Life-history and feeding behaviour of *Rhopalosiphum padi* and *Sitobion avenae* on wheat hosts grown under contrasting CO_2 and nitrogen regimes

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Abstract
Interactions between abiotic and biotic factors are a source of ecosystem complexity that needs direct experimental assessment. We investigated plant-aphid interactions in wheat grown in a chamber in a 2^2 factorial: [CO_2] (400 and 700 ppm) and nitrogen (high, low). At the onset of plant response to treatments, we established two assays with the cereal aphids *Rhopalosiphum padi* and *Sitobion avenae*. Assay 1 focused on fitness and assay 2 focused on feeding behaviour, and in both aphids were forced to feed on single leaf laminae. Assay 3 measured fitness of *R. padi* where insects were free to move along the whole plant. Experimental setting had a large impact on aphids. Where insects were constrained to single leaf laminae, high nitrogen reduced fitness and discouraged phloem feeding. Where insects could freely move throughout the plant, high nitrogen enhanced aphid fitness. The responses to [CO_2] were consistent between assays, but the interaction between nitrogen and [CO_2] varied with experimental setting. The number of *R. padi* adults in assay 3 varied 10-fold with plant growing conditions and correlated negatively with molar concentration of sugars in stem. This is consistent with theory and has two implications. First, the common interpretation that high nitrogen favours insect fitness because protein-rich animal bodies have to build from nitrogen-poor plant food needs to be expanded to account for the effect of nitrogen mediated by labile carbohydrates: lower plant nitrogen associates with higher concentration of labile carbohydrates, which can cause osmotic stress in aphids. Second, the common interpretation of water-soluble carbohydrates playing a buffering role for grain growth in wheat needs expanding to account for the putative role of carbohydrates in plant defence against aphids.

Keywords interactions; C:N ratio; osmotic; stress; water-soluble carbohydrates, amino acids

Introduction

Wheat, which contributes about 20 % of the total dietary calories and proteins worldwide, is critical to food security and aphids (Hemiptera, Aphidoidea) together with the viruses they transmit are its most important pests. Hence, the focus on wheat-aphid interactions in this study. The decoupling of trophic webs is a conspicuous, ecologically significant effect of global change. Air CO_2 concentration [CO_2], ambient temperature, availability of water and nitrogen influence the plant phenotype with bottom-up consequences for the fitness and behaviour of herbivorous arthropods. An important feature of multiple stresses is that they are often non-additive; largely unpredictable interactions are a fundamental cause of ecosystem complexity (Wootton 2002).

The concentration of nitrogen in plants correlates negatively with the concentration of labile carbohydrates (Sadras *et al.* 2020). Typically, plants grown under elevated [CO_2] feature lower nitrogen content and higher carbohydrate content than their counterparts under low [CO_2] (Moreno-Delafuente *et al.* 2020). Theory and empirical evidence indicate that aphid fitness responds non-linearly to the concentration of labile carbohydrates in plants, with low concentration impairing feeding and high concentration compromising insect osmoregulation (Sadras *et al.* 2020). Lagging theory means the early proposition to solve high-level interactions empirically remains valid. Here we report the shifts in life-history and feeding behaviour of *Rhopalosiphum padi* and *Sitobion avenae* in response to changes in the profiles of labile carbohydrates and amino acids of wheat plants grown under the factorial combination of two [CO_2] and two rates of nitrogen supply.

