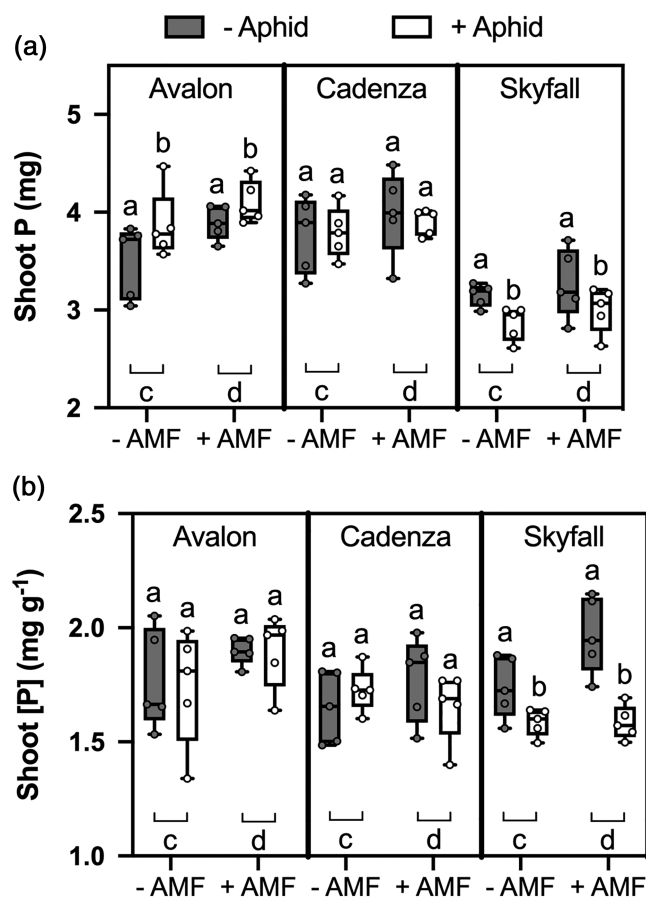
4 | DISCUSSION

Despite growing understanding of how abiotic factors influence mycorrhizal responsiveness of host plants, the impact of biotic drivers,
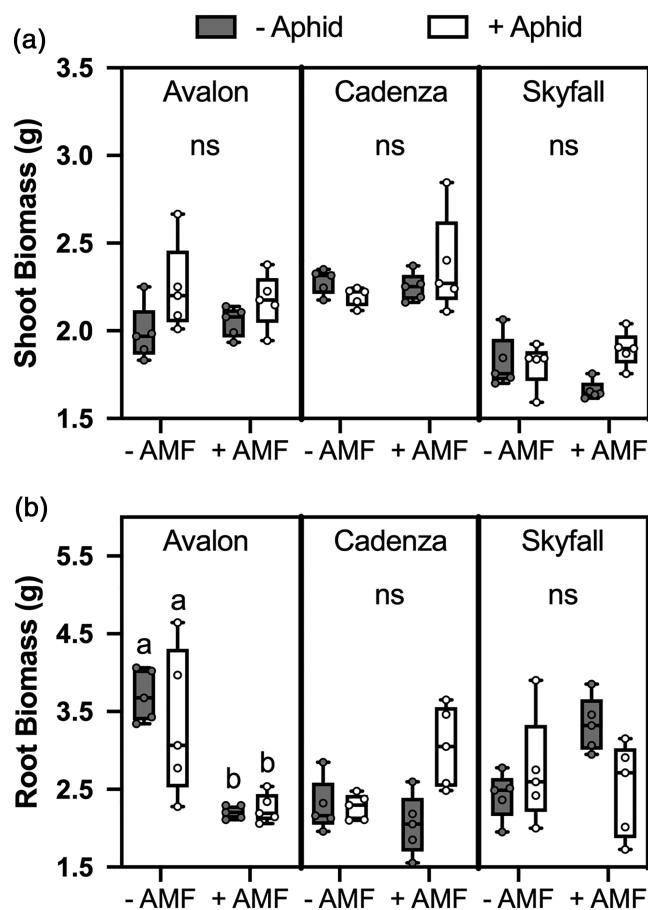
FIGURE 3 Biomass of three wheat cultivars inoculated or not with an arbuscular mycorrhizal fungus (+AMF or −AMF, respectively) and grown in the presence or absence of cultivar-specific aphid loads (+Aphid or −Aphid, respectively). Aphid loads (number of aphids per gramme of dry shoot weight) were used as the factor to represent aphid treatment. (a) Shoot biomass (dry weight); (b) root biomass (dry weight). Boxes extend from the 25th to the 75th percentile. Middle lines represent median values, and whiskers extend to minimum and maximum data points (closed or open markers, $n = 5$). Different letters indicate significant differences between treatment means (where $p < 0.05$, Tukey HSD tests). 'ns' indicates no significant differences. Different letters and 'ns' apply to specific cultivars and not between cultivars. Differences between cultivars are not shown in the figure.

FIGURE 4 Phosphorus uptake by three wheat cultivars inoculated or not with an arbuscular mycorrhizal fungus (+AMF or −AMF, respectively) and grown in the presence and absence of aphids (+Aphid or −Aphid, respectively). Aphid loads (number of aphids per gramme of dry shoot weight) were used as the factor to represent aphid treatment. (a) Total shoot phosphorus (P); (b) shoot P concentration [P]. Boxes extend from the 25th to the 75th percentile. Middle lines represent median values, and whiskers extend to minimum and maximum data points (closed or open markers, $n = 5$). Different letters indicate significant differences between treatment means within each cultivar (where $p < 0.05$, Tukey HSD tests). Different letters apply within cultivars, not between cultivars. Differences between cultivars are not shown. Letters below bars denote differences between AMF treatment, while the letters above the bars refer to differences between aphid treatments.

such as insect herbivory, on plant growth responses to colonisation by arbuscular mycorrhizal (AM) fungi has remained under-explored, in both managed and unmanaged ecosystems. Here, we investigated how herbivory by aphids (*Rhopalosiphum padi*) modulates responses of wheat to colonisation by the AM fungus *Rhizophagus irregularis*. We found that aphid feeding, which may limit plant C availability, had no impact on AM colonisation of wheat, nor plant growth promotion by the AM fungus. Instead, aphid load differed between three wheat cultivars commonly grown in the UK, which also varied in their capacity to maintain or increase shoot biomass when exposed to aphids, although this trend was not statistically significant. AM colonisation did not increase aphid abundance, with herbivory rather impacting shoot nutrient status differently between cultivars.

