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Effects of elevated atmospheric CO_2 concentrations on insects and pathogens of spring wheat (*Triticum aestivum* L. cv. Triso) and oilseed rape (*Brassica napus* cv. Campino)

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1. Summary

It is suggested that plants, herbivore insects and pathogens will be affected by rising atmospheric CO_2. The working hypothesis of this study was that elevated CO_2 will affect plant composition and will thus exert influence on plant-insect interactions by changing the nutritive value for insects feeding on phloem sap.

To test this hypothesis, experiments were carried out on wheat and oilseed rape in two different systems: controlled environment chambers (climate chamber system) and an open field exposure system with natural climatic and soil conditions (Mini FACE system).

The abundance of detrimental insects from different feeding guilds and plant damage by parasitic organisms were examined in a Mini FACE system, while the consequences of elevated CO_2 on aphid performance and potential correlations to phloem sap composition of host plants were observed in controlled environment chambers. The concentrations of amino acids and carbohydrates in the phloem of host plants were analysed by high–performance liquid chromatography (HPLC), using a fluorescence detector for amino acids and the evaporative light scattering detector for carbohydrates.

In a Mini-FACE system, phenological development of spring wheat and OSR was not significantly changed due to CO_2 enrichment. However, elevated CO_2 induced changes in plant chemistry (increased carbon:nitrogen ratio and defensive compounds), which resulted in changes in population densities of some pest species. In order to monitor alterations in insect population density, two different methods were applied: direct counts (method 1) and using of yellow sticky traps (method 2). These methods showed both increases and decreases of insect numbers due to elevated CO_2, depending on species and on the period of observation. In spring wheat, high levels of CO_2 significantly increased the abundances of *Oulema melanopus* (method 1) and *Phyllotreta vittula* (method 2) during the booting stage and thrips species at the stage of fruit development (method 1), while decreases were observed for *Delia coarctata* at tillering, *Chaetocnema aridula* at stem elongation and *Haplodiplosis marginata* at the stage

of fruit development (method 2). In OSR, elevated CO_2 significantly increased the abundance of thrips species at inflorescence emergence (2009) and flowering stages (2007), *Meligethes aeneus* at the fruit stage (2007), *Athalia rosae* and *Aleyrodes proletella* at flowering and *Delia radicum* during the whole vegetation period (2009) using method 2, while significant decreases were established in the abundances of *M. aeneus* at the inflorescence emergence and flowering stages (2009) using method 1 and cicadas (2007) and *M. aeneus* (2009) at the fruit stage and *Dasyneura brassicae* during the whole vegetation period (2009) using method 2.

Concerning plant pathogens, leaves of spring wheat were only slightly and not significantly damaged by *Erysiphe graminis*, *Puccinia striiformis*, *Puccinia recondita* and *Septoria tritici* during the 2006/2008 years in all treatments. Also the OSR was not significantly damaged by *Peronospora parasitica*. The frequency and severity of disease infestation on spring wheat and OSR was not significantly impacted by elevated CO_2.

In controlled-environment chambers, the phenology, above ground biomass and RGR of OSR were not significantly impacted due to elevated CO_2. And although the phenology of spring wheat was not influenced by raised CO_2, significant increases were observed for plant above ground biomass and RGR. The aphid presence significantly reduced the aboveground biomass and RGR of spring wheat, while no effects due to aphids were observed in OSR.

High-CO_2 treatment differently impacted the performance of aphids. Slight and non-significant increases due to elevated atmospheric CO_2 conditions were observed for the aphid relative developmental stages and intrinsic rates of increase, while the weight and RGR were significantly increased for *Rhopalosiphum padi* and decreased for *Myzus persicae*.

In order to clear CO_2-impacts on the insect performance, phloem sap from host plants was analysed for the composition and concentration of amino acids and carbohydrates. Significant increases under elevated CO_2 were observed for fructose (BBCH 12, BBCH 30) and glucose (BBCH 30) in spring wheat, while no CO_2 effects were found for carbohydrates in the phloem

sap of OSR. The concentration of fructose in spring wheat was not significantly related to RGR of *R. padi* under ambient (BBCH 30) and elevated CO_2 (BBCH 12).

Although the concentrations of total amino acids in the phloem sap of both host plants were not significantly changed due to elevated CO_2, the RGR of *R. padi* was significantly related to their concentration (BBCH 12). In the phloem of spring wheat, significant increases due to elevated CO_2 were observed for the concentrations of Lys, Leu, Ile, Phe, Val, Tyr, Ala, Thr, Ser, Asn and Glu (BBCH 12) and for Ala, Arg, GABA and Leu (BBCH 30), while the concentration of Orn was significantly decreased in the phloem sap of both plants. At ambient conditions, the RGR of *R. padi* was significantly related to the concentrations of Gly (BBCH 12), Gln and Phe (BBCH 30) in spring wheat, whereas the RGR of *M. persicae* showed significant relation to Tyr and Lys (BBCH 14) in OSR. Additionally, significant relationships were observed between RGR of *M. persicae* and the concentrations of αAA, Tyr, Trp, Phe and Leu (BBCH 30) in the phloem sap of OSR under elevated CO_2.

In summary, although the phenological development of spring wheat and OSR was not affected due to elevated CO_2, significant changes were found for the concentration of carbohydrates in the phloem sap of spring wheat and individual amino acids in both host plants. These alterations in plant chemistry affected the performance and abundance of herbivore insects.

2. Zusammenfassung

Es muss vermutet werden, dass die Erhöhung der CO_2-Konzentration in der Atmosphäre einen wesentlichen Einfluss auf Nutzpflanzen, pflanzenfressende Insekten und das Auftreten von pflanzlichen Pathogenen hat. Die Arbeitshypothesen der vorliegenden Arbeit besagen, dass erhöhte CO_2-Konzentrationen die Zusammensetzung der pflanzlichen Gewebe beeinflusst und sich dadurch Veränderungen in den Interaktionen zwischen Pflanzen und herbivoren Insekten ergeben, welche durch veränderte Nährwerte des Phloemsaftes für die Insekten hervorgerufen werden. Um diese Hypothese zu testen, wurden Versuche mit Sommerweizen und Raps in zwei unterschiedlichen Systemen durchgeführt: in einem Klimakammersystem unter kontrollierten Klimabedingungen und in einem Freilandexpositionssystem unter natürlichen klimatischen Bedingungen (Mini-FACE-System).

In den Freilandexperimenten wurden die Abundanz von Schädlingen aus verschiedenen Nahrungsgruppen und die daraus resultierenden Schädigungen an den Pflanzen durch die parasitischen Organismen untersucht. In den Klimakammerexperimenten wurden die Auswirkungen erhöhter atmosphärischer CO_2-Konzentrationen auf die Vitalität gezielt angesetzter Blattlausarten sowie potenzielle Korrelationen zwischen Blattlausvitalität und Phloemsaftzusammensetzung der Wirtpflanzen untersucht. Die Messungen der Phloemsaftzusammensetzung in Hinblick auf Aminosäuren- und Kohlenhydrat-Konzentration erfolgte mittels Hochleistungsflüssigchromatographie, die mit einem Fluoreszenzdetektor für die Erfassung der Aminosäuren bzw. einem Verdampfer/Streuungsdetektor für die Kohlenhydratanalytik.

