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ORIGINAL ARTICLE

Effects of elevated atmospheric CO_2 concentrations on phloem sap composition of spring crops and aphid performance

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The concentration and composition of free amino acids and carbohydrates in the phloem sap of wheat and oilseed rape (OSR) and the effects on the performance of aphids (*Rhopalosiphum padi* and *Myzus persicae*) were determined under atmospheric carbon dioxide (CO_2) enrichment. The analysis of phloem sap showed that carbohydrates and amino acid levels of the host plants were significantly affected by elevated CO_2 level. Among carbohydrate concentrations in the phloem sap, significant increases were observed in fructose and glucose in spring wheat under CO_2 enrichment, whereas no changes were observed in OSR. These changes in plant chemistry affected the performance of herbivorous insects (i.e. aphids) in varying ways, positively affecting the relative growth rate (RGR) of *R. padi* in spring wheat and negatively affecting the RGR of *M. persicae* on OSR.

Keywords: CO_2 enrichment; aphid; spring crops; amino acid; sugar

1. Introduction

Atmospheric carbon dioxide (CO_2) concentration is currently 387 μl l^{-1} and predicted to reach 550 μl l^{-1} by 2050 (Meehl et al. 2007). In accordance, the physiological and growth characteristics of plant species will also be affected (Poorter & Navas 2003). CO_2 enrichment has been shown to promote above-ground biomass by 12% in spring wheat (Högy et al. 2009) and 21% in summer oilseed rape (OSR; Högy et al. 2010). Canopy height and the production of reproductive organs of OSR were significantly increased under elevated CO_2, indicating an acceleration of the plant development (Franzaring, Högy, et al. 2008). Accordingly, the plant metabolism is also altered in wheat and OSR, leading to changes in the composition of generative plant parts (Högy & Fangmeier 2008; Högy et al. 2009, 2011) and most likely alterations to the feeding behavior of herbivorous insects. As, under natural conditions, phloem feeders are feeding on live cells, they are true parasites of their host plants. In such a way, they are highly sensitive indicators of any changes of plant performance whenever phloem constituents are involved (Bezemer & Jones 1998). Phloem-feeding aphids are reported to respond differently to changes in the nutritional quality characteristics of plants under elevated CO_2 (Newman et al. 2003; Wang et al. 2006; Prichard et al. 2007; Sun & Ge 2011).

In general, the phloem sap of plants contains high concentrations of carbohydrates (800–1800 mM) and relatively low concentrations of minerals and amino acids (60–200 mM) (Klingauf 1987; Sandström & Moran 2001; Douglas 2006). Host plants of high food quality are characterized by a rise in the ratio of amino acids to carbohydrates (Mittler & Meikle 1991). Consistently, the carbon to nitrogen ratio is increased in the phloem sap due to elevated CO_2, resulting in a diminished nutritional value of host plants and negative impacts on phloem-feeding insects due to limitations in nitrogen supply (Awmack & Leather 2002; Stiling & Cornelissen 2007; Sudderth et al. 2005). Therefore, in order to meet the amino acid requirement, aphids increase ingestion of assimilates from the phloem, leading to an increase in crop damage (Marks & Lincoln 1996; Sun, Cheng et al. 2009).

The development and growth rate of aphids can be influenced by the availability of amino acids in the phloem sap of host plants (Wilkinson & Douglas 2003), which determines their reproductive capability and abundance (Mittler & McNeill 1967). According to Dadd (1985), essential amino acids for aphids are histidine (His), threonine (Thr), tryptophan (Trp), methionine (Met), valine (Val), phenylalanine (Phe), isoleucine (Ile) and lysine (Lys). However, the demand of individual amino acids differs with the aphid species (Emden & Bashford 1971; Sandström & Moran 1999, 2001). While *Myzus persicae* Sulz. needs Met and γ-amino butyric acid (GABA), the amino acids Thr, His and alanine (Ala) are important for *Rhopalosiphum padi* Koch (Kazemi & Emden 1992).

In general, concentrations of amino acids in the phloem sap of host plants were found to be highly variable and they slightly tended to decrease under elevated CO_2 (Docherty et al. 1997). Sicher (2008)

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observed that the concentration of the total soluble amino acids in barley leaves was reduced by 59% under CO_2 treatment. Some aphid species have been shown to respond differently to elevated CO_2 on different host plants (Bezemer et al. 1999). According to Awmack et al. (1997), elevated CO_2 affected the performance of aphids (*Aulacorthum solani* [Kaltenbach]) by increasing the production of nymphs by 16% on bean (*Vicia faba* L.) and accelerating the development time by 10% on tansy (*Tanacetum vulgare* L.). Additionally, Awmack et al. (1996) found that elevated CO_2 increased the fecundity of *Sitobion avenae* F. on winter wheat.

In the life cycle of aphids, numerous roles are also played by carbohydrates, which store and transport the structural components and provide the chemical energy for longevity, fecundity and mobility (Rhodes et al. 1996). According to Avigad and Dey (1997), sucrose makes up 80–85% of the organic components of the phloem sap. It is the most important transportable sugar in most plant species and the most effective phagostimulant for herbivorous insects (Hawker 1985). Cabrera et al. (1995) argued that the development of aphid nymphs may also be affected by sucrose. However, compared to the major necessary nutrients, the amino acids, sucrose is not a limiting factor.