Methods

Potted wheat (cv Pedrosa) was grown in chambers in a factorial combining two [CO_2] (ambient: 400 ppm, elevated: 700 ppm) and two nitrogen rates (high N, full Hoagland solution; low N, 20% nitrogen Hoagland). We performed three independent aphid assays. Assay 1 measured life-history traits of *R. padi* and *S. avenae* confined to single leaves (n = 10) with the method of Fereres et al. (1989). Assay 2 evaluated feeding behaviour of *R. padi and S. avenae* with electric penetration graphs (EPG Systems Wageningen, The Netherlands); $n \geq 16$. Assay 3 (n = 3) measured the reproduction of *R. padi* on whole plants. We measured amino acids (Riga *et al.* 2019) and sugars (Nadezdha *et al.* 2013) in plants sampled before assay 1, and immediately after assays 2 and 3. We used ANOVA to test the effects of nitrogen, [CO_2] and their interaction on plant and aphid traits; we report *p*-value as a continuous quantity, and Shannon information transform [$s = -\log_2(p)$] as a measure of the information against the tested hypothesis.

Results

Plant traits. At 21 days after sowing (DAS), we recorded treatment effects for the first time: leaves were longer under high nitrogen (p = 0.0032, s = 8.3) and high [CO_2] (p = 0.0007, s = 10.5), with no interaction between nitrogen and [CO_2] (p = 0.70, s = 0.5). Dry matter also increased additively (interaction p = 0.31, s = 1.7) with high nitrogen (p = 0.022, s = 5.5) and high [CO_2] (p = 0.0009, s = 10.1). Percentage of water in shoot increased additively (interaction p = 0.64, s = 0.6) with high nitrogen ($p < 0.0001$, $s > 13.3$) and high [CO_2] (p = 0.027, s = 8.5).

Figure 1 shows the effects of nitrogen, [CO_2] and their interaction on the concentration of sugars and amino acids in plants at 28 DAS, immediately before the establishment of aphid assays 1 and 2. Low nitrogen (p = 0.0004, s = 11.3) and high [CO_2] (p = 0.014, s = 6.2) increased the total concentration of sugars additively (interaction: p = 0.24, s = 2.0) (Fig. 1A). Treatments did not affect the total concentration of amino acids (Fig. 1B); hence, the molar ratio of sugars to amino acids increased from 2.5 in plants with high nitrogen to 12.6 in their counterparts with low nitrogen (p = 0.0008, s = 10.3). [CO_2] (p = 0.30, s = 2.9) and the interaction between nitrogen and [CO_2] (p = 0.12, s = 1.4) did not affect the sugar to amino acid ratio. The concentration of sugars ranked fructose > glucose > maltose, with sucrose below detection level. Plants grown with high nitrogen had lower concentration of fructose, glucose and maltose than their counterparts with low nitrogen (Fig. 1D-F). Fructose and glucose increased with high [CO_2], and maltose did not respond to [CO_2]. All three sugars were unresponsive to the interaction between [CO_2] and nitrogen (Fig. 1D-F). Some amino acids were unresponsive to all three sources of variation including glycine, aspartic acid, glutamic acid, tyrosine, hydroxiproline, methylhistidine, alanine, and tryptophan. Serine responded to both nitrogen and the interaction between nitrogen and [CO_2], increasing concentration in response to high nitrogen at high [CO_2] and decreasing concentration in response to low nitrogen at high [CO2] (Fig. 1G). Proline increased with both high nitrogen and high [CO_2], with no response to interaction (Fig. 1H). Valine, arginine, amino butyric acid, phenylalanine, threonine and leucine all increased with high nitrogen, and were unresponsive to both [CO_2] and the interaction (Fig. 1I).