4.1 | Aphid feeding does not impact AM colonisation or mycorrhiza-mediated growth of wheat

In our study, aphids had no effect on AM colonisation of wheat (Figure 2a–c) or on extraradical hyphal lengths in surrounding soils (Figure 2d). This was true across all cultivars tested and represents the first research into how cereal aphids impact root-internal and root-external AM fungal biomass across multiple wheat varieties. As obligate biotrophs (Smith & Read, 2010), AM fungi rely solely on plant C for the development and renewal of resource uptake and exchange structures (Roth & Paszkowski, 2017). As such, it may be possible to indirectly infer the degree to which host plants are supplying C to AM fungi by
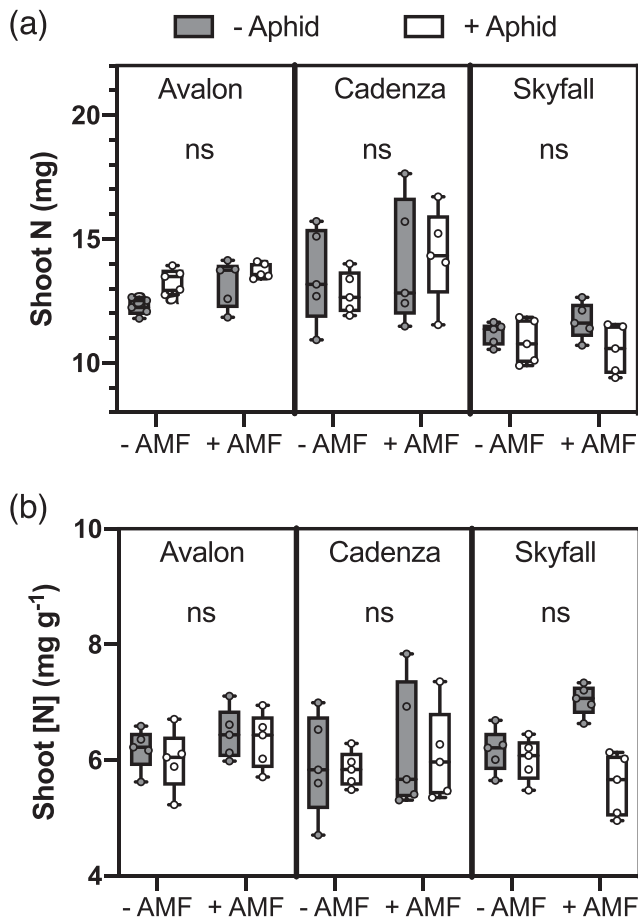
FIGURE 5 Nitrogen uptake by three wheat cultivars inoculated or not with an arbuscular mycorrhizal fungus (+AMF or −AMF, respectively) and grown in the presence and absence of cultivar-specific aphid loads (+Aphid or −Aphid, respectively). (a) Total shoot nitrogen (N); (b) shoot N concentration [N]. Boxes extend from the 25th to the 75th percentile. Middle lines represent median values, and whiskers extend to minimum and maximum data points (closed or open markers, $n = 5$). 'Ns' indicates no significant differences within cultivars and not between cultivars. Differences between cultivars are not shown in the figure.

using the extent of fungal colonisation in roots and hyphal lengths in adjacent soils (Müller et al., 2017). Similarly, potential capacity for fungal-acquired nutrient transfer to host plants may be indirectly deduced from colonisation of plant roots and/or from the type of intracellular fungal structures present (Johnson, 1993), particularly in multi-trophic contexts (Wearn & Gange, 2007). Our findings suggest that mycorrhizal function in wheat was not affected by aphids, regardless of crop cultivar. It is important to note, however, that mycorrhizal infection does not always reflect resource exchange dynamics between mycorrhizal symbionts (Nagy et al., 2009; Smith et al., 2003). Previous research using isotope tracers to track carbon-for-nutrient exchange between mycorrhizal partners demonstrated that aphids can reduce C supply from wheat to an AM fungus without reducing root colonisation (Charters et al., 2020). Thus, without employing such methods, it is not possible to rule out this eventuality in our experiment and, if reciprocated with reduced plant P/N uptake, could explain our finding that

inoculating plants with *R. irregularis* did not promote shoot growth in the three wheat cultivars tested (Figure 3a). It is also conceivable that longer exposure periods, or greater aphid loads, may reveal cultivar-specific effects of aphid feeding on AM colonisation of wheat, which ought to be studied, although the 2-week exposure used here was comparable with previous work in which aphids did alter mycorrhizal colonisation of non-crop hosts (Meier & Hunter, 2018).

Neutral plant growth responses to AM colonisation have been reported previously in wheat (Hetrick et al., 1992). AM fungi may also have no effect on plant growth if they provide little-to-no nutritional benefit in exchange for plant C, thereby inflicting a net C 'cost' on their hosts (Smith & Smith, 2013). However, we found some evidence for an active mycorrhizal nutrient uptake pathway, where colonisation by *R. irregularis* reduced root biomass of cv. Avalon (Figure 3b), but shoot P and N content and concentrations were unchanged (Figures 4a and 5a). These findings add to the understanding that AM fungi may supply plants with soil nutrients without any impact on plant growth (Li et al., 2006; Smith et al., 2003), perhaps reflecting reduced plant nutrient uptake directly via the roots (Smith & Smith, 2011).