Overall, elevated CO_2 changes the concentration of carbohydrates in crops. Bezemer & Jones (1998) observed that the concentration of individual carbohydrates in wheat leaves was increased by 47% due to elevated CO_2. Another study on soybeans (*Glycine max* L. Merr. cv. 'Bragg') showed that the foliar concentrations of sucrose and reducing sugars were significantly increased by 108 and 33%, respectively, at 800 $\mu l\ l^{-1}$ CO_2 (Vu et al. 1989).

Such increases in the concentrations of carbohydrates cause changes in aphid performance. According to Zhang et al. (2003), high-CO_2 treatment (550 $\mu l\ l^{-1}$) increased the concentration of soluble carbohydrates in the leaves of wheat, leading to an increase in the population growth of *R. padi*. However, these authors did not assess the absolute or relative concentrations of amino acids; the interpretation thus still leaves some open questions. Newman et al. (1999) reported that *R. padi* responded to higher concentration of carbohydrates in the leaves of tall fescue (*Festuca arundinacea*) with a decrease in population density under elevated CO_2 (700 $\mu l\ l^{-1}$). In both cases, it revealed a close relationship between aphid population size and nutrient availability.

The aim of this study was to analyze the effects of elevated atmospheric CO_2 concentrations on the composition of phloem nutrients in spring wheat (*Triticum aestivum* L. cv. 'Triso') and OSR (*Brassica napus* cv. 'Campino') and the resulting consequences for herbivores such as green peach aphid (*Myzus persicae* Sulz.) and bird cherry-oat aphid (*Rhopalosiphum padi* L.). CO_2-induced changes of phloem sap nutrients such as carbohydrates (sucrose, glucose and fructose) and free amino acids were analyzed in order to identify the effects on host plant suitability and performance of phloem-feeding insects. Research on alterations in the nutritional quality of phloem sap in spring wheat and OSR and related growth characteristics of *R. padi* and *M. persicae* under CO_2 enrichment has not yet been performed.

2. Materials and methods

2.1. Cultivation of plants and experimental conditions

The experiments were performed from 16 June to 13 August 2008 with spring wheat and from 27 May to 17 August 2009 with OSR at the Universität Hohenheim, Germany. A pot experiment was conducted in six controlled environment chambers (Vötsch Bioline®), the first three chambers with ambient CO_2 (400 $\mu l\ l^{-1}$) and another three with elevated CO_2 (600 $\mu l\ l^{-1}$). Seeds were sown in pots (one seed per pot, Ø 18 cm, volume 1.5 L) filled with a mixture of substrate (Fruhstorfer Erde Typ LD 80, Industrie-Erdenwerk Archut, Lauterbach, Germany) and sand (9:1). Germination took place under 18 hours light and 6 hours dark at 22°C and 80% relative humidity. Mean irradiation level during the 18 hours light was 1100 $\mu mol\ m^{-2}\ s^{-1}$ as photosynthetically active radiation. Out of the 16 host plants in each chamber, 10 were chosen for aphid infestation and 6 for phloem sap analysis on sugar concentration and amino acid composition. Plants were fertilized weekly with 50 ml of 0.3% nutrient solution (Wuxal®, Aglukon GmbH) and irrigated daily using 50 ml water. In order to ensure results were not chamber specific, climate profiles and host plants were rotated weekly between chambers. Supplementary information about chamber characteristics is given in Franzaring, Holz, et al. (2008).

2.2. Aphid rearing and growth parameters

In order to introduce aphids in a synchronized long-term cultivation, Petri dishes were used as small plexiglass cages (Ø 3.5 cm). Aphid cultivation was performed in controlled conditions at 20°C, 60–70% relative humidity and long-day terms with a lighting duration of 16 hours to approximately 1.600 lux. The synchronization aphids were placed for 5 hours on spring wheat (BBCH stage 12, Zadoks et al. 1974) and OSR (BBCH stage 14, Weber & Bleiholder 1990) to produce larvae. Afterwards, adult aphids were removed and only five newly born larvae (L_1) remained in the cages (5 larvae per cage, 1 cage per plant, 1 plant per pot and 10 pots per chamber). In order to calculate the relative growth rate (RGR) of aphids, the youngest excess larvae and subsequently adult pre-reproductive aphids were weighed on a precision balance (Sartorius analytic 4504 MP8).

RGR of aphids was calculated according to Howard and Dixon (1995).

2.3. Sampling of phloem sap

According to King and Zeewart (1974), samples of phloem sap from spring wheat and OSR were collected both at leaf development stage before aphid infestation (three plants per chamber, BBCH 12, 14) and 44 and 48 days after infestation (three plants per chamber, which were used as control plants, BBCH 30), respectively. In both cases, host plants used for analysis were not infested with aphids. Plants were cut, transferred to vials containing a solution of 20 mM ethylenediaminetetraacetic acid (EDTA, adjusted to pH 7.0 with NaOH) and incubated in darkness at 20°C to reduce water loss due to transpiration. After 3 hours, plants were removed from the vials and the phloem sap fraction was frozen at 25°C until chemical analyses (Hellwald 1989).