Die phänologische Entwicklung von Weizen und Raps im Freilandsystem wurde durch die CO_2-Erhöhung nicht wesentlich verändert. Der CO_2-Anstieg bewirkte jedoch Änderungen in der chemischen Struktur der Pflanzen (ein gestiegenes Kohlenstoff-Stickstoffverhältnis und eine Anreicherung von Abwehrkomponenten), was zu Änderungen in der Populationsdichte

einiger Schädlingsarten führte. Um die Änderungen in der Populationsdichte zu beobachten, wurden zwei verschiedene Methoden benutzt: Zählung der Schädlinge direkt auf den Pflanzen (Methode 1) und Fang von Schädlingen auf sogenannten Gelbtafeln (Methode 2). Mit diesen beiden Methoden konnten sowohl Steigerung als auch Verringerungen der Insektenanzahl durch erhöhtes CO_2 nachgewiesen werden, abhängig von den betreffenden Insektenarten und dem Beobachtungszeitraum. In Beständen von Sommerweizen stieg die Anzahl von *Oulema melanopus* (Methode 1) und *Phyllotreta vittula* (Methode 2) während des Zeitraums des Ährenschiebens deutlich und die Anzahl von Individuen aus der Gruppe der Thripsarten im Stadium der Fruchtentwicklung (Methode 2). Gleichzeitig wurde eine Abnahme von *Delia coarctata* während der Bestockung, von *Chaetocnema aridula* im Stadium des Schossens und von *Haplodiplosis marginata* während der Fruchtentwicklung (Methode 2) beobachtet.

Bei Raps stieg die Anzahl von Individuen der Thripsarten im Stadium der Blütenstandausbildung (2009) und während der Blüte (2007), außerdem stieg die Anzahl von *Meligethes aeneus* in der Fruchtentwicklung (2007), von *Athalia rosae* und *Aleyrodes proletella* in der Blütezeit und von *Delia radicum* während der ganzen Vegetationsperiode an (2009, Methode 2). Es wurde ein deutlicher Abfall der Anzahl von *M. aeneus* im Stadium der Blütenstandausbildung und der Blüte (2009, Methode 1) registriert sowie von Zikaden (2007) und *M. aeneus* (2009) während der Fruchtentwicklung und von *Dasyneura brassicae* während der ganzen Vegetationsperiode (2009, Methode 2).

Hinsichtlich der Schadbilder durch Krankheitserreger waren Weizenblätter durch *Erysiphe graminis*, *Puccinia striiformis*, *Puccinia recondita* und *Septoria tritici* in den Untersuchungsjahren 2006 und 2008 auf allen Untersuchungsflächen leicht geschädigt. Erhöhtes CO_2 hatte keinen besonderen Einfluss auf Häufigkeit und Stärke von Pflanzenkrankheiten bei Weizen und Raps.

Unter kontrollierten Klimabedingungen wurden weder die oberirdische Biomasse, die relative Wachstumsrate (RGR) noch die Phänologie von Raps durch CO_2 signifikant beeinflusst. Bei

Sommerweizen trat kein CO_2-Effekt auf die Phänologie auf, wohl aber eine deutliche Zunahme von Biomasse und RGR. Der Besatz mit Blattläusen reduzierte sowohl die oberirdische Biomasse als auch die relative Wachstumsrate des Sommerweizens signifikant. Im Gegenteil dazu wurde kein Einfluss von Blattläusen auf den Raps festgestellt.

Die CO_2-Erhöhung beeinflusste auf unterschiedliche Art und Weise die Vitalität von Blattläusen. Eine leichte Zunahme wurde in den relativen Entwicklungsstadien und in der intrinsischen Entwicklungsrate der Blattläuse beobachtet. Das Gewicht und die Wachstumsrate von *Rhopalosiphum padi* nahmen bedeutend zu, die von *Myzus persicae* jedoch ab.

Um mögliche Wirkungsmechanismen der CO_2-Auswirkungen auf die Vitalität der Insekten zu klären, wurde der Phloemsaft der Wirtpflanzen auf Zusammensetzung und Konzentration von Aminosäuren und Kohlenhydraten analysiert. Dabei konnte ein deutlicher Anstieg der Fruktose- (BBCH 12, BBCH 30) und der Glukose-Konzentrationen (BBCH 30) in Sommerweizen festgestellt werden. Während es keine signifikanten Veränderungen von Kohlenhydraten im Phloemsaft von Raps gab. Die Fruktose-Konzentration in Sommerweizen korrelierte nicht signifikant mit der relativen Wachstumsrate von *R. padi* unter normalen CO_2 (BBCH 30) und unter erhöhtem CO_2 (BBCH 12).

Obwohl die Gesamtkonzentration an Aminosäuren im Phloemsaft von beiden Wirtpflanzen durch erhöhtes CO_2 nicht stark verändert wurde, korrelierte die relative Wachstumsrate von *R. padi* signifikant mit der Gesamtkonzentration (BBCH 12). Im Phloemsaft von Sommerweizen wurde unter CO_2-Erhöhung ein starker Anstieg von Lys, Leu, Ile, Phe, Val, Tyr, Ala, Thr, Ser, Asn und Glu (BBCH 12) und Ala, Arg, GABA und Leu (BBCH 30) beobachtet. Allerdings nahm die Konzentration von Orn im Phloemsaft von beiden Wirtpflanzen stark ab. Unter normalen CO_2-Konzentrationen war die relative Wachstumsrate von *R. padi* mit den Konzentrationen von Gly (BBCH 12) bzw. Gln, Phe (BBCH 30) in Sommerweizen korreliert, während in Raps die relative Wachstumsrate von *M persicae* eine starke Verbindung mit Tyr

und Lys (BBCH 14) zeigte. Ergänzend wurde signifikante Zusammenhänge zwischen der relativen Wachstumsrate von *M. persicae* und den Konzentrationen von αAA, Tyr, Trp, Phe and Leu (BBCH 30) im Phloemsaft des Rapses unter erhöhten CO_2-Konzentrationen beobachtet.

Zusammengefasst fanden keine wesentlichen Veränderungen der phänologischen Entwicklung von Sommerweizen und Raps durch erhöhtes Kohlendioxid statt. Es gab aber deutliche Effekte auf die Konzentration von Kohlenhydraten im Phloemsaft von Sommerweizen und von individuellen Aminosäuren in beiden Wirtpflanzen. Diese Änderungen im Chemismus der Pflanzen beeinflussten die Vitalität und das Vorkommen von herbivoren Insekten.

3. General Introduction

Carbon dioxide (CO_2) concentrations in the atmosphere have increased yearly by 1.8 µl l^{-1} on average (Kingsolver 1996). The concentration of CO_2 has increased by 36% since 1750 and is continuing to rise due to human activity (EPA 2007; IPCC 2007). The current atmospheric CO_2 concentration is at an average of approximately 385 µl l^{-1} (Keeling *et al.* 2009). According to Prather *et al.* (2001), CO_2 concentration is predicted to rise to 550 µl l^{-1} by 2050. Inevitably, such striking changes will influence agricultural crops, causing effects such as increasing photosynthesis and photosynthate production (Groninger *et al.* 1996; Makino & Mae 1999; Ainsworth & Rogers 2007) and reducing transpiration and stomatal conductivity (Cure & Acock 1986; Ainsworth & Rogers 2007). Wand *et al.* (1999) analysed existing experimental data and found that the photosynthesis of C_3 and C_4 plants in the high-CO_2 conditions was increased on average by 33% and 25%, respectively. Contrary to this, a reduction was observed for transpiration (23%, Cure & Acock 1986) and stomatal conductivity (22%, Ainsworth & Rogers 2007). In addition, doubled CO_2 levels decreased photorespiration and led to acclimation of the photosynthetic apparatus with lower ribulose-1,5-bisphosphate carboxylase-oxygenase (RuBisCO) concentrations, still leading to an increase in net photosynthesis between 25 and 75% (Stitt 1991).