Although in-line with some previous studies (Wilkinson et al., 2019), the same levels of colonisation between wheat cultivars in response to aphid exposure contrasts with results from other tri-trophic systems, in which aphids induced variable effects on AM colonisation in closely related wild plants (Meier & Hunter, 2018). This variability reiterates the fact that these responses cannot be generalised across study systems and are thus context-dependent (Chitty & Gange, 2021). Modern crop breeding, which focuses chiefly on improving above-ground traits of wheat under optimal conditions for achieving high yields (Martinez et al., 2020), may provide an explanation. Over time, artificial selection has inadvertently restricted root characteristics of elite wheat (Voss-Fels et al., 2017), resulting in smaller root systems (den Herder et al., 2010), lower root-to-shoot ratios (Siddique et al., 1990) and reduced mycorrhizal responsiveness when compared to older varieties (Zhang et al., 2018), with modern wheat cultivars also known to supply AM fungi with fewer plant C resources than more derived plants (Field et al., 2012; Thirkell et al., 2019, 2021). Nevertheless, given the relative lack of studies into poorly responsive plant-AM fungal interactions (Watts-Williams et al., 2019), efforts to address this bias will be crucial in improving our understanding of AM symbioses in both managed and unmanaged ecosystems. Aphids have been shown to reduce AM colonisation in other domesticated plants such as broad bean (Babikova et al., 2014), which—like wheat—has also been the subject of selective breeding efforts (Maalouf et al., 2018). Thus, rather than the functional group of the plant, variable aphid feeding rates (Puterka et al., 2017) and/or differing plant C requirements between AM fungi (Lendenmann et al., 2011) may determine colonisation responses to herbivory, which warrants further study.

4.2 | AM colonisation did not impact aphid abundance

Inoculating wheat with the AM fungus *R. irregularis* did not impact aphid performance (Figure 1) in any of the cultivars selected,
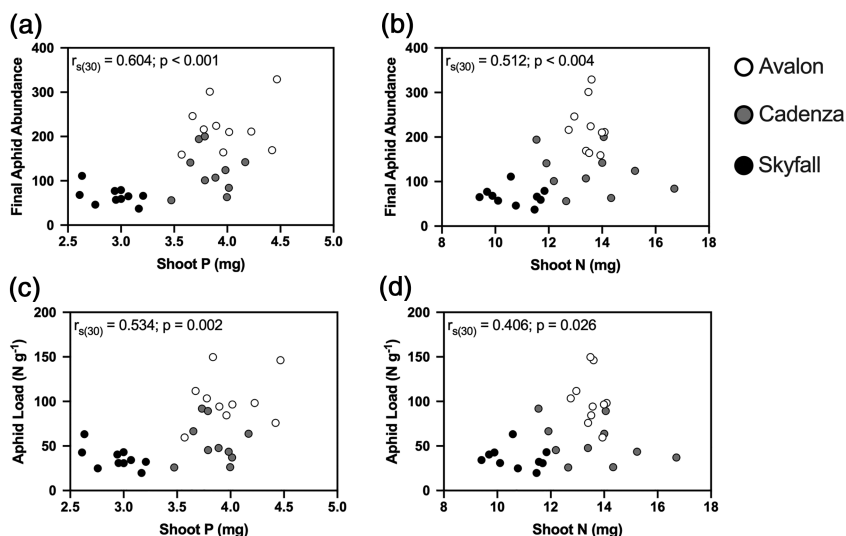
FIGURE 6 Correlations between shoot nutrient status and bird cherry-oat aphid performance. (a) Shoot phosphorus (P) vs. final aphid abundance; (b) shoot nitrogen (N) vs. final aphid abundance; (c) shoot P vs. aphid load; (d) shoot N vs. aphid load. Pearson's correlation coefficient (r) indicated in each panel

reinforcing previous findings using the same AM fungal isolate and a different wheat variety (Abdelkarim et al., 2011). Intriguingly, colonisation by *Gigaspora margarita* reduced aphid numbers in the same study, suggesting outcomes for aphids may be influenced by AM fungal identity. These differences could be driven by the variable rates at which AM fungi colonise plant roots (Hart & Reader, 2002), as the stage of AM colonisation can determine how mycorrhizal fungi impact aphid development (Tomczak & Müller, 2017), perhaps due to the triggering of different phytohormonal pathways at different phases of root infection (Cameron et al., 2013). In studying the effect of mycorrhizal colonisation on aphid numbers alone, it is conceivable that fungal-mediated effects on other aspects of aphid performance were missed, such as relative growth rate (Tomczak & Müller, 2017, 2018), survivorship (Volpe et al., 2018) and feeding behaviour (Simon et al., 2017). Over longer time periods—such as a full growing season—or in non-crop systems, these subtle changes may ultimately impact pest numbers and thus herbivore pressure, meaning these traits should be considered in subsequent research into this economically important yet poorly studied tri-partite system. Such effects may also have implications for disease management, as aphids vector ∼275 plant viruses (Fereres & Moreno, 2009) with *R. padi* responsible for the transmission of the most acute strain of barley yellow dwarf virus, RPV (*R. padi* virus) (Girvin et al., 2017).

4.3 | Wheat responses to aphids vary by cultivar

Aphid abundance and load were, however, different between the three wheat cultivars tested (Figure 1), suggesting perhaps variable levels of resistance to these phloem-feeding pests. While no elite wheat cultivar has been explicitly bred for reduced susceptibility to aphid attack (Simon et al., 2021), evidence for *R. padi* resistance exists in ancestral wheat lines (Simon et al., 2021) and land race collections (Aradottir et al., 2017), as well as in some modern Chinese and

Brazilian cultivars (Correa et al., 2020; Zhang et al., 2016). The variation in aphid numbers and load between cultivars may relate to differences in the nutritional (Figures 4 and 5), morphological (Figure 3) and genomic traits of the wheat cultivars studied. Cvs. Skyfall and Cadenza supported the lowest numbers of *R. padi* aphids (Figure 1). Cv. Skyfall is believed to harbour genetic resistance to insect pests such as the orange wheat blossom midge (*Sitodiplosis mosellana*) (AHDB, 2021), and cv. Cadenza carries resistance alleles against other biotic stressors, such as biotrophic pathogens (Bass et al., 2006; Ma et al., 2015). In contrast, cv. Avalon hosted the greatest number of aphids and is considered susceptible to wheat diseases like yellow stripe rust (Gardiner et al., 2020). Bird cherry-oat aphids are host-alternating, meaning they over-winter on a primary host plant (*Prunus padus*) before colonising their secondary hosts (grasses) in spring (Blackman & Eastop, 2000). Winter wheat varieties such as cv. Avalon, which undergo foundational stages during winter before yielding in the early summer, may be rendered more susceptible to aphid infestation by climate change; warmer temperatures could shorten *R. padi* generation times (Finlay & Luck, 2011) and see them spend more time on crops over winter, meaning greater emphasis on breeding for defence traits of winter wheat varieties is now needed (Bass et al., 2006; Gardiner et al., 2020).