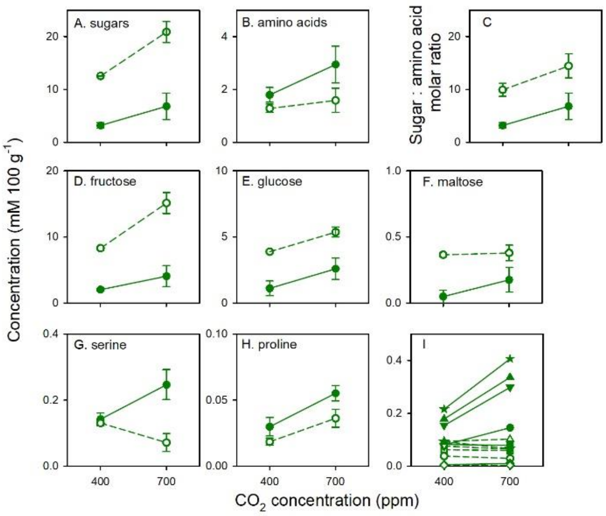

Figure 1. Effects of nitrogen (high: closed symbol, low: open symbol) and [CO_2] on the concentration of sugars and amino acids in wheat plants at 28 days after sowing, immediately before the establishment of aphid assays 1 and 2. (A) Total sugars, (B) total amino acids, (C) sugar : amino acid ratio, (D) fructose, (E) glucose, (F) maltose, (G) serine, (H) proline; in (I) circles are valine, squares are arginine, triangles are amino butyric acid, triangles down are phenylalanine, lozenges are threonine and stars are leucine. Error bars are two standard errors of the mean and have been omitted for clarity in I.

Aphid assay 1. A 2-3 day old adult was placed on the third leaf of each test plant at 28 DAS. After 48 hours?, *S. avenae* had laid more nymphs than *R. padi* ($p < 0.0001$, $s > 13.3$), and high nitrogen reduced the number of nymphs in relation to their counterparts under low nitrogen by 44% in *S. avenae* ($p = 0.006$, $s = 7.3$) and by 68% in *R. padi* ($p < 0.0001$, $s > 13.3$), with no effect of [CO_2] ($p = 0.57$, $s = 0.8$) or interaction between nitrogen and [CO_2] ($p = 0.57$, $s = 0.8$). Of these newly-born *R. padi nymphs*, none survived on leaves with high nitrogen. *S. avenae* nymphs survived to reproductive stage irrespective of treatment, but high nitrogen halved their weight in relation to those raised in low nitrogen leaves ($p < 0.0001$, $s > 13.3$). Adult weight at the onset of reproduction did not change with [CO_2] ($p = 0.26$, $s = 1.9$) or interaction between nitrogen and [CO_2] ($p = 0.76$, $s = 0.4$) in *S. avenae*, whereas *R. padi* adults raised in plants under high [CO_2] were 40% heavier than their counterparts under low [CO_2] ($p = 0.013$, $s = 6.3$).

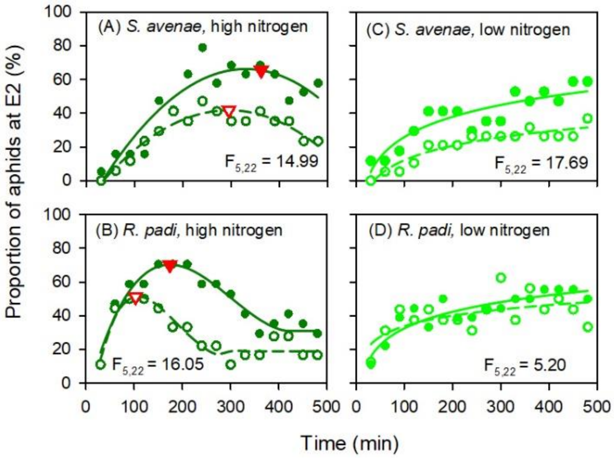

Figure 2. Proportion of aphids passively feeding on phloem sap (waveform E2) as affected by growing conditions of wheat plants in 8-h EPG assays. Polynomials were fitted to high nitrogen ($0.55 < R^2 < 0.90$) and logarithmic functions to low nitrogen ($0.39 < R^2 < 0.90$). Open symbols are low [CO_2] and closed

Aphid assay 2. Irrespective of aphid species and [CO_2], the proportion of aphids feeding on phloem sap (E2) in the high nitrogen treatment increased initially, reached a peak, and declined at later stages of the assay (Fig. 2A & B). In contrast, the proportion of aphids at E2 increased at diminishing rates but showed no peak or decline under low nitrogen (Fig. 2C & D). Comparisons of curves showed [CO_2] effect for both *S. avenae* ($F_{5,22} = 14.99$, $p < 0.00001$, $s > 16.6$) and *R. padi* ($F_{5,22} = 16.05$, $p < 0.00001$, $s > 16.6$) under high nitrogen (Fig. 2AB). This was reflected in delayed and higher peak for the proportion of aphids at E2 (arrowheads in Fig. 2AB) at elevated $[CO_2]$ than at low $[CO_2]$. *R. padi* peaked earlier (103-174 min) than *S. avenae* (295-362 min).