The alterations in the plants due to elevated CO_2 were also observed in the physiological structure of the photosynthetic apparatus (Alfermann 2001) and in the morphology of leaves, changing the size and the number of stomata per square millimetre of the leaf area (Beerling & Chaloner 1994; Morison 1998). According to Franks & Beerling (2009), stomata density was negatively impacted by elevated CO_2, while a positive correlation was found between the stomata size and elevated CO_2. Thus, the size of stomata showed an increase of 16.8% in maize (*Zea mays* L. ssp. mays), 10.5% in rice (*Oryza sativa* cv. Pusa Basmati) and 14.1% in silver birch (*Betula pubescens*). Contrary to Franks & Beerling (2009), Radoglou & Jarvis

(1993) observed a significant increase of stomata density in broad bean (*Vicia faba*) under high-CO_2 concentrations.

Elevated CO_2 may change the physiological metabolism of plants by increasing the carbon (C) concentration in the tissue of leaves. In turn, increased C accelerates the growth of plants and results in greater biomass accumulation (Amthor 1995). According to Billes *et al.* (1993), the total plant production increased by 34% due to elevated CO_2, resulting in an increase of dry mass production by 41% for shoots and 23% for roots. Supporting this, rising CO_2 increased the aboveground biomass (17%, Ainsworth & Rogers 2007) and belowground biomass (30%, de Graaff *et al.* 2006) in a FACE experiment. A literature survey on data from 156 plant species showed an increase on average by 37% in the vegetative growth under elevated CO_2, of which an increase for wheat varied between 7 and 97% (Poorter 1993). In addition, the vegetative growth of C_3 plants from 250 species rose with an average of 47% in enriched CO_2 environment (Poorter *et al.* 1996).

Many studies proved that the increases in the size and canopy density and also the alteration in the physiology and morphology of agricultural crops may lead to the progression of various diseases (Manning & Tiedemann 1995; Chakraborty *et al.* 1998; Kobayashi *et al*. 2006). The low light levels and reduced air circulation in dense plant canopies result in higher relative humidity, which promote the growth and sporulation of many plant pathogens (Eastburn *et al*. 2010). According to Lake & Wade (2009), elevated CO_2 increased the aggressiveness of powdery mildew (*Erysiphe cichoracearum*) on the mouse-ear cress (*Arabidopsis thaliana* L.), leading to an enhancement in stomata density and guard cell length. Many other studies showed accelerated disease development due to elevated CO_2, increasing sheath blight *(Rhizoctonia solani* Kunh) on rice (Kobayashi *et al.* 2006), anthracnose pathogen (*Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc.) on shrubby stylo (Chakraborty & Datta 2003) and stem rust (*Puccinia graminis* f. sp. tritici Eriks. & Henn) on wheat plants (Mitchell 1979).

Elevated CO_2 concentrations may significantly influence herbivorous insects by changing the quality or quantity of host plants. Changes take place in the concentrations of nitrogen (N), water, carbohydrates and secondary compounds in the plant tissues (Bezemer & Jones 1998; Coviella & Truble 1999; Awmack & Leather 2002; Pritchard *et al.* 2007**)**. In general, the concentration of N decreases under elevated CO_2, while the carbohydrate concentration increases. Changes in N and carbohydrate levels correlate with the performance of insects, altering their fecundity, population size, food consumption and development time (Evans 1938; Iheagwam 1974; Manning & Tiedemann 1995; Bezemer & Jones 1998; Newman *et al.* 2003; Chen *et al.* 2004). According to Ainsworth (2008) and Ainsworth & Long (2005), elevated CO_2 decreased N content per unit leaf mass by 13%, while the concentration of non-structural carbohydrates (sugar and starches) per unit leaf area was increased on average by 30-40%. This caused different responses in herbivorous insects depending on their feeding guilds, such as an increased population size (phloem-feeders), increased food consumption by 30% (leaf-chewers), decreased pupal weights (leaf-miners) and reduced development time by 17% (phloem-feeders, Bezemer & Jones 1998).

Pimentel (2009) estimated the existence of 50.000 plant pathogens and 70.000 pest species, of which 10% were considered major pests. Another review from Price (2002), estimated the existence of 360.000 insect species, mainly herbivorous ones. Both insects and plant pathogens pose a high risk for agriculture, causing losses in crop yield. Among crops, the total global potential loss due to pests varied from about 50% in wheat to more than 80% in cotton production (Oerke 2006). More specifically, the responses are estimated at losses of 26-29% for wheat, soybean and cotton, 31% for maize, 37% for rice and 40% for potatoes. The worldwide potential loss due to fungal and bacterial pathogens is estimated at 16% in wheat (Oerke & Dehne 2004), while Tiedemann *et al*. (2008) reported the loss in wheat from 20 to 50%, depending on the kind of the pathogen. In oilseed rape (OSR), the possible potential

losses are estimated as up to 50% by deleterious effect of single pathogens (Tiedemann *et al*. 2008).

Previous studies showed very different effects of elevated CO_2 on pests and parasitic organisms, impacting them either positively or negatively, depending on weather conditions or the kind of pest species.

The focal point of the present study was to observe the infestation with pests and parasitic organisms under elevated CO_2 in two crop species, being spring wheat (*Triticum aestivum* L. cv. Triso) and OSR (*Brassica napus* cv. Campino), which have great agricultural importance. Spring wheat is grown on all continents and is the most important cereal crop in France, the USA, China, India and Russia (Oerke 2006). The world harvested area of wheat and OSR in 2009 was estimated at 225.4 million hectare (ha) and 31 million ha, respectively (FAO, 2010). Of this about 3.2 million ha of spring wheat and 1.47 million ha of OSR were planted in Germany. The worldwide production of wheat and OSR in 2009 was 682 million tonnes and 61.6 million tonnes, respectively. Of this total, the estimate for Germany was about of 25.2 million tonnes for spring wheat and 6.3 million tonnes for OSR.

We hypothesized that:

- Elevated CO_2 concentrations would affect plants, increasing their biomass and density, which would alter the microclimate and development of plant pathogens.
- Elevated CO_2 would alter the chemical composition of plant tissue and phloem sap constituents, reducing feed quality for herbivores insects and causing changes in their population dynamics.
- Elevated CO_2 would impact the performance (developmental time, weight, relative growth rate, etc.) of phloem-feeding insects due to changes in plant biochemical composition, due to the causal relationship existing between the composition of phloem constituents and the performance of insects.

In order to prove these hypotheses, two basic approaches were employed: a Mini FACE (free-air CO_2 enrichment) system (Research Station for Plant Breeding, Stuttgart, Germany) with "natural conditions" and a climate chamber system (Institute for Landscape and Plant Ecology of Hohenheim University, Germany) with "standardised" conditions.

The Mini FACE system allowed natural or agricultural ecosystems to be fumigated with elevated CO_2 in field conditions, which helped to indicate plant responses to elevated CO_2 under future real-world conditions. The experiment was focused on the consequences of CO_2 elevation on disease abundance, monitoring of detrimental species of pests from different feeding guilds and the resulting pressure they may exert on plants.