Interestingly, despite hosting the greatest number of aphids and therefore perhaps being least resistant, cv. Avalon appeared to be the most tolerant cultivar we tested, with shoot biomass seemingly increasing when aphids were present compared to when they were absent (Figure 3a). Tolerance defines the ability of plants to withstand herbivore damage and/or compensate for it, which is less 'costly' for plants in terms of C resource investment than chemical or physical defence (Tao et al., 2016) and may not induce insect virulence (Crespo-Herrera et al., 2014). This trend in cv. Avalon may suggest a trade-off between tolerance and resistance against aphids (Hu et al., 2016). Compensatory plant growth in response to herbivory may off-set the effects of tissue damage or the loss of plant phloem and perhaps enables greater regrowth following aphid removal. This

may be due to the external biotic C sink (i.e., the aphids) stimulating higher rates of photosynthesis, as has been recorded in other cereals (e.g., *Sorghum bicolor*), which ultimately leads to increased yields (Kucharik et al., 2016; Liere et al., 2015). Over-compensatory growth responses have not been shown in wheat before, meaning it is now critical to determine the density of aphid infestations at which this can occur, and when yield begins to be adversely affected, potentially leading to more precise application of synthetic pesticides and greater sustainability in agroecosystems.

4.4 | Wider considerations

Pressure from pests is expected to grow as climate change worsens (Deutsch et al., 2018). For sap-feeding pests, such as aphids, this threatens crops and non-crops through direct feeding and virus transmission, but also through indirect effects via tri-trophic interactions such as those investigated here. Given that AM fungi are known to influence plant–plant competitive outcomes (Cameron, 2010; Wagg et al., 2011), our research has shown that, in economically and socially significant multi-trophic systems, the mechanisms by which these outcomes are realised may be more complex than previously thought. Our findings show that although AM fungal colonisation and AM-mediated plant growth responses were not affected by aphid feeding, there were differences between cultivars in terms of tolerance of aphids and in the benefits gained by host plants from AM colonisation in terms of nutrient acquisition and growth. We were not able to collect data from our experiment relating directly to impacts of aphids on wheat yield, and this should form part of any future studies, particularly those conducted in field environments. Understanding what causes cultivar-specific outcomes and how they ultimately impact plant growth promotion represent key research goals for future work.

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CONFLICT OF INTEREST

The authors have no conflict of interest.

AUTHOR CONTRIBUTIONS

MDC, SMS and KJF conceived and designed the experiments; MDC performed the experiments; MDC and EKD analysed the data and MDC wrote the first draft of the manuscript. All authors contributed to development of the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available via Dryad (at https://doi.org/10.5061/dryad.tmpg4f51q).

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REFERENCES

Abdelkarim, M., Ownley, B., Klingeman, W., & Gwinn, K. (2011). Effect of arbuscular mycorrhizae on aphid infestation of wheat. *Phytopathology*, *101*, S2–S2.

AHDB. (2021). AHDB Recommended List Winter Wheat 2021/2022. Available online: https://ahdb.org.uk/knowledge-library/recommended-lists-for-cereals-and-oilseeds-rl

Ahmad, S., Veyrat, N., Gordon-Weeks, R., Zhang, Y., Martin, J., Smart, L., Glauser, G., Erb, M., Flors, V., & Frey, M. (2011). Benzoxazinoid metabolites regulate innate immunity against aphids and fungi in maize. *Plant Physiology*, *157*, 317–327. https://doi.org/10.1104/pp.111.180224

Ali, J. G., & Agrawal, A. A. (2012). Specialist versus generalist insect herbivores and plant defense. *Trends in Plant Science*, *17*, 293–302. https://doi.org/10.1016/j.tplants.2012.02.006

Aradottir, G. I., Martin, J. L., Clark, S. J., Pickett, J. A., & Smart, L. E. (2017). Searching for wheat resistance to aphids and wheat bulb fly in the historical Watkins and Gediflux wheat collections. *Annals of Applied Biology*, *170*(2), 179–188. https://doi.org/10.1111/aab.12326

Babikova, Z., Gilbert, L., Bruce, T., Dewhirst, S. Y., Pickett, J. A., & Johnson, D. (2014). Arbuscular mycorrhizal fungi and aphids interact by changing host plant quality and volatile emission. *Functional Ecology*, *28*, 375–385. https://doi.org/10.1111/1365-2435.12181

Barto, E. K., & Rillig, M. C. (2010). Does herbivory really suppress mycorrhiza? A meta-analysis. *Journal of Ecology*, *98*, 745–753. https://doi.org/10.1111/j.1365-2745.2010.01658.x

Bass, C., Hendley, R., Adams, M. J., Hammond-Kosack, K. E., & Kanyuka, K. (2006). The Sbm1 locus conferring resistance to soil-borne cereal mosaic virus maps to a gene-rich region on 5DL in wheat. *Genome*, *49*, 1140–1148. https://doi.org/10.1139/g06-064

Bennett, A. E., Alers-Garcia, J., & Bever, J. D. (2005). Three-way interactions among mutualistic mycorrhizal fungi, plants, and plant enemies: Hypotheses and synthesis. *The American Naturalist*, *167*, 141–152.

Blackman, R. L., & Eastop, V. F. (2000). *Aphids on the world's crops: An identification and information guide*. John Wiley & Sons Ltd.