2.4. Carbohydrate analysis

In the phloem sap, the concentrations of sucrose, glucose and fructose were analyzed by high-performance liquid chromatography (HPLC) using a Perkin Elmer Pump on a Shodex Asahipak NH_2 P-50 column (5 µm, 250 × 4.6 mm) at 30°C. Gradient elution buffers were acetonitrile (elution A) and twice-distilled water with 2% acetonitrile (elution B). The flow rate was constant at 1.0 ml min^{-1}. Carbohydrates were detected by using an evaporative light scattering detector (ELSD, Sedere) at 40°C.

2.5. Amino acid analysis

In order to define the composition of amino acids, exudates were analyzed by HPLC using a fluorescence detector (Jasco FP-1520.S). The lyophilized phloem sap (1 ml) was dissolved in 0.3 ml water and transferred into microtubes to centrifuge for 4 min (12,000 rpm). The supernatant was diluted with 25% methanol. Pre-column derivatization took place with *o*-phthalaldehyde reagent (OPA reagent; 1.6 µl OPA, 1 µl methanol and 0.4 µl 2-mercaptoethanol made up to 7 µl with borate buffer [boric acid solution 0.1 M, pH 10.4]) by using an autosampler (Varian Model 410).

Reversed phase HPLC analysis was performed at 28°C using a Varian Pro Star Pump and a Varian Pursuit XRS C-18 column (3 µm, 150 × 4.6 mm). Elution buffers were phosphate buffer (pH 6.8, 1 mM) with 10% MeOH (elution A) and MeOH (elution B). The flow rate was constant at 0.7 ml min^{-1}. The fluorescence excitation and emission wavelength were set at 330 and 440 nm, respectively. Peak identification of amino acids was confirmed by standard addition and quantified by an eternal standard with 17 amino acids (Ala, Arg, aspartic acid (Asp), cysteine, glutamine (Gln), glycine (Gly), His, tyrosine (Tyr), Ile, Leu, Lys, Met, Phe, proline, serine (Ser), Thr, Val) each at a concentration of 250 µmol ml^{-1}.

2.6. Statistical analyses

The CO_2 effects on phloem sap regarding carbohydrates and amino acid composition of spring wheat and OSR and the performance of *R. padi* and *M. persicae* were tested using PASW Statistics 18 (version 18, SPSS). The CO_2 effects were analyzed by analysis of variance (ANOVA). The results were expressed as percentage changes (%, elevated *versus* ambient CO_2) and significant CO_2 effects were presented as level of probability (p). The relationships between concentrations of carbohydrates and total or individual amino acids and the performance of aphids were calculated by using linear regression analysis.

3. Results

3.1. Concentrations of carbohydrates in the phloem sap

The concentrations of sucrose, glucose and fructose were examined in the phloem sap of spring wheat and OSR. In wheat, significant increases were found for fructose (50.5%, BBCH 12; 86%, BBCH 30) and glucose (62%, BBCH 30) in the high-CO_2 treatment (Figure 1). The concentration of sucrose was increased at BBCH 12 and decreased at BBCH 30; however, these CO_2 effects were not statistically significant. In OSR, the concentration of sucrose was not significantly increased due to elevated CO_2, while glucose and fructose were below the detection limit (Table 1).

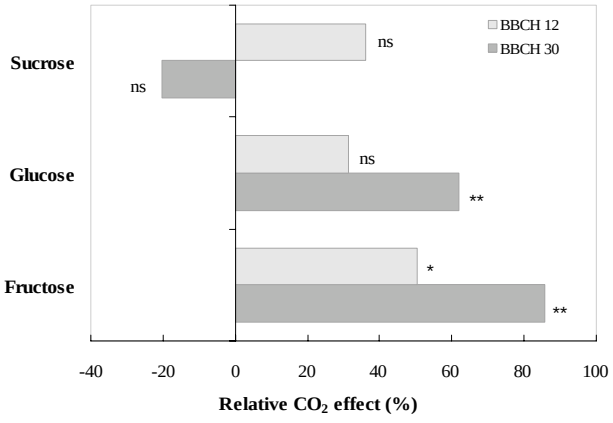

Figure 1. CO_2-induced changes (ambient = 100) of sucrose, glucose and fructose concentrations in phloem sap of spring wheat at leaf development (BBCH 12) and stem elongation (BBCH 30) stages in 2008. The results of the ANOVA are denoted by asterisks ($^{ns}p > 0.05$, $*p < 0.05$, $**p < 0.01$).

Table 1. Individual amino acid and carbohydrate concentrations (μg/mg) in spring wheat (SW, 2008) and oilseed rape (OSR, 2009) under ambient and elevated CO_2 concentrations in controlled-environment chambers.