The artificial infestation of the plants with diseases was not planned in this field experiment and the observations made were purely on the spreading of the pathogens as they occurred naturally. Preference was given to obligatory biotrophic pathogens, which came into narrow connection with the living cells of host plants, extracting the nutrients from them and producing externally or internally their own microscopic spores. This concerns the classes of Ascomycetes (powdery mildew, *Erysiphe graminis* f. sp. tritici; septoria leaf blotch, *Mycosphaerella graminicola* (Fuckel) J. Schröt in Cohn (anamorph: *Septoria tritici* Roberge ex Desmaz)), Basidiomycetes (yellow rust, *Puccinia striiformis* Westend f. sp. tritici; brown rust, *Puccinia recondita* f. sp. tritici) and Oomycetes (downy mildew, *Peronospora parasitica* Pers. ex Fr.). The effects of elevated CO_2 on the plant phenology, abundance and plant damage by parasitic organisms are found in chapter 2.

From an ecological and physiological standpoint, the examination of plant-insect-interaction may help predict future alterations in behaviour due to globally elevated CO_2. For this purpose, the impact of elevated CO_2 concentrations on the performance of phloem-feeding insects (i.e. aphids) was observed in controlled-environment chambers (chapter 3). This experiment was conducted with bird cherry-oat aphid (*Rhopalosiphum padi* L.) on spring wheat and green peach aphid (*Myzus persicae* Sulz.) on OSR. The development of the aphids

on host plants was observed from the nymph to the adult stage under elevated CO_2, making record of the relative developmental stage, population growth rate and relative growth rate of insects. All these growth parameters have causal connections to the biochemical composition of plant phloem sap, which is susceptible to changes due to elevated CO_2. In order to prove the existing connection and the resulting consequences on *R. padi* and *M. persicae*, the composition of phloem nutrients such as carbohydrates (sucrose, glucose and fructose) and amino acids were analysed in the phloem sap of host plants (chapter 4).

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Pest and disease abundance and dynamics in wheat and oilseed rape as affected by elevated atmospheric CO_2 concentrations

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Abstract. Future atmospheric CO_2 concentrations are predicted to increase, and directly affect host plant phenology, which, in turn, is assumed to mediate the performance of herbivorous insects indirectly as well as the abundance and epidemiology of plant diseases. In a 4-year field experiment, spring wheat (*Triticum aestivum* L. cv. Triso) and spring oilseed rape (*Brassica napus* L. cv. Campino) were grown using a mini- free-air CO_2 enrichment (FACE) system, which consisted of a control (CON), an ambient treatment (AMB) and FACE treatments. The CON and AMB treatments did not receive additional CO_2, whereas the FACE plots were moderately elevated by 150 μL L^{-1} CO_2. The impact of elevated CO_2 was examined with regard to plant phenology, biomass, leaf nitrogen and carbon, abundance of insect pest species and their relative population growth by either direct counts or yellow sticky traps. Occurrence and damage of plants by pathogens on spring wheat and oilseed rape were directly assessed. Disease infestations on plants were not significantly different between ambient and elevated CO_2 in any of the years. Plant phenology, aboveground biomass, foliar nitrogen and carbon concentrations were also not significantly affected by CO_2 enrichment. In contrast, the abundance of some species of insects was significantly influenced by elevated CO_2, showing either an increase or a decrease in infestation intensity.

Additional keywords: *Brassica* spp., CO_2 enrichment, plant–insect interactions.

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Introduction

Atmospheric CO_2 concentration is predicted to reach 550 μL L^{-1} at the middle of this century (Intergovernmental Panel on Climate Change 2007). This increase has been shown to affect plant physiology, morphology, development, growth and reproduction (Bazzaz 1990; Poorter and Navas 2003; Högy *et al*. 2009). According to Franzaring *et al*. (2008) and Högy *et al*. (2010), spring oilseed rape (OSR, *Brassica napus* L. cv. Campino) grown under elevated CO_2 showed an increase in biomass, dry weight, stem and shoot length, and leaf area. Aboveground biomass increased by 11.8% in spring wheat (*Triticum aestivum* L. cv. Triso) under CO_2 enrichment, the latter resulting from a higher number of tillers per plant (Högy *et al*. 2009). An increase in atmospheric CO_2 levels alters the chemical composition of the plant tissue and phloem sap constituents, potentially causing a reduction in food quality for herbivores as determined by the contents of fibre, starch, water, sugars, allelochemicals and nitrogen in host plant leaves (Curtis *et al*. 1989; Johnson and Lincoln 1991; Brown 1995). Food consumption of leaf-chewing larvae increased by 20–80% under elevated CO_2, which was interpreted as a mechanism to compensate for a decreased N concentration (Bezemer and Jones 1998). Consequently, increased consumption resulted in boosted feeding damage by herbivores and detritus conversion by detritivores (Lincoln *et al*. 1993; Hughes and Bazzaz 1997; Stadler 1999). According to Stiling *et al*. (2009), elevated CO_2 resulted in prolonged preimaginal development time, decreased adult weight (5%) and relative growth rate (8.3%) as well as increased mortality rate of leafminers on oak species (*Quercus myrtifolia* Willd, *Q. chapmanii* Sargent and *Q. geminata* Samll).

Phloem feeders, feeding on live cell contents, can be considered as true plant parasites reacting rapidly to changes in nutritive quality such as a reduction in biochemical compounds (e.g. proteins) or an increase in the carbohydrates in the phloem. Alterations in the concentrations or composition of N-containing substances in the phloem, such as amino acids, may affect phloem-feeding insects in their development, growth rate and population growth (Newman *et al*. 2003). According to Awmack *et al*. (1997), the performance of potato aphid (*Aulacorthum solani* Kalt.) was enhanced on broad bean (*Vicia faba* L.) and tansy (*Tanacetum vulgare* L.) by elevated CO_2. Moreover, the nature of the response was different on each plant species. Thus, on tansy, preimaginal development of aphids was 10% shorter but there was no difference in the rate of nymph reproduction, whereas on broad beans, the duration of preimaginal development was not affected by elevated CO_2 but the rate of nymph reproduction was increased by 16%. Chen and Parajulee (2005) observed some positive effects of elevated CO_2 on

Aphis gossypii Glover, i.e. an increase in fecundity, mean relative growth and consumption rate. Chen *et al.* (2004) reported that the offspring of the grain aphid (*Sitobion avenae* F.) was increased by 13% at 550 μL L^{-1} CO_2 and by 19% in the 750 μL L^{-1} CO_2 treatment. Elevated CO_2 affects the population dynamics of most insect species (Newman *et al.* 2003; Dermody *et al.* 2008). Also, Chen *et al.* (2004) reported a significant increase in the population size of *Sitobion avenae* F. of ~15% and 22% under 550 μL L^{-1} and 750 μL L^{-1} CO_2, respectively.

According to Dahlman *et al.* (1991), elevated CO_2 affected the host–pathogen interactions by changing the physiology of the host plants (ryegrass, *Lolium perenne* L.). In particular, the increase in foliar carbohydrate concentrations under elevated CO_2 promotes the development of biotrophic plant pathogens such as rust diseases (Drandarevski 1969). Moreover, the reduction of leucine-rich protein in the vegetative organs, which directly affects the defence reaction of plants against pathogens, as well as a reduction in salicylic acid and soluble phenolic substances due to elevated CO_2 increases pathogen aggressiveness, leading to greater pathogenic damage (Goicoechea *et al.* 2004).