Cabral, C., Wollenweber, B., António, C., Rodrigues, A. M., & Ravnskov, S. (2018). Aphid infestation in the phyllosphere affects primary metabolic profiles in the arbuscular mycorrhizal hyphosphere. *Scientific Reports*, *8*, 14442. https://doi.org/10.1038/s41598-018-32670-1

Cameron, D. D. (2010). Arbuscular mycorrhizal fungi as (agro) ecosystem engineers. *Plant and Soil*, *333*, 1–5. https://doi.org/10.1007/s11104-010-0361-y

Cameron, D. D., Neal, A. L., van Wees, S. C. M., & Ton, J. (2013). Mycorrhiza-induced resistance: More than the sum of its parts? *Trends in Plant Science*, *18*, 539–545. https://doi.org/10.1016/j.tplants.2013.06.004

Charters, M. D., Sait, S. M., & Field, K. J. (2020). Aphid herbivory drives asymmetry in carbon for nutrient exchange between plants and an arbuscular mycorrhizal fungus. *Current Biology*, *30*(10), 1801–1808. https://doi.org/10.1016/j.cub.2020.02.087

Chitty, R. P., & Gange, A. C. (2021). Reciprocal interactions between aphids and arbuscular mycorrhizal fungi across plant generations. *Arthropod-Plant Interactions*, *101*, 33–43. https://doi.org/10.1007/s11829-021-09875-9

Correa, L. D. J., Maciel, O. V. B., Bücker-Neto, L., Pilati, L., Morozini, A. M., Faria, M. V., & Da-Silva, P. R. (2020). A comprehensive analysis of wheat resistance to Rhopalosiphum padi (Hemiptera: Aphididae) in

Brazilian wheat cultivars. *Journal of Economic Entomology*, *113*(3), 1493–1503. https://doi.org/10.1093/jee/toaa059

Cotton, T. A. (2018). Arbuscular mycorrhizal fungal communities and global change: An uncertain future. *FEMS Microbiology Ecology*, *94*, fiy179. https://doi.org/10.1093/femsec/fiy179

Crespo-Herrera, L., Akhunov, E., Garkava-Gustavsson, L., Jordan, K. W., Smith, C. M., Singh, R., & Åhman, I. (2014). Mapping resistance to the bird cherry-oat aphid and the greenbug in wheat using sequence-based genotyping. *Theoretical and Applied Genetics*, *127*, 1963–1973. https://doi.org/10.1007/s00122-014-2352-5

den Herder, G., van Isterdael, G., Beeckman, T., & de Smet, I. (2010). The roots of a new green revolution. *Trends in Plant Science*, *15*, 600–607. https://doi.org/10.1016/j.tplants.2010.08.009

Deutsch, C. A., Tewksbury, J. J., Tigchelaar, M., Battisti, D. S., Merrill, S. C., Huey, R. B., & Naylor, R. L. (2018). Increase in crop losses to insect pests in a warming climate. *Science*, *361*, 916–919. https://doi.org/10.1126/science.aat3466

Douglas, A. (2006). Phloem-sap feeding by animals: Problems and solutions. *Journal of Experimental Botany*, *57*, 747–754. https://doi.org/10.1093/jxb/erj067

Ellouze, W., Hamel, C., Depauw, R. M., Knox, R., Cuthbert, R. D., & Singh, A. K. (2016). Potential to breed for mycorrhizal association in durum wheat. *Canadian Journal of Microbiology*, *62*, 263–271. https://doi.org/10.1139/cjm-2014-0598

Fereres, A., & Moreno, A. (2009). Behavioural aspects influencing plant virus transmission by homopteran insects. *Virus Research*, *141*, 158–168. https://doi.org/10.1016/j.virusres.2008.10.020

Field, K. J., Cameron, D. D., Leake, J. R., Tille, S., Bidartondo, M. I., & Beerling, D. J. (2012). Contrasting arbuscular mycorrhizal responses of vascular and non-vascular plants to a simulated Palaeozoic CO_2 decline. *Nature Communications*, *3*, 835. https://doi.org/10.1038/ncomms1831

Finlay, K. J., & Luck, J. E. (2011). Response of the bird cherry-oat aphid (*Rhopalosiphum padi*) to climate change in relation to its pest status, vectoring potential and function in a crop-vector-virus pathosystem. *Agriculture, Ecosystems and Environment*, *144*, 405–421. https://doi.org/10.1016/j.agee.2011.08.011

Frew, A., & Price, J. N. (2019). Mycorrhizal mediated plant-herbivore interactions in a high CO_2 world. *Functional Ecology*, *33*(8), 1376–1385. https://doi.org/10.1111/1365-2435.13347

Gardiner, L.-J., Bansept-Basler, P., El-Soda, M., Hall, A., & O'Sullivan, D. M. (2020). A framework for gene mapping in wheat demonstrated using the Yr7 yellow rust resistance gene. *PLoS ONE*, *15*, e0231157. https://doi.org/10.1371/journal.pone.0231157

Gehring, C. A., & Whitham, T. G. (1994). Interactions between aboveground herbivores and the mycorrhizal mutualists of plants. *Trends in Ecology & Evolution*, *9*, 251–255. https://doi.org/10.1016/0169-5347(94)90290-9

Girousse, C., Faucher, M., Kleinpeter, C., & Bonnemain, J.-L. (2003). Dissection of the effects of the aphid *Acyrthosiphon pisum* feeding on assimilate partitioning in *Medicago sativa*. *New Phytologist*, *157*, 83–92. https://doi.org/10.1046/j.1469-8137.2003.00659.x

Girvin, J., Whitworth, R. J., Rojas, L. M. A., & Smith, C. M. (2017). Resistance of select winter wheat (*Triticum aestivum*) cultivars to *Rhopalosiphum padi* (Hemiptera: Aphididae). *Journal of Economic Entomology*, *110*, 1886–1889. https://doi.org/10.1093/jee/tox164

Hart, M. M., & Reader, R. J. (2002). Taxonomic basis for variation in the colonization strategy of arbuscular mycorrhizal fungi. *New Phytologist*, *153*, 335–344. https://doi.org/10.1046/j.0028-646X.2001.00312.x

Hartley, S. E., & Gange, A. C. (2009). Impacts of plant symbiotic fungi on insect herbivores: Mutualism in a multitrophic context. *Annual Review of Entomology*, *54*, 323–342. https://doi.org/10.1146/annurev.ento.54.110807.090614

Hetrick, B., Wilson, G., & Cox, T. (1992). Mycorrhizal dependence of modern wheat varieties, landraces, and ancestors. *Canadian Journal of Botany*, *70*, 2032–2040. https://doi.org/10.1139/b92-253

Hoeksema, J. D., Chaudhary, V. B., Gehring, C. A., Johnson, N. C., Karst, J., Koide, R. T., Pringle, A., Zabinski, C., Bever, J. D., & Moore, J. C. (2010). A meta-analysis of context-dependency in plant response to inoculation with mycorrhizal fungi. *Ecology Letters*, *13*, 394–407. https://doi.org/10.1111/j.1461-0248.2009.01430.x