	Ambient CO_2				Elevated CO_2			
	BBCH 12	BBCH 14	BBCH 30		BBCH 12	BBCH 14	BBCH 30	
Components	SW	OSR	SW	OSR	SW	OSR	SW	OSR
Amino acid concentrations								
Aspartic acid	2817.3	208.4	2126.6	1429.7	3463.0	172.0	2528.3	614.7
Glutamic acid	9836.6	263.0	3733.5	1782.2	13334.6	262.8	2743.3	813.8
α-Amino-adipic acid	nd	nd	26.6	3.5	nd	nd	37.2	1.6
Asparagine	1267.3	178.0	3161.1	294.7	1999.6	151.1	4329.0	207.1
Serine	3463.0	80.7	1908.0	368.2	4873.4	74.3	1965.7	175.5
Glutamine	11427.6	228.8	5203.5	1898.0	13602.5	198.8	6207.3	987.6
Histidine	126.3	21.7	340.0	109.4	140.3	13.3	412.3	58.1
Citrulline	40.8	0	6.5	3.2	0	0	4.0	2.3
Glycine	155.1	13.8	247.8	20.1	291.1	11.0	281.7	19.6
Threonine	741.0	24.2	870.8	255.8	1539.6	39.7	841.5	154.8
Arginine	1697.1	54.2	1090.8	251.5	1874.8	31.2	2314.7	91.8
Alanine	3066.1	27.4	1318.7	76.7	4476.6	26.0	2248.3	100.3
γ-Amino butyric acid	1273.1	23.0	1514.1	86.1	1721.6	22.3	2893.5	115.7
Tyrosine	157.3	13.2	216.8	55.0	322.0	13.0	302.0	39.0
Tryptophan	140.1	4.5	124.8	19.6	50.3	5.8	163.0	12.1
Methionine	229.6	2.1	110.1	28.8	280.6	0	123.3	12.4
Valine	477.3	49.2	490.8	100.0	762.3	36.5	709.3	98.1
Phenylalanine	427.2	62.7	301.6	111.4	560.3	60.0	375.1	79.8
Isoleucine	153.0	32.0	264.1	102.3	321.2	24.4	376.4	86.4
Leucine	257.8	22.8	362.8	88.1	421.2	19.3	545.7	57.0
Ornithine	596.0	14.2	78.2	36.1	355.1	18.0	91.4	15.6
Lysine	241.1	45.0	490.2	250.4	459.0	34.0	497.0	197.0
Carbohydrate concentrations								
Fructose	417.0	nd	510.3	nd	627.7	nd	947.7	nd
Glucose	475.6	nd	656.7	nd	625.3	nd	1063.4	nd
Sucrose	1041.0	98.3	960.3	123.3	1418.0	242.0	767.0	250.2

nd, not detectable.

3.2. *Concentrations of amino acids in the phloem sap*

The phloem sap of OSR grown at elevated CO_2 showed non-significant decreases in the total amino acids. In contrast, the phloem sap of spring wheat showed an increase in the total amino acid concentration at elevated CO_2; however, it was not statistically significant (Figure 2).

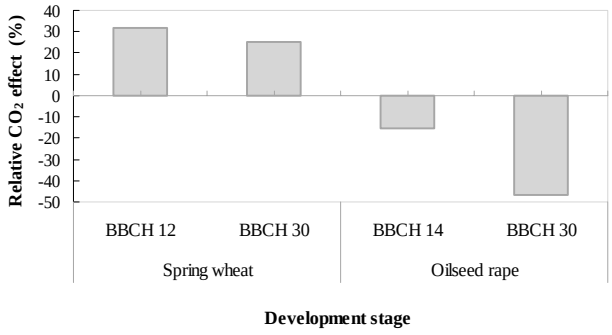

Figure 2. Relative CO_2 effects (%; elevated *versus* ambient; ambient = 100) on the concentration of total amino acids in the phloem sap of spring wheat and oilseed rape at leaf development and stem elongation stages.

In total, 22 individual amino acids were detected in the phloem sap of spring wheat and OSR, respectively. Acidic amino acids like glutamic acid (Glu) and Asp, together with their amides asparagine (Asn) and Gln, constituted the largest fraction of the total amino acids. In wheat, all concentrations of individual amino acids were increased due to elevated CO_2 except for Trp and ornithine (Orn) at BBCH 12 and Thr, citrulline (Cit) and Glu at BBCH 30 (Figure 3; Table 2). Significant increases due to elevated CO_2 were observed for the concentrations of Lys (90.3%), Leu (63.3%), Ile (110.1%), Phe (31.0%), Val (60.0%), Tyr (104.7%), Ala (46.0%), Thr (107.8%), Ser (40.7%), Asn (57.8%) and Glu (35.6%) at BBCH 12 and for Arg (112.2%), Ala (70.5%), Leu (50.4%) and GABA (91.1%) at BBCH 30. In contrast, elevated CO_2 significantly decreased the concentration of Orn (40.4%) in the phloem sap of spring wheat. Both α-aminoadipic acid (αAA) and Cit could not be determined at BBCH 12 in spring wheat.

In OSR, almost all amino acids showed a decrease, with the exception of Orn, Trp, Tyr, Thr (BBCH 14) and GABA and Ala (BBCH 30), whose