The increase in the biomass and density of host plants associated with elevated CO_2 is assumed to modify the microclimate and development of plant diseases such as a powdery mildew (*Erysiphe graminis* DC) and brown rust (*Puccinia recondita* Roberge ex Desmaz) in wheat; and white stem disease (*Sclerotinia sclerotiorum* (Lib.) de Bary), beet rhizomania (*Polymyxa betae* Keskin), black spot (*Alternaria brassicae* (Berk.) Sacc.), and stem or root rot (*Phoma lingam* (Tode ex Fr.) Desm.; teleomorph: *Leptosphaeria maculans* (Desm.) Ces. and de Not) in OSR (Drandarevski 1969; Manning and Tiedemann 1995; Patterson *et al.* 1999; Keller 2002). As information on the effects of elevated CO_2 concentrations on both the spread of fungal diseases and pests, representing the most important biotic stressor categories in crop production, is still fragmentary, more detailed studies are necessary.

Monitoring of pests and diseases under elevated CO_2 is a relatively modern approach. Only a few studies have been done utilising free-air CO_2 enrichment (FACE) facilities for the observation of diseases on spring wheat (pathogens *Fusarium pseudograminearum*, Melloy *et al.* 2010 and *Puccinia triticina* Erikss. and Henn; Chakraborty *et al.* 2011), on rice (*Oryza sativa* L.) (pathogen: *Rhizoctonia solani* Kunh; Kobayashi *et al.* 2006), on soybean (*Glycine max* L.) (pathogens: *Peronospora manshurica* L. and *Septoria* brown spot; Eastburn *et al.* 2010) and on red maple (*Acer rubrum* L.) (pathogen: *Phyllosticta minima*; McElrone *et al.* 2005). The consequences of CO_2 elevation on pest and disease abundance and the resulting pressure they may exert on spring wheat and OSR under FACE field conditions were the focus of this study. The objectives were to (i) assess and describe the effects of elevated CO_2 on the phenology, abundance and plant damage by parasitic organisms like powdery mildew (*Erysiphe graminis* f. sp. tritici), yellow rust (*Puccinia striiformis* Westend f. sp. tritici), brown rust (*Puccinia recondita* f. sp. tritici), septoria leaf blotch (*Mycosphaerella graminicola* (Fuckel) J. Schröt in Cohn (anamorph: *Septoria tritici* Roberge et Desmaz)) on spring wheat, and downy mildew (*Peronospora parasitica* (Pers. ex Fr.)) on OSR; and (ii) monitor the abundance of insect pests and their population dynamics under elevated CO_2.

Materials and methods

CO_2 exposure

The experiments were performed over a 4-year period from 2006 to 2009 on the Research Station for Plant Breeding Heidfeldhof, situated in the south of Stuttgart, Germany (9°11′28″E, 48°42′51″N; 395 m above sea leavel). The mini-FACE system used consisted of 15 circular plots, 2 m in diameter, and three different CO_2 treatments. Elevated CO_2 (ELE) was supplied in five FACE plots (plus 150 μL L^{-1}). Five ambient plots (AMB) were supplied with the same technical infrastructure as the ELE plots but with no additional CO_2 fumigation. Additionally, five control plots (CON) with neither CO_2 fumigation nor racks were set up to identify effects caused by the technical equipment. In the high-CO_2 treatment, pure CO_2 (Westfalen Gas, Münster, Germany) was added continuously during the entire vegetation period. Variation coefficients between seasonal CO_2 concentrations determined in the five plots were small and amounted to 1.9–5.1% in the years 2006–09 (representing real differences in CO_2 concentrations between the plots from 10 μL L^{-1} to 31 μL L^{-1}). Nevertheless, the average seasonal CO_2 concentrations in the FACE treatments differed between years due to the different crop species and related canopy structures so that the set concentration value of 550 μL L^{-1} was not reached exactly at all times. Seasonal (from sowing to harvest) 24 h CO_2 concentrations were 529, 494, 558 and 613 μL L^{-1} in the years 2006–09, respectively. The overall 4-year mean CO_2 concentration of 549 μL L^{-1}, however, was comparable to the set concentration. A more detailed description of the operational principles and the performance of the FACE system are given in Erbs and Fangmeier (2006).

Cultivation and phenological development of plants

Spring wheat (*Triticum aestivum* L. cv. Triso; 200 plants m^{-2} in 2006 and 360 plants m^{-2} in 2008; 13 rows with 15-cm row spacing) and OSR (*Brassica napus* L. cv. Campino; 70 plants m^{-2}; 13 rows with 15-cm row spacing in 2007 and 2009) were cultivated on clay-loam soil.

Phenological development of plants was determined using the Biologische Bundesanstalt and Chemische Industrie scale (BBCH scale; Tottman and Broad 1987; Weber and Bleiholder 1990). All development stages were based on observations on the main stem. Examination was carried out from leaf development (BBCH 10) until senescence (BBCH 90).

Plots with OSR were fertilised annually with 130 kg N ha^{-1} (NH_4NO_3), 60 kg P ha^{-1}, 60 kg K ha^{-1}, 18 kg Mg ha^{-1} and 4 kg S ha^{-1} at leaf development stage (BBCH 14). Potassium (60 kg ha^{-1}) was applied at the stage of tiller formation (BBCH 25) and shortly before flowering (BBCH 57) (Högy *et al.* 2010). Plots with spring wheat were fertilised annually with 140 kg N ha^{-1} (NH_4NO_3), 30 kg P $ha^{-1}$and 60 kg K ha^{-1} in total at tillering (BBCH 25), stem elongation (BBCH 36) and inflorescence emergence (BBCH 43). Additionally, 0.45 kg Mg ha^{-1}, 0.36 kg S ha^{-1}, 0.03 kg B ha^{-1} and 0.03 kg Mn ha^{-1} were applied at tillering (Högy *et al.* 2009, 2012).

Environmental conditions and soil characteristics

Meteorological data (air temperature, relative humidity, precipitation and global radiation) of the years 2006–09 was recorded by the Institute of Physics and Meteorology and Institute for Landscape and Plant Ecology (University of Hohenheim; Table 1). The mean air temperatures from April to August were 15.3°C (2006), 15.6°C (2007), 15.4°C (2008) and 16.0°C (2009). Soil moisture and temperature were measured at a 15 cm depth during the growing season using reflectometry (TDR, IMKO GmbH Karlsruhe, Germany) and thermocouples (UP, Deckenpfronn, Germany). The N and C contents in the soil were determined in the autumn before the experiment was performed, using an elemental analyser (Vario EL, Elementar Analysensysteme, Hanau, Germany). Average C contents were 1.6% with a C : N ratio of 8.8, which did not significantly change over time (Franzaring *et al*. 2010).

Biomass production and determination of nitrogen and carbon concentrations in leaves

In order to determine the foliage biomass at ambient and elevated CO_2, leaves of spring wheat and OSR were harvested at the central area (1 m^2) of each plot at the flowering stage (BBCH 65–69), dried at 60°C (3 days) to constant weight in a drying oven and weighed on a balance (A 120 S, Triad Scientific, Manasquan, NJ, USA). According to ISO 10694 (International Standards Organisation 1995), the concentrations of foliage C and N were analysed using an isotope-ratio mass spectrometer (IRMS, Thermo Finnigan MAT, Bremen, Germany) in 2006 and 2007 and a Vario EL, elemental analyser (Elementar Analysensysteme) in 2008 and 2009 (Högy *et al*. 2010). The concentrations of foliage C and N were measured at the flowering stage (BBCH65–69) because consumption of plant tissues at this stage is usually higher than in other stages.