Hoysted, G. A., Bell, C. A., Lilley, C., & Urwin, P. E. (2018). Aphid colonisation affects potato root exudate composition and the hatching of a soil borne pathogen. *Frontiers in Plant Science*, *9*, 1278. https://doi.org/10.3389/fpls.2018.01278

Hu, X.-S., Liu, Y.-J., Wang, Y.-H., Wang, Z., Yu, X.-L., Wang, B., Zhang, G.-S., Liu, X.-F., Hu, Z.-Q., Zhao, H.-Y., & Liu, T.-X. (2016). Resistance of wheat accessions to the English grain aphid *Sitobion avenae*. *PLoS ONE*, *11*(6), e0156158. https://doi.org/10.1371/journal.pone.0156158

Jiang, Y., Wang, W., Xie, Q., Liu, N., Liu, L., Wang, D., Zhang, X., Yang, C., Chen, X., & Tang, D. (2017). Plants transfer lipids to sustain colonization by mutualistic mycorrhizal and parasitic fungi. *Science*, *356*(6343), 1172–1175. https://doi.org/10.1126/science.aam9970

John, M. K. (1970). Colorimetric determination of phosphorus in soil and plant materials with ascorbic acid. *Soil Science*, *109*, 214–220. https://doi.org/10.1097/00010694-197004000-00002

Johnson, N. C. (1993). Can fertilization of soil select less mutualistic mycorrhizae? *Ecological Applications*, *3*(4), 749–757. https://doi.org/10.2307/1942106

Johnson, N. C., Wilson, G. W. T., Wilson, J. A., Miller, R. M., & Bowker, M. A. (2015). Mycorrhizal phenotypes and the law of the minimum. *New Phytologist*, *205*, 1473–1484. https://doi.org/10.1111/nph.13172

Johnson, N. C., Wolf, J., Reyes, M. A., Panter, A., Koch, G. W., & Redman, A. (2005). Species of plants and associated arbuscular mycorrhizal fungi mediate mycorrhizal responses to CO_2 enrichment. *Global Change Biology*, *11*, 1156–1166. https://doi.org/10.1111/j.1365-2486.2005.00967.x

Kaplan, I., Sardanelli, S., Rehill, B. J., & Denno, R. F. (2011). Toward a mechanistic understanding of competition in vascular-feeding herbivores: An empirical test of the sink competition hypothesis. *Oecologia*, *166*, 627–636. https://doi.org/10.1007/s00442-010-1885-9

Klironomos, J. N. (2003). Variation in plant response to native and exotic arbuscular mycorrhizal fungi. *Ecology*, *84*, 2292–2301. https://doi.org/10.1890/02-0413

Koricheva, J., Gange, A. C., & Jones, T. (2009). Effects of mycorrhizal fungi on insect herbivores: A meta-analysis. *Ecology*, *90*, 2088–2097. https://doi.org/10.1890/08-1555.1

Kucharik, C. J., Mork, A. C., Meehan, T. D., Serbin, S. P., Singh, A., Townsend, P. A., Whitney, K. S., & Gratton, C. (2016). Evidence for compensatory photosynthetic and yield response of soybeans to aphid herbivory. *Journal of Economic Entomology*, *109*, 1177–1187. https://doi.org/10.1093/jee/tow066

Kula, A. A. R., Hartnett, D. C., & Wilson, G. W. T. (2005). Effects of mycorrhizal symbiosis on tallgrass prairie plant–herbivore interactions. *Ecology Letters*, *8*, 61–69. https://doi.org/10.1111/j.1461-0248.2004.00690.x

Larson, K. C., & Whitham, T. G. (1997). Competition between gall aphids and natural plant sinks: Plant architecture affects resistance to galling. *Oecologia*, *109*, 575–582. https://doi.org/10.1007/s004420050119

Leather, S., Walters, K. A., & Dixon, A. G. (1989). Factors determining the pest status of the bird cherry-oat aphid, *Rhopalosiphum padi* (L.)(Hemiptera: Aphididae), in Europe: A study and review. *Bulletin of Entomological Research*, *79*, 345–360. https://doi.org/10.1017/S0007485300018344

Leigh, N., Hodge, A., & Fitter, A. H. (2009). Arbuscular mycorrhizal fungi can transfer substantial amounts of nitrogen to their host plant from organic material. *New Phytologist*, 181(1), 199–207. https://doi.org/10.1111/j.1469-8137.2008.02630.x

Lendenmann, M., Thonar, C., Barnard, R. L., Salmon, Y., Werner, R. A., Frossard, E., & Jansa, J. (2011). Symbiont identity matters: Carbon and phosphorus fluxes between Medicago truncatula and different arbuscular mycorrhizal fungi. *Mycorrhiza*, 21, 689–702. https://doi.org/10.1007/s00572-011-0371-5

Li, H., Smith, S. E., Holloway, R. E., Zhu, Y., & Smith, F. A. (2006). Arbuscular mycorrhizal fungi contribute to phosphorus uptake by wheat grown in a phosphorus-fixing soil even in the absence of positive growth responses. *New Phytologist*, 172, 536–543. https://doi.org/10.1111/j.1469-8137.2006.01846.x

Li, H., Zhu, Y., Marschner, P., Smith, F., & Smith, S. (2005). Wheat responses to arbuscular mycorrhizal fungi in a highly calcareous soil differ from those of clover, and change with plant development and P supply. *Plant and Soil*, 277, 221–232. https://doi.org/10.1007/s11104-005-7082-7

Liere, H., Kim, T. N., Werling, B. P., Meehan, T. D., Landis, D. A., & Gratton, C. (2015). Trophic cascades in agricultural landscapes: Indirect effects of landscape composition on crop yield. *Ecological Applications*, 25, 652–661. https://doi.org/10.1890/14-0570.1

Loxdale, H. D., Edwards, O., Tagu, D., & Vorburger, C. (2017). Population genetic issues: New insights using conventional molecular markers and genomics tools. In *Aphids as crop pests*. CAB International. https://doi.org/10.1079/9781780647098.0050