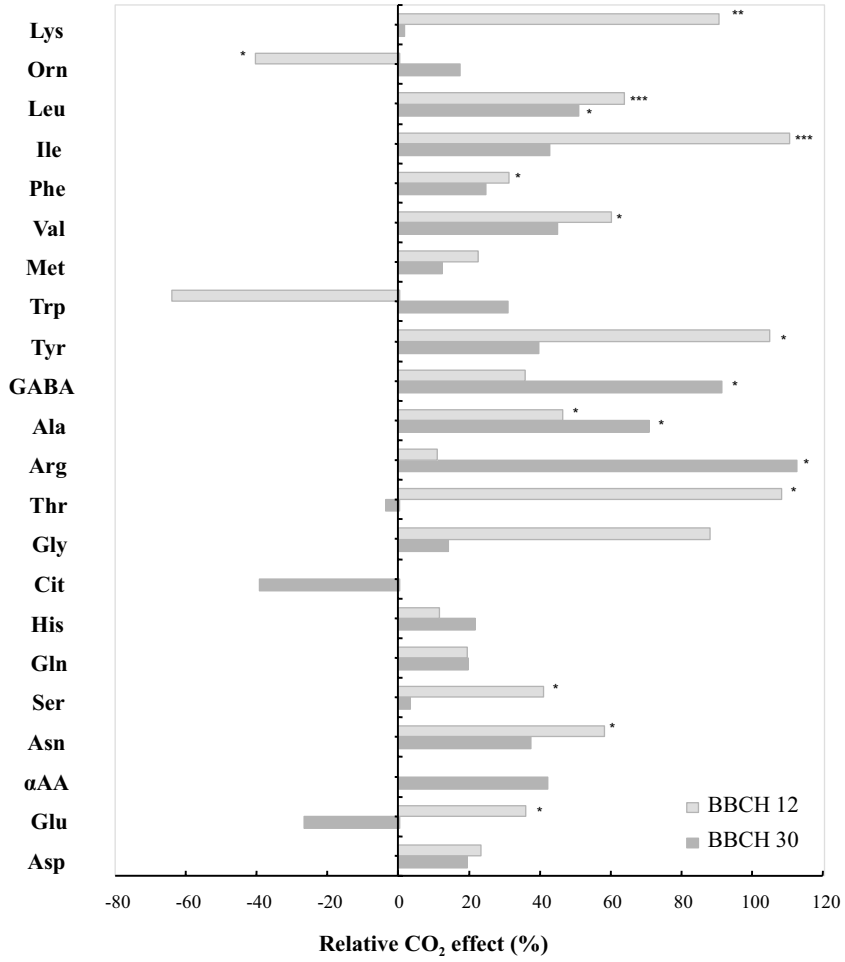

Figure 3. Changes in amino acid concentrations of phloem sap of spring wheat at leaf development (BBCH 12) and stem elongation (BBCH 30) stages under elevated CO_2 (ambient = 100). Given are the mean value and the standard deviation of three replicates. The results of the ANOVA are denoted by asterisks (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

concentrations were non-significantly increased under elevated CO_2 (Table 2). There were no significant CO_2 effects on the concentrations of individual amino acids in the phloem sap of OSR, except for a significant decrease of Orn (56.8%) at BBCH 30.

3.3. Correlation between RGR of aphids and carbohydrates and total amino acids

The RGR of *M. persicae* feeding on OSR achieved 0.08 ± 0.00 $(\mu g/\mu g/day)^1$ at ambient CO_2 and 0.07 ± 0.00 $(\mu g/\mu g/day)^1$ at 600 ppm CO_2. The RGR of *M. persicae* was smaller than that of *R. padi* on spring wheat, which achieved 0.11 ± 0.003 $(\mu g/\mu g/day)^1$ at 400 ppm CO_2 and 0.13 ± 0.01 $(\mu g/\mu g/day)^1$ at 600 ppm CO_2. The RGR of *R. padi* was significantly increased by 18.2% under elevated CO_2, while it was decreased by 12.5% for *M. persicae* (data not shown). The correlations between RGR of *R. padi* and concentrations of fructose and total amino acids in the phloem sap of spring wheat were not statistically significant under ambient CO_2 (BBCH 12) and in the high-CO_2 treatment (BBCH 30; Table 3). However, a significant CO_2 effect was found for the correlation between RGR of *R. padi* and the concentration of total amino acids (BBCH 12). RGR of *R. padi* was significantly correlated with the concentration of fructose in spring wheat under ambient (BBCH 30) and elevated CO_2 (BBCH 12). Unfortunately, it was impossible to detect glucose and fructose in the samples of OSR. The relationships between RGR of *M. persicae* and sucrose or total amino acids in the phloem sap of OSR were not statistically significant.

3.4. Correlation between RGR of aphids and individual amino acids

In wheat, significant correlations were limited to the RGR of *R. padi* and the concentration of Gly (BBCH 12) and Gln and essential Phe (BBCH 30) under ambient CO_2 (Table 4). In OSR, significant correlations were found for the RGR of *M. persicae* and Tyr and essential Lys under ambient CO_2 (BBCH 14). In the high-CO_2 treatment, significant correlations were observed between RGR of *M. persicae* and αAA, Tyr and essential amino acids such as Trp, Phe and Leu at BBCH 30 (Table 5).

Table 2. Percentage changes (%, elevated *versus* ambient CO_2) in the concentrations of individual amino acids and carbohydrates in phloem of spring wheat (2008) and oilseed rape (2009) at leaf development (BBCH 12, 14) and stem elongation (BBCH 30) stages.