Table 1. Environmental conditions in the years 2006–09 (April–August)

Data were recorded by the Institute of Physics and Meteorology (†) and Institute for Landscape and Plant Ecology (‡) of the University of Hohenheim

Parameters	Month	Years			
		2006	2008	2007	2009
Temperature, °C†	April	8.7	8.2	13.6	12.1
	May	13.6	15.4	15.1	14.8
	June	17.3	17.4	14.7	16.0
	July	22.3	18.3	17.6	18.2
	August	15.3	17.7	17.0	19.0
Seasonal mean air temperatures, °C†	April–August	15.3	15.4	15.6	16.0
Relative humidity, %†	April	71.6	75.5	55.1	67.0
	May	66.9	65.3	66.8	73.9
	June	66.6	74.4	62.8	72.7
	July	63.3	70.4	72.4	73.6
	August	78.0	74.7	75.3	72.2
Global radiation, W m^{-2}†	April	155.2	153.9	269.8	190.5
	May	208.5	250.9	218.6	233.7
	June	278.8	241.6	236.8	248.0
	July	282.4	237.0	229.1	233.2
	August	167.4	199.9	191.7	230.1
Sum of precipitation, mm‡	April	57.2	52.5	50.2	35.3
	May	101.0	102.8	162.8	128.4
	June	31.0	126.2	167.2	93.3
	July	99.8	69.0	81.14	126.0
Water content across all plots, % vol‡	April	29.6	26.1	27.0	13.5
	May	30.2	21.0	29.0	21.0
	June	24.0	20.1	24.1	11.7
	July	16.0	10.7	16.6	18.5

Monitoring of pests and diseases

Insect pest and disease abundance was monitored on spring wheat and OSR, at weekly intervals from leaf emergence until plant maturity. As the focus of this study was on the insect pests and diseases associated with of aboveground plant biomass, root feeding organisms, soilborne diseases and root diseases were not considered. Pest abundance was assessed directly by counting numbers per plant (M_1) and indirectly by counting the total number of individuals on yellow sticky traps (Bayer Crop Science GmbH, Monheim am Rhein, Germany, 7.3 × 19.8 cm; M_2). For the M_1 method and determination of the abundance of plant diseases, 10 plants were marked in the central area (1 m^2) of each plot. For the M_2 method, one yellow trap was used per plot, hung 10 cm above the canopies in the middle of each plot and replaced weekly. In 2006, method M_2 was not used. Commencing in 2007, both M_1 and M_2 methods were applied. This alteration in observation methods took place as the sticky traps enabled a wider variety of crawling and flying pest species to be caught. Taxonomical identification of insect species was made by morphological characteristics (Garbe *et al*. 1999; Dunford and Long 2002), using a stereomicroscope (Stemi DV4, Carl Zeiss, Jena, Germany) with high resolution (32× detail magnification, 8×–32×–18 eyepiece micrometer).

Visual monitoring of the whole plant from the upper to the lower leaves was made to assess plant pathogen incidence and disease levels. Preference was given to obligate biotrophic pathogens, which deprive the live plant cells of nutrients and may be easily detected on the surface of green leaves. For the determination of plant pathogens, leaves of wheat and OSR (2 cm^2) were cut from the main stem and microscopically observed for the presence of spores. Visual monitoring of plant diseases was done weekly during the whole vegetation period. The calculated damage of leaf surface is given as a percentage.

In order to determine plant damage, the disease frequency of infestation (FI) and the disease severity of infestation (SI) were examined. According to Verreet (1985), plants were rated for FI on a four-point scale (0 = no disease; 1 = 1–30%; 2 = 30–60%; 3 = 60–90%; 4 >90%) and for SI on a seven-point scale (1 = 0–0.9%; 2 = 0.91–1.9%; 3 = 1.91–2.9%; 4 = 2.91–3.9%; 5 = 3.91–4.9%; 6 = 4.91–5.9%; 7 = 5.91–6.9%).

Statistical analyses

Data from AMB and ELE treatments with five replicates were subjected to statistical analyses for plant parameters (development, biomass, nitrogen and carbon concentrations), frequency and intensity of disease infestation on plants and the

abundance of different pest species using PASW Statistics ver. 18 (SPSS, Chicago, IL, USA). Because the data were normally distributed no transformation was applied. The data from the CON treatment were excluded as their differences compared with the AMB treatment were not statistically significant. Effects of CO_2 treatments were identified by ANOVA. The relationships between the concentrations of foliage C and N, and the abundance of insects were calculated by using linear regression analysis.

Results

Effects of elevated CO_2 on plant phenology, leaf biomass, and carbon and nitrogen concentrations

The phenology of spring wheat and OSR was examined from leaf development (BBCH 10) until ripening (BBCH 80). In spring wheat, the phenological development under elevated CO2 was retarded (BBCH 10, 40), delayed (BBCH 20) and postponed by 7 days (BBCH 30) in 2006, and delayed (BBCH 40) and retarded (BBCH 80) in 2008 (Table 2). In OSR, phenological development was retarded (BBCH 20, 30) and delayed (BBCH 60, 80) under elevated CO_2 in 2007 and delayed (BBCH 20) in 2009. The effects of elevated CO_2 on crop phenology were generally small and not statistically significant. Leaf biomass and foliar C and N concentrations were not significantly affected by elevated CO_2 concentration (Table 3).

Pests on spring wheat

In 2006, only the abundance of bird cherry-oat aphids (*Rhopalosiphum padi* L. (Homoptera: Aphididae)) was monitored on spring wheat using method M_1; however, no CO_2 effects were found (data not shown). In 2008, the

Table 2. Duration of phenological phases after sowing of spring wheat in 2006–08 and oilseed rape in 2007–09
All development stages are based on observations on the main stem. Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie (BBCH) codes used to quantify the growth stages in cereals are as follows: BBCH 10, leaf development; BBCH 20, tillering; BBCH 30, stem elongation; BBCH 40, booting; BBCH 50, inflorescence emergence or heading; BBCH 60, flowering; BBCH 70, dough development; BBCH 80, ripening. For oilseed rape the codes are: BBCH 10, leaf development; BBCH 20, formation of side shoots; BBCH 30, stem elongation; BBCH 50, inflorescence emergence; BBCH 60, flowering; BBCH 70, development of fruit; BBCH 80, ripening. AMB, ambient CO_2 concentration; ELE, elevated CO_2 concentration

Crop	Year	Growth stage	BBCH code	Duration of phenological phase (days)	
				AMB	ELE
Spring wheat	2006	Leaf development	10	49	25
		Tillering	20	77	70
		Stem elongation	30	28	14
		Booting	40	7	1
		Inflorescence emergence	50	1	1
		Flowering	60	1	7
		Dough development fruit	70	14	14
		Ripening	80	1	1
Spring wheat	2008	Leaf development	10	21	21
		Tillering	20	28	28
		Stem elongation	30	28	28
		Booting	40	7	14
		Inflorescence emergence	50	7	7
		Flowering	60	1	1
		Dough development fruit	70	14	14
		Ripening	80	21	14
Oilseed rape	2007	Leaf development	10	24	24
		Formation of side shoots	20	13	6
		Stem elongation	30	6	1
		Inflorescence emergence	50	6	6
		Flowering	60	6	14
		Development of fruit	70	27	27
		Ripening	80	28	35
Oilseed rape	2009	Leaf development	10	36	36
		Formation of side shoots	20	42	50
		Stem elongation	30	22	22
		Inflorescence emergence	50	15	15
		Flowering	60	20	20
		Development of fruit	70	7	7
		Ripening	80	35	35

Table 3. Biomass (g DW per plant), carbon and nitrogen concentration (% DW) in leaves of spring wheat (2006–08) and oilseed rape (2007–09) at Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie (BBCH) 65–69 at ambient (AMB) and elevated (ELE) CO_2 concentrations
n.s., not significant ($P > 0.05$)