Ma, J., Wingen, L. U., Orford, S., Fenwick, P., Wang, J., & Griffiths, S. (2015). Using the UK reference population Avalon× Cadenza as a platform to compare breeding strategies in elite Western European bread wheat. *Molecular Breeding*, 35, 70. https://doi.org/10.1007/s11032-015-0268-7

Maalouf, F., Hu, J., O'Sullivan, D. M., Zong, X., Hamwieh, A., Kumar, S., & Baum, M. (2018). Breeding and genomics status in faba bean (Vicia faba). *Plant Breeding*, 138(4), 465–473. https://doi.org/10.1111/pbr.12644

Macedo, T. B., Peterson, R. K., Weaver, D. K., & Ni, X. (2009). Impact of Diuraphis noxia and *Rhopalosiphum padi* (Hemiptera: Aphididae) on primary physiology of four near-isogenic wheat lines. *Journal of Economic Entomology*, 102, 412–421. https://doi.org/10.1603/029.102.0154

Martinez, A. F., Lister, C., Freeman, S., Ma, J., Berry, S., Wingen, L., & Griffiths, S. (2020). Resolving a QTL complex for height, heading, and grain yield on chromosome 3A in bread wheat. *BioRxiv* https://doi.org/10.1101/2020.02.14.947846

Meier, A. R., & Hunter, M. D. (2018). Arbuscular mycorrhizal fungi mediate herbivore-induction of plant defenses differently above and belowground. *Oikos*, 127, 1759–1775. https://doi.org/10.1111/oik.05402

Milcu, A., Bonkowski, M., Collins, C. M., & Crawley, M. J. (2015). Aphid honeydew-induced changes in soil biota can cascade up to tree crown architecture. *Pedobiologia*, 58, 119–127. https://doi.org/10.1016/j.pedobi.2015.07.002

Müller, A., Ngwene, B., Peiter, E., & George, E. (2017). Quantity and distribution of arbuscular mycorrhizal fungal storage organs within dead roots. *Mycorrhiza*, 27, 201–210. https://doi.org/10.1007/s00572-016-0741-0

Munkvold, L., Kjøller, R., Vestberg, M., Rosendahl, S., & Jakobsen, I. (2004). High functional diversity within species of arbuscular mycorrhizal fungi. *New Phytologist*, 164, 357–364. https://doi.org/10.1111/j.1469-8137.2004.01169.x

Murphy, J., & Riley, J. P. (1962). A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta*, 27, 31–36. https://doi.org/10.1016/S0003-2670(00)88444-5

Nagy, R., Drissner, D., Amrhein, N., Jakobsen, I., & Bucher, M. (2009). Mycorrhizal phosphate uptake pathway in tomato is phosphorus-repressible and transcriptionally regulated. *New Phytologist*, 181, 950–959. https://doi.org/10.1111/j.1469-8137.2008.02721.x

Pellegrino, E., Öpik, M., Bonari, E., & Ercoli, L. (2015). Responses of wheat to arbuscular mycorrhizal fungi: A meta-analysis of field studies from 1975 to 2013. *Soil Biology and Biochemistry*, 84, 210–217.

Petermann, J. S., Müller, C. B., Weigelt, A., Weisser, W. W., & Schmid, B. (2010). Effect of plant species loss on aphid–parasitoid communities. *Journal of Animal Ecology*, 79, 709–720. https://doi.org/10.1111/j.1365-2656.2010.01674.x

Piñera-Chavez, F. J., Berry, P. M., Foulkes, M. J., Sukumaran, S., & Reynolds, M. P. (2021). Identifying quantitative trait loci for lodging-associated traits in the wheat doubled-haploid population Avalon × Cadenza. *Crop Breeding & Genetics*, 61, 2371–2386. https://doi.org/10.1002/csc2.20485

Puterka, G. J., Nicholson, S. J., & Cooper, W. (2017). Survival and feeding rates of four Aphid species (Hemiptera: Aphididae) on various sucrose concentrations in diets. *Journal of Economic Entomology*, 110, 1518–1524. https://doi.org/10.1093/jee/tox168

RAGT. (2018). RGT Skyfall G1 Winter Wheat. Available at: https://ragt-seeds.co.uk/en-gb/nos-varietes/rgt-skyfall-winter-wheat

Rosikiewicz, P., Bonvin, J., & Sanders, I. R. (2017). Cost-efficient production of in vitro Rhizophagus irregularis. *Mycorrhiza*, 27, 477–486. https://doi.org/10.1007/s00572-017-0763-2

Roth, R., & Paszkowski, U. (2017). Plant carbon nourishment of arbuscular mycorrhizal fungi. *Current Opinion in Plant Biology*, 39, 50–56. https://doi.org/10.1016/j.pbi.2017.05.008

Savary, R., Masclaux, F. G., Wyss, T., Droh, G., Corella, J. C., Machado, A. P., Morton, J. B., & Sanders, I. R. (2018). A population genomics approach shows widespread geographical distribution of cryptic genomic forms of the symbiotic fungus Rhizophagus irregularis. *ISME Journal*, 12, 17–30. https://doi.org/10.1038/ismej.2017.153

Siddique, K., Belford, R., & Tennant, D. (1990). Root: Shoot ratios of old and modern, tall and semi-dwarf wheats in a Mediterranean environment. *Plant and Soil*, 121, 89–98. https://doi.org/10.1007/BF00013101

Simon, A. L., Caulfield, J. C., Hammond-Kosack, K. E., Field, L. M., & Aradottir, G. I. (2021). Identifying aphid resistance in the ancestral wheat Triticum monococcum under field conditions. *Scientific Reports*, 11, 13495. https://doi.org/10.1038/s41598-021-92883-9

Simon, A. L., Wellham, P. A. D., Aradottir, G. I., & Gange, A. C. (2017). Unravelling mycorrhiza-induced wheat susceptibility to the English grain aphid Sitobion avenae. *Scientific Reports*, 7, 46497. https://doi.org/10.1038/srep46497

Smith, F. A., & Smith, S. E. (2011). What is the significance of the arbuscular mycorrhizal colonisation of many economically important crop plants? *Plant and Soil*, 348, 63–79. https://doi.org/10.1007/s11104-011-0865-0

Smith, F. A., & Smith, S. E. (2013). How useful is the mutualism-parasitism continuum of arbuscular mycorrhizal functioning? *Plant and Soil*, 363, 7–18. https://doi.org/10.1007/s11104-012-1583-y

Smith, S. E., & Read, D. J. (2010). *Mycorrhizal symbiosis*. Academic press.