	Growth stages			
	BBCH 12	BBCH 14	BBCH 30	
Components	Spring wheat	Oilseed rape	Spring wheat	Oilseed rape
Amino acid concentrations				
Aspartic acid	23.0	−17.5	18.8	−57.0
Glutamic acid	35.6	−21.4	−26.5	−54.3
α-Amino-adipic acid	nd	nd	41.8	−52.2
Asparagine	57.8	−15.0	37.0	−29.7
Serine	40.7	−8.0	3.0	−52.3
Glutamine	19.0	−13.1	19.3	−48.0
Histidine	11.1	−39.0	21.3	−47.0
Citrulline	0	0	−39.2	−30.1
Glycine	87.7	−20.8	13.7	−2.6
Threonine	107.8	64.3	−3.4	−39.5
Arginine	10.5	−42.5	112.2	−63.5
Alanine	46.0	−5.2	70.5	30.7
γ-Amino butyric acid	35.2	−2.8	91.1	34.4
Tyrosine	104.7	−3.2	39.3	−29.1
Tryptophan	−64.0	30.8	30.6	−38.3
Methionine	22.2	0	12.0	−56.8
Valine	60.0	−25.8	44.5	−1.7
Phenylalanine	31.0	−4.8	24.4	−28.3
Isoleucine	110.1	−23.7	42.5	−15.6
Leucine	63.3	−15.0	50.4	−35.2
Ornithine	−40.4	26.3	17.0	−56.8
Lysine	90.3	−24.4	1.4	−21.3
Carbohydrate concentrations				
Fructose	50.5	nd	85.7	nd
Glucose	31.5	nd	62.0	nd
Sucrose	36.2	46.0	−20.1	2.9

nd, not detectable.

4. Discussion

4.1. Concentrations of carbohydrates in the phloem sap and relationships to RGR of aphids

In general, carbohydrates in the phloem sap of host plants were increased under elevated CO_2, except for sucrose in spring wheat at BBCH 30. Significant increases were observed for concentrations of fructose (BBCH 12, BBCH 30) and glucose (BBCH 30) in the phloem sap of spring wheat. Knowledge of CO_2-induced impacts on the chemical composition

Table 3. Relationships (r with p) between relative growth rate (RGR) of aphids *(Rhopalosiphum padi, Myzus persicae)* and concentrations of individual carbohydrates and total amino acids of spring wheat (BBCH 12 and 30) and oilseed rape (BBCH 14 and 30) in ambient and high-CO_2 treatments.

		Carbohydrates			
CO_2 treatment	Development stage of plants	Sucrose	Fructose	Glucose	Total amino acids
Spring wheat					
400 μl l^{-1}	BBCH 12	0.812 (0.397)	0.771 (0.439)	0.956 (0.191)	0.734 (0.475)
	BBCH 30	0.450 (0.703)	0.998 (0.027)	0.989 (0.097)	0.925 (0.248)
600 μl l^{-1}	BBCH 12	0.985 (0.112)	0.996 (0.041)	0.991 (0.084)	0. 998 (0.024)
	BBCH 30	0.153 (0.902)	0.495(0.670)	0.048 (0.969)	0.681 (0.523)
Oilseed rape					
400 μl l^{-1}	BBCH 14	0.832 (0.374)	nd	nd	0.579 (0.607)
	BBCH 30	0.991 (0.086)	nd	nd	0.013 (0.992)
600 μl l^{-1}	BBCH 14	0.889 (0.303)	nd	nd	0.639 (0.559)
	BBCH 30	0.903 (0.283)	nd	nd	0.791 (0.419)

Notes: r = correlation coefficient; p = level of probability; $p > 0.05$ = not significant; nd = not detectable.

Table 4. Correlations of RGR (*R. padi*) and individual amino acid concentrations in spring wheat (essential amino acids [Dadd 1985] are given in bold characters).

	Ambient CO_2				Elevated CO_2			
	BBCH 12		BBCH 30		BBCH 12		BBCH 30	
Amino acids	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Aspartic acid	0.955	0.078	0.820	0.388	0.790	0.420	0.981	0.123
Glutamic acid	0.853	0.350	0.994	0.070	0.955	0.192	0.740	0.469
α-Amino-adipic acid	nd	nd	0.946	0.210	nd	nd	0.447	0.705
Asparagine	0.107	0.932	0.942	0.217	0.687	0.517	0.990	0.092
Serine	0.440	0.710	0.984	0.114	0.701	0.506	0.876	0.332
Glutamine	0.604	0.587	0.998	0.031	0.992	0.080	0.182	0.883
Histidine	0.075	0.953	0.874	0.323	0.949	0.205	0.916	0.263
Citrulline	0.826	0.382	0.946	0.210	nd	nd	0.666	0.536
Glycine	0.997	0.034	0.996	0.054	0.305	0.802	0.947	0.208
Threonine	0.014	0.991	0.169	0.892	0.476	0.684	0.857	0.345
Arginine	0.734	0.475	0.775	0.436	0.182	0.883	0.030	0.981
Alanine	0.330	0.786	0.173	0.889	0.694	0.512	0.371	0.758
γ-Amino butyric acid	0.510	0.659	0.897	0.292	0.329	0.787	0.577	0.609
Tyrosine	0.597	0.592	0.907	0.277	0.582	0.604	0.873	0.324
Tryptophan	0.449	0.704	0.828	0.379	0.655	0.545	0.829	0.378
Methionine	0.593	0.596	0.961	0.178	0.544	0.634	0.807	0.402
Valine	0.822	0.386	0.766	0.445	0.906	0.279	0.810	0.399
Phenylalanine	0.417	0.726	0.999	0.011	0.908	0.276	0.810	0.399
Isoleucine	0.276	0.822	0.911	0.270	0.915	0.264	0.879	0.316
Leucine	0.670	0.533	0.385	0.308	0.908	0.275	0.857	0.344
Ornithine	0.268	0.827	0.871	0.327	0.009	0.994	0.840	0.365
Lysine	0.002	0.999	0.803	0.407	0.49	0.682	0.990	0.089

Notes: r = correlation coefficient; p = level of probability for linearity; $p > 0.05$ = not significant; nd = not detectable.