Years	BBCH code	Crop traits	CO_2 treatment[A]		*P*-values
			AMB	ELE	(ANOVA)
2006	BBCH 69	Leaf biomass	0.29 ± 0.04	0.29 ± 0.01	n.s.
		Leaf nitrogen	3.00 ± 0.47	2.95 ± 0.28	n.s.
		Leaf carbon	43.22 ± 0.30	43.56 ± 0.23	n.s.
2007	BBCH 65–69	Leaf biomass	1.00 ± 0.39	1.65 ± 0.68	n.s.
		Leaf nitrogen	2.88 ± 0.35	2.76 ± 0.46	n.s.
		Leaf carbon	39.7 ± 1.06	40.13 ± 1.81	n.s.
2008	BBCH 65–69	Leaf biomass	0.68 ± 0.19	0.58 ± 0.13	n.s.
		Leaf nitrogen	3.20 ± 0.15	3.03 ± 0.27	n.s.
		Leaf carbon	42.13 ± 0.22	42.08 ± 0.42	n.s.
2009	BBCH 69	Leaf biomass	2.19 ± 0.77	2.60 ± 0.46	n.s.
		Leaf nitrogen	3.05 ± 0.34	2.47 ± 0.28	n.s.
		Leaf carbon	39.42 ± 0.43	39.18 ± 0.50	n.s.

[A]Values represent means and s.e. across replicates, the level of statistical significance according to one-way ANOVA; $n = 5$.

abundance of thrips species (Thysanoptera: Thripidae), cereal leaf beetles (*Chaetocnema aridula* (Gyll.), *Oulema melanopus* (L.) (Coleoptera: Chrysomelidae)), click beetle (*Agriotes sputator* L. (Coleoptera: Elateridae)), cereal ground beetle (*Zabrus tenebrioides* Goeze (Coleoptera: Carabidae)), shield bug (*Aelia acuminata* L. (Hemiptera: Pentatomidae)) and *R. padi* were observed using method M_1. Under elevated CO_2, the abundance of *O. melanopus* and thrips species was significantly increased at BBCH 59 and BBCH 71, respectively (Table 4). The abundance of *C. aridula*, *A. sputator*, *A. acuminata*, *R. padi* and *Z. tenebrioides* was not significantly affected by elevated CO_2.

On spring wheat, using the M_2 method, orange wheat blossom midge (*Sitodiplosis mosellana* Géhin (Diptera: Cecidomyiidae)), saddle gall midge (*Haplodiplosis marginata* (von Roser) (Diptera: Cecidomyiidae)), barley leaf beetle (*Phyllotreta vittula* (Redt.) (Coleoptera: Chrysomelidae)), green cicada (*Cicadella viridis* (L.) Müller (Hemiptera: Cicadellidae)) and wheat bulb fly (*Delia coarcata* (Fallén) (Diptera: Anthomyidae)) were observed. Significant reductions in population density under elevated CO_2 were observed for *D. coarcata* at BBCH 22 and BBCH 23, for *C. aridula* at BBCH 31 and *H. marginata* at BBCH 83, and the abundance of *P. vittula* was significantly increased at BBCH 41 (Table 5).

Pests on oilseed rape

In 2007 and 2009, thrips species (Thysanoptera: Thripidae), turnip sawfly (*Athalia rosae* (L.) (Hymenoptera: Tenthredinidae)), green cicada (*Cicadella viridis* (L.) Müller (Hemiptera: Cicadellidae)), pollen beetle (*Meligethes aeneus* F. (Coleoptera: Nutidulidae)), spring cabbage fly (*Delia radicum* L. (Diptera: Anthomyidae)), cabbage whitefly (*Aleyrodes proletella* L. (Hemiptera: Aleyrodidae)), green peach aphid (*Myzus persicae* (Sulz.) (Hemiptera: Aphididae)) and brassica pod midge (*Dasyneura brassicae* Winnertz (Diptera: Cecidomyidae)) were observed in OSR.

In 2007, a significant increase in the abundance of thrips species (BBCH 71, M_2) was observed under elevated CO_2, whereas the abundance of *M. aeneus* (BBCH 77, M_1) and cicadas (BBCH 81, M_2) decreased (Table 5).

In 2009, a significant decreases in the abundance of *M. aeneus* were again observed under elevated CO_2 at BBCH 55 and BBCH 67 using method M_1 and at BBCH 80 using M_2 (Table 6). The results of method M_2 show that elevated CO_2 resulted in a significant increase in the abundance of thrips species (*A. rosae*, *D. radicum*, *M. aeneus* and *A. proletella*). Significant increases due to elevated CO_2 were observed in the abundance of *A. rosae* and thrips species at BBCH 55, *A. proletella* at BBCH 67 and *D. radicum* during the whole cultivation period, with maximum numbers of insects being 5.6 ± 0.5 (AMB) and 10.4 ± 1.1 (ELE) at BBCH 67. Elevated CO_2 significantly decreased the infestation by *D. brassicae* during the whole cultivation period, with maximum numbers of insects reaching 11.2 ± 1.3 at ambient CO_2 and 3.2 ± 0.8 at elevated CO_2 (BBCH 80).

Linear regression analysis between the concentrations of foliar C and N, and the abundance of insects

No significant relationships were found between the abundance of insects and the concentration of foliar C of spring wheat (2006–008) and OSR (2007–09) in either of the CO_2 treatments (data not shown). However, relationships were observed between the concentrations of N and the abundance of *A. proletella* (M_2), *M. aeneus* (M_1) and *D. radicum* (M_2) in OSR in 2009 (74 days after sowing, DAS) under elevated CO_2 (Table 7).

Pathogens

Under elevated CO_2, the leaves of spring wheat were only slightly damaged by powdery mildew (*E. graminis*), yellow rust (*P. striiformis*) and brown rust (*P. recondita*) in 2006, and by septoria leaf blotch (*S. tritici*), *E. graminis* and *P. recondita* in

Table 4. Abundance of insect species per plant (method M_1) and per trap (method M_2) in spring wheat during the whole vegetation period under ambient (AMB) and elevated (ELE) CO_2 treatments in 2008

Method M_1 uses direct counts on plants; method M_2 captures insects on adhesive traps. Results of the statistical analysis (ANOVA) are presented as *P*-values (n.s., not significant; $P \leq 0.05$, significant); $n = 5$. BBCH, Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie

Species of insect	Days after sowing	Growth stages BBCH code	AMB	ELE	CO_2 effect *P*-values (ANOVA)
			Average numbers of individuals with s.e.		
Method M_1					
Oulema melanopus	52	31	0.2 ± 0.1	0.2 ± 0.0	n.s.
	59	41	0.3 ± 0.1	0.4 ± 0.2	n.s.
	66	53	0.1 ± 0.1	0.2 ± 0.1	n.s.
	73	59	0.1 ± 0.1	0.4 ± 0.2	0.05
Rhopalosiphum padi	59	41	0.3 ± 0.1	0.8 ± 0.9	n.s.
	66	53	1.3 ± 0.5	1.1 ± 0.3	n.s.
	73	59	0.4 ± 0.3	0.6 ± 0.4	n.s.
	80	71	0.4 ± 0.1	0.6 ± 0.6	n.s.
	87	83	0.2 ± 0.1	0.7 ± 0.8	n.s.
Thrips species	80	71	1.0 ± 0.1	1.6 ± 0.2	0.05
	87	83	1.5 ± 0.1	2.0 ± 0.6	n.s.
	94	83	2.7 ± 1.9	4.4 ± 0.6	n.s.
	101	84	0.7 ± 0.8	0.2 ± 0.2	n.s.
Zabrus tenebrioides	73	59	0.2 ± 0.2	0.2 ± 0.2	n.s.
Chaetocnema aridula	79	71	0.1 ± 0.1	0.0 ± 0.0	n.s.
Agriotes sputator	87	83	0.0 ± 0.1	0.2 ± 0.1	n.s.
	87	83	0.1 ± 0.1	0.1 ± 0.1	n.s.
Method M_2					
Delia coarcata	36	22	36.4 ± 4.3	2.8 ± 0.8	0.05
	44	23	12.8 ± 1.3	4.6 ± 0.5	0.05
	51	31	7.8 ± 3.5	5.2 ± 4.5	n.s.
	58	41	6.6 ± 3.5	5.6 ± 2.8	n.s.
	65	53	14.4 ± 4.1	9.6 ± 5.5	n.s.
C. aridula	51	31	7.6 ± 0.8	1.4 ± 0.5	0.01
	58	41	9.0 ± 6.6	4.2 ± 1.7	n.s.
	65	53	12.0 ± 7.3	7.4 ± 5.3	n.s.
	72	59	35.6 ± 7.5	27.6 ± 23.9	n.s.
Phyllotreta vittula	58	41	1.4 ± 0.5	4.6 ± 1.1	0.05
	65	53	8.0 ± 4.5	8.8 ± 6.9	n.s.
	72	59	9.6 ± 4.3	7.6 ± 5.5	n.s.
	79	71	20.0 ± 10.5	17.6 ± 6.3	n.s.
	86	83	6.0 ± 3.3	3.8 ± 3.7	n.s.
Haplodiplosis marginata	93	83	3.2 ± 0.4	1.2 ± 0.4	0.05
Sitodiplosis mosellana	93	83	2.4 ± 0.5	0.4 ± 0.5	n.s.
	100	84	0.6 ± 0.5	0.4 ± 0.5	n.s.

Table 5. Average numbers of *Meligethes aeneus* in oilseed rape using method M_1 and *Cicadella viridis* and thrips species using M_2 under ambient (AMB) and elevated (ELE) CO_2 treatments in 2007

The Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie (BBCH) code represents the growth stages of oilseed rape. Results of statistical analysis (ANOVA) are presented as *P*-values ($P \leq 0.05$ = significant); $n = 5$. For an explanation of M_1 and M_2, refer to Table 4

Species of insect	Method	Days after sowing	BBCH code	AMB	ELE	CO_2 effect *P*-values (ANOVA)
				Average numbers of pests with s.e.		
M. aeneus	M_1	78	77	0.6 ± 0.1	0.3 ± 0.1	0.01
Thrips species	M_2	63	71	133.6 ± 10.1	190.6 ± 27.6	0.01
C. viridis	M_2	91	81	2.0 ± 0.7	0.4 ± 0.1	0.05

Table 6. Occurrence of individuals of insect species per plant (method M_1) and per trap (method M_2) in oilseed rape during the whole vegetation period under ambient (AMB) and elevated (ELE) CO_2 treatments in 2009

For an explanation of M_1 and M_2, refer to Table 4. Results of the statistical analysis (ANOVA) are presented as *P*-values (n.s., not significant; $P \leq 0.05$, significant); $n = 5$. BBCH, Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie

Species of insect	Days after sowing	Growth stages BBCH code	AMB	ELE	CO_2 effect *P*-values (ANOVA)
			Average numbers of individuals with s.e.		
Method M_1					
Meligethes aeneus	46	50	0.6 ± 1.2	1.0 ± 1.2	n.s.
	52	55	3.6 ± 0.7	1.8 ± 0.5	0.01
	60	62	4.3 ± 3.3	3.7 ± 3.4	n.s.
	66	66	4.1 ± 3.4	3.2 ± 3.2	n.s.
	74	67	10.1 ± 1.9	7.5 ± 1.3	0.05
	80	71	2.5 ± 1.2	4.3 ± 3.0	n.s.
	88	77	1.2 ± 1.1	2.2 ± 1.6	n.s.
Method M_2					
Athalia rosae	52	55	2.2 ± 0.4	5.0 ± 0.7	0.05
	60	62	2.2 ± 0.4	4.2 ± 1.3	0.05
	67	66	4.6 ± 0.5	6.0 ± 1.4	n.s.
Delia radicum	52	55	3.4 ± 0.5	7.8 ± 1.7	0.001
	60	62	4.2 ± 0.4	8.6 ± 1.1	0.001
	67	66	4.8 ± 0.8	9.8 ± 0.8	0.01
	74	67	5.6 ± 0.5	10.4 ± 1.1	0.01
	95	80	3.2 ± 0.4	6.2 ± 0.8	0.05
	102	81	2.2 ± 1.7	3.8 ± 1.3	n.s.
Dasyneura brassicae	52	55	1.8 ± 0.8	0.2 ± 0.4	0.01
	60	62	1.6 ± 0.5	0.2 ± 0.4	0.05
	67	66	1.0 ± 0.7	1.6 ± 1.1	n.s.
	74	67	0.4 ± 0.5	0.2 ± 0.4	0.05
	95	80	5.2 ± 0.8	1.4 ± 0.8	0.01
	108	81	11.2 ± 1.3	3.2 ± 0.8	0.05
Thrips species	52	55	41.4 ± 1.7	69.2 ± 1.3	0.001
	60	62	71.2 ± 28.2	89.4 ± 20.1	n.s.
	67	66	15.6 ± 4.2	18.8 ± 11.2	n.s.
	74	67	30.6 ± 10.9	20.4 ± 8.7	n.s.
	95	80	48.2 ± 29.5	30.4 ± 16.6	n.s.
Aleyrodes proletella	74	67	1.2 ± 0.4	4.6 ± 0.5	0.001
	95	80	2.4 ± 2.0	1.0 ± 1.4	n.s.
M. aeneus	74	67	16.4 ± 8.1	17.0 ± 6.0	n.s.
	95	80	47.4 ± 27.7	18.4 ± 5.7	n.s.
	102	80	7.0 ± 1.2	3.1 ± 0.7	0.05

2008. The FI and SI of these diseases were not significantly affected under CO_2 enrichment (Table 8).

In 2007, no disease symptoms were observed in OSR in any of the treatments. Although, in 2009, downy mildew (*P. parasitica*) appeared on OSR from 89 DAS until 94 DAS at the ripening stage (BBCH 80), the SI and FI of this disease were not significantly affected by elevated CO_2 (Table 8).

Discussion

In our study, the phenological development and aboveground biomass of spring wheat and OSR were not significantly affected under CO_2 enrichment, which was not expected, as crops are often advanced in their life cycle. In contrast, Atwell *et al*. (1999) showed that CO_2 enrichment (700 µL L^{-1}) enhanced the development of wheat (*Triticum aestivum* L.) and rice (*Oryza sativa* L.), significantly accelerating the visual appearance of successive leaves and shortening the flowering time. A slight enhancement of phenological development under elevated CO_2 (494 µmol mol^{-1}) was also observed in OSR (Franzaring *et al*. 2008) and maize (*Zea mays* L.) (Leakey 2009). According to Garbutt *et al*. (1990), *Amaranthus retroflexus* L. flowered significantly earlier under elevated CO_2 (700 µL L^{-1} vs 350), whereas *Setaria faberi* Herrm flourished significantly later