Smith, S. E., Smith, F. A., & Jakobsen, I. (2003). Mycorrhizal fungi can dominate phosphate supply to plants irrespective of growth responses. *Plant Physiology*, 133(1), 16–20. https://doi.org/10.1104/pp.103.024380

Tao, L., Ahmad, A., de Roode, J. C., & Hunter, M. D. (2016). Arbuscular mycorrhizal fungi affect plant tolerance and chemical defences to herbivory through different mechanisms. *Journal of Ecology*, 104, 561–571. https://doi.org/10.1111/1365-2745.12535

Thirkell, T. J., Cameron, D. D., & Hodge, A. (2016). Resolving the 'nitrogen paradox' of arbuscular mycorrhizas: Fertilization with organic matter brings considerable benefits for plant nutrition and growth. *Plant, Cell & Environment*, 39, 1683–1690. https://doi.org/10.1111/pce.12667

Thirkell, T. J., Campbell, M., Driver, J., Pastok, D., Merry, B., & Field, K. J. (2021). Cultivar-dependent increases in mycorrhizal nutrient acquisition by barley in response to elevated CO_2. *Plants, People, Planet*, 3, 553–566. https://doi.org/10.1002/ppp3.10174

Thirkell, T. J., Charters, M. D., Elliott, A. J., Sait, S. M., & Field, K. J. (2017). Are mycorrhizal fungi our sustainable saviours? Considerations for achieving food security. *Journal of Ecology*, 105, 921–929. https://doi.org/10.1111/1365-2745.12788

Thirkell, T. J., Grimmer, M., James, L., Pastok, D., Allary, T., Elliott, A., Paveley, N., Daniell, T., & Field, K. J. (2022). Variation in mycorrhizal growth response among a spring wheat mapping population shows potential to breed for symbiotic benefit. *Food and Energy Security*, 11, e370. https://doi.org/10.1002/fes3.370

Thirkell, T. J., Pastok, D., & Field, K. J. (2019). Carbon for nutrient exchange between arbuscular mycorrhizal fungi and wheat varies according to cultivar and changes in atmospheric carbon dioxide concentration. *Global Change Biology*, 26(3), 1725–1738. https://doi.org/10.1111/gcb.14851

Tomczak, V. V., & Müller, C. (2017). Influence of arbuscular mycorrhizal stage and plant age on the performance of a generalist aphid. *Journal of Insect Physiology*, 98, 258–266. https://doi.org/10.1016/j.jinsphys.2017.01.016

Tomczak, V. V., & Müller, C. (2018). Plant species, mycorrhiza, and aphid age influence the performance and behaviour of a generalist. *Ecological Entomology*, 43, 37–46. https://doi.org/10.1111/een.12461

Ueda, K., Tawaraya, K., Murayama, H., Sato, S., Nishizawa, T., Toyomasu, T., Murayama, T., Shiozawa, S., & Yasuda, H. (2013). Effects of arbuscular mycorrhizal fungi on the abundance of foliar-feeding insects and their natural enemy. *Applied Entomology and Zoology*, 48, 79–85. https://doi.org/10.1007/s13355-012-0155-1

van der Heijden, M. G., Martin, F. M., Selosse, M.-A., & Sanders, I. R. (2015). Mycorrhizal ecology and evolution: The past, the present, and the future. *New Phytologist*, 205, 1406–1423. https://doi.org/10.1111/nph.13288

Volpe, V., Chitarra, W., Cascone, P., Volpe, M. G., Bartolini, P., Moneti, G., Pieraccini, G., di Serio, C., Maserti, B., Guerrieri, E., & Balestrini, R. (2018). The association with two different arbuscular mycorrhizal fungi differently affects the water stress tolerance in tomato. *Frontiers in Plant Science*, 9, 1480. https://doi.org/10.3389/fpls.2018.01480

Voss-Fels, K. P., Qian, L., Parra-Londono, S., Uptmoor, R., Frisch, M., Keeble-Gagnère, G., Appels, R., & Snowdon, R. J. (2017). Linkage drag constrains the roots of modern wheat. *Plant, Cell & Environment*, 40, 717–725. https://doi.org/10.1111/pce.12888

Wagg, C., Jansa, J., Stadler, M., Schmid, B., & van der Heijden, M. G. A. (2011). Mycorrhizal fungal identity and diversity relaxes plant–plant competition. *Ecology*, 92(6), 1303–1313. https://doi.org/10.1890/10-1915.1

Watts-Williams, S. J., Jewell, N., Brien, C., Berger, B., Garnett, T., & Cavagnaro, T. (2019). Using high-throughput phenotyping to explore growth responses to mycorrhizal fungi and zinc in three plant species. *Plant Phenomics*, 2019, 1–12. https://doi.org/10.34133/2019/5893953

Wearn, J. A., & Gange, A. C. (2007). Above-ground herbivorlly causes rapid and sustained changes in mycorrhizal colonization of grasses. *Oecologia*, 153, 959–971. https://doi.org/10.1007/s00442-007-0789-9

Wilkinson, T. D., Miranda, J.-P., Ferrari, J., Hartley, S. E., & Hodge, A. (2019). Aphids influence soil fungal communities in conventional agricultural systems. *Frontiers in Plant Science*, 10, 895. https://doi.org/10.3389/fpls.2019.00895

Zhang, M., Qiao, X., Peng, X., & Chen, M. (2016). Variation of resistance and susceptibility in wheat cultivars to different populations of *Rhopalosiphum padi* (Hemiptera: Aphididae) in China. *Journal of Asia-Pacific Entomology*, 19, 307–311. https://doi.org/10.1016/j.aspen.2016.03.006

Zhang, S., Lehmann, A., Zheng, W., You, Z., & Rillig, M. C. (2018). Arbuscular mycorrhizal fungi increase grain yields: A meta-analysis. *New Phytologist*, 222(1), 543–555. https://doi.org/10.1111/nph.15570

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.