Table 5. Correlations of RGR of *M. persicae* and individual amino acid concentrations in oilseed rape (essential amino acids [Dadd 1985] are given in bold characters).

	Ambient CO_2				Elevated CO_2			
	BBCH 14		BBCH 30		BBCH 14		BBCH 30	
Amino acids	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Aspartic acid	0.965	0.170	0.359	0.766	0.667	0.536	0.828	0.379
Glutamic acid	0.984	0.116	0.160	0.897	0.853	0.349	0.716	0.492
α-Amino-adipic acid	nd	nd	0.341	0.778	nd	nd	0.999	0.014
Asparagine	0.256	0.835	0.193	0.877	0.769	0.442	0.817	0.391
Serine	0.228	0.854	0.212	0.864	0.278	0.820	0.170	0.891
Glutamine	0.570	0.614	0.232	0.851	0.369	0.760	0.748	0.462
Histidine	0.174	0.888	0.648	0.551	0.589	0.599	0.442	0.709
Citrulline	nd	nd	0.341	0.778	nd	nd	0.878	0.317
Glycine	0.838	0.368	0.474	0.686	0.089	0.943	0.776	0.435
Threonine	0.250	0.839	0.314	0.797	0.158	0.899	0.741	0.469
Arginine	0.067	0.957	0.065	0.958	0.803	0.406	0.627	0.568
Alanine	0.258	0.834	0.324	0.790	0.784	0.427	0.841	0.364
γ-Amino butyric acid	0.561	0.621	0.245	0.843	0.924	0.250	0.631	0.565
Tyrosine	0.998	0.024	0.110	0.930	0.824	0.384	0.995	0.043
Tryptophan	0.282	0.818	0.550	0.629	0.750	0.460	0.994	0.048
Methionine	0.985	0.112	0.547	0.632	nd	nd	0.213	0.863
Valine	0.380	0.752	0.415	0.727	0.373	0.757	0.969	0.158
Phenylalanine	0.178	0.886	0.075	0.952	0.572	0.612	0.994	0.047
Isoleucine	0.914	0.266	0.223	0.857	0.557	0.624	0.981	0.127
Leucine	0.980	0.128	0.065	0.959	0.566	0.617	0.996	0.040
Ornithine	0.088	0.944	0.612	0.581	0.925	0.248	0.825	0.383
Lysine	0.999	0.010	0.140	0.910	0.659	0.542	0.951	0.201

Notes: r = correlation coefficient; p = level of probability for linearity; $p > 0.05$ = not significant; nd = not detectable.

of phloem sap in plants, except for carbohydrate and amino acids concentrations, is limited. Ainsworth et al. (2007) and Krumbein et al. (2010) reported that the concentration of sucrose under CO_2 enrichment was significantly increased in leaves of soybean (*Glycine max*) and broccoli (*Brassica oleracea* var. 'Italica') by 8.4 and 60%, respectively. Moreover, a significant increase in the foliar concentration of glucose by 60% was observed under elevated CO_2 in broccoli (Krumbein et al. 2010). Some studies observed that spring wheat grown in elevated CO_2 conditions contained significantly more water soluble carbohydrates, fructans, starch and non-structural carbohydrates (TNC) in the leaves (Conroy et al. 1993). However, Högy (1994) observed that elevated CO_2 had no significant impact on the concentration of sucrose in leaves of spring wheat and potato (*Solanum tuberosum* L.). In addition, Leakey et al. (2006) observed that the concentrations of sucrose, fructose and glucose in maize leaves remained unchanged under elevated CO_2.

Both an increased CO_2 concentration and the feeding habits of insects on host plants may affect the concentration of carbohydrates in crops. Supporting this, Cabrera et al. (1995) argued that barley (*Hordeum vulgare* cv. Aramir) infested with the greenbug (*Schizaphis graminum* [Rondani]) showed a total decrease in soluble carbohydrates by 52%, of which a proportion of 49% derived from sucrose.

Our results show that elevated CO_2 can positively affect the concentration of carbohydrates in the phloem sap of host plants. A positive response in carbohydrate production under elevated CO_2 was expected. It was also expected that CO_2 enrichment would indirectly affect the performance of aphids (development time, RGR, survival, fecundity) through direct effects on chemical composition of host plants. The effects of elevated CO_2 on aphid performance were essentially different between the two species, with one being negatively and the other positively affected. More specifically, high-CO_2 treatment significantly decreased the RGR of *M. persicae* on OSR, while the RGR of *R. padi* was significantly increased under elevated CO_2 in spring wheat. Supporting our results with *M. persicae*, Watt et al. (1995) argued that herbivorous insect responded to increased levels of CO_2 by reducing their growth rates. In the present study, no relationship was found between the RGR of *M. persicae* and the concentration of sucrose in the phloem sap of OSR under elevated CO_2. However, a significant relationship was found between the RGR of *R. padi* and the fructose concentration (BBCH 12) under elevated CO_2. Supporting our results with *R. padi*, Douglas et al. (2006) observed that the RGR of pea aphid (*Acyrthosiphon pisum* [Harris]) was significantly related to sucrose concentration in the diet.