Aphid assay 3. Nitrogen increased fitness of *R. padi* ($p < 0.0001$, $s > 13.3$), with a stronger effect on total number of insects at high $[CO_2]$ (interaction $p = 0.005$, $s = 7.6$). We observed, but did not measure, a larger proportion of aphids on stems at the bottom of the plant and total number of adults correlated negatively with the molar concentration of sugars in stems (Figure 3a). In an unreplicated field comparison of 28 cereal cultivars (15 bread wheat, 5 durum wheat, 8 barley), plant damage caused by Russian aphid (*Diuraphis noxia)* declined with increasing concentration of WSC (Fig. 3b).

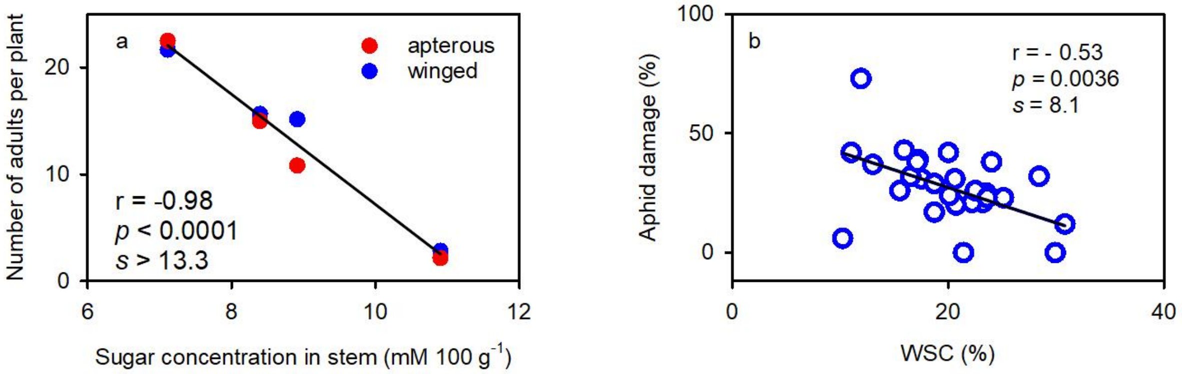

Fig. 3 (a) Correlation between total number of *R. padi* adults (winged and apterous) and molar concentration of sugars in stem in Assay 3. (b) Correlation between Russian aphid damage to cereals and shoot concentration of WSC in field crops. The lines are least-square regressions.

Conclusion

Where aphids were constrained to a single leaf, high nitrogen reduced fitness and discouraged phloem feeding. Where insects were allowed to move throughout the plant, high nitrogen enhanced aphid fitness. The responses to $[CO_2]$ were consistent between assays. The number of *R. padi* adults in assay 3 varied 10-fold with plant growing conditions and correlated negatively with molar concentration of sugars in stem. This is consistent with theory (Sadras *et al.* 2020) and has two implications. First, the common interpretation that high nitrogen favours insect fitness because protein-rich animal bodies have to build from nitrogen-poor plant food needs to be expanded to account for the effect of nitrogen mediated by labile carbohydrates: lower plant nitrogen associates with higher concentration of labile carbohydrates, which can cause osmotic stress in aphids. Second, the common interpretation of water-soluble carbohydrates playing a buffering role for grain growth in wheat needs expanding to account for the putative role of carbohydrates in plant defence against aphids, that needs field evaluation.

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