4.2. The content of amino acids in the phloem sap of plants under elevated CO_2 and relation to RGR of aphids

In phloem sap of spring wheat, the concentrations of nearly all individual amino acids were increased under elevated CO_2. In accordance, Sicher (2010) observed a significant increase by 20% in the concentration of Asp in the leaflets of soybean (*Glycine max*) under CO_2 enrichment. According to Saijo et al. (1989) and Ke et al. (1993), significant increases in the concentration of GABA were observed in the tissues of tomatoes and crisphead lettuce in air enriched with 5–20% CO_2. In our study, Glu and Gln were the predominant free amino acids in the phloem of wheat, which parallels the results of Sicher (2010) with spring wheat. Such a response in the production of individual amino acids under elevated CO_2 was not expected in the present study and no clear explanation can be suggested for this result.

In OSR, elevated CO_2 had no impact on the concentrations of individual and total amino acids except for Orn. In agreement, studies on maize and soybean showed that the total free amino acids in the leaves were unchanged under high-CO_2 treatment (Leakey et al. 2006; Rogers et al. 2006). In contrast, Sun, Jing et al. (2009) found that amino acid concentrations were lower in phloem of cotton plants grown at elevated CO_2. Similar results were obtained by Bertrand and Bigras (2006), who mentioned that the concentration of amino acids in needles of black spruce (*Picea mariana* (Mill.) B.S.P.) was decreased under 710 $\mu l\ l^{-1}$ CO_2.

In our study, concentrations of Gly and Met were very low in the phloem sap of both crop species. Supporting this, Sicher (2010) argued that the concentration of Gly was lowered under elevated CO_2 in the leaves of wheat and soybean. Additionally, Weibull and Melin (1990) observed low concentrations of Gly and Met in *Brassica* plants; moreover, the amino acid pattern closely resembled that of cereals.

There is factual evidence of an existing relationship between amino acid composition and performance of phloem-feeding herbivores (Weibull 1988; Sandström & Pettersson 1994). According to Karley et al. (2002), the correlation between RGR of two aphid species (*Myzus persicae* and *Macrosiphum euphorbiae* [Thomas]) and amino acid composition in potato plants was robust. Furthermore, the insects responded differently to alterations in amino acid concentrations in the phloem sap of host plants under elevated CO_2 (Prichard et al. 2007). In our study, RGR of *M. persicae* was significantly related to the concentrations of αAA, Tyr and essential amino acids like Trp, Phe and Leu (BBCH 30) under elevated

CO_2. In the study of Emden and Bashford (1971) with leaves of Brussels sprout (*Brassica oleracea* Gemmifera Group), the RGR of *M. persicae* was significantly related to the concentrations of Met and GABA. In detail, the increase of Met had a positive impact on the RGR of *M. persicae*, while the increase of non-protein GABA indicated a negative effect (Emden & Bashford 1971). However, our results showed no significant relationship between the RGR of *M. persicae* and concentrations of Met and GABA in the phloem sap of OSR as stated earlier. This comparison proves that the same aphid species (i.e. *Myzus persicae*) has specific amino acid requirements and may respond differently on different plant species, significantly correlating with such individual amino acids as Met and GABA in one case, and showing no significant relationship with them in another case.

In spring wheat, RGR of *R. padi* was significantly related positively to the total amino acids under elevated CO_2 (BBCH 12). Moreover, the RGR of *R. padi* was significantly increased with an increase of amino acids under elevated CO_2. In agreement, Weibull (1987) observed that RGR of *R. padi* was significantly increased as total amino acids were raised and vice versa. According to Kidd et al. (1990), the CO_2-induced increase in the concentration of amino acids by 47% in pine trees resulted in an increase in growth rate of conifer aphids (*Schizolachnus pineti* F., *Cinara pini* L.) by 31%. Contrary to this, Docherty et al. (1997) observed that RGR of aphids (*Drepanosiphum platanoidis* Schrank and *Periphyllus testudinaceus* Ferni) were not related to the concentration of total amino acids in *Acer pseudoplantanus* L. under elevated CO_2 as the RGR of aphids remained unaffected although the total amino acids were significantly decreased. Other observations by Sandström and Pettersson (1994) confirmed no significant correlations between the performance of pea aphid (*Acyrthosiphon pisum*) and the concentration of total free amino acids in the phloem of pea (*Pisum sativum* L.), broad bean (*Vicia faba* cv. Major), alfalfa (*Medicago sativa* cv. Sverre) and red clover (*Trifolium pratense* L. cv. Hermes II). Additionally, Sandström (2000) argued that the RGR of *R. padi* was not related to the concentration of total amino acids in the leaves of bird cherry (*Prunus padus* L.) and barley (*Hordeum vulgare* L.).

In conclusion, the working hypothesis of this study was that elevated CO_2 would affect the chemical composition of wheat and OSR and thus exert an influence on plant–insect interactions by altering the nutritive value for insects feeding on phloem sap. It was confirmed that the CO_2 effects on RGR were opposite between the two biotrophic systems, with an increase for *R. padi* and a decrease for *M. persicae*. From the experiments within the two biotrophic systems under controlled conditions, no general conclusions could be derived since they both responded differently to CO_2 enrichment. Rather, much more information on a variety of different biotrophic systems appears necessary to be able to gain a mechanistic understanding of the underlying processes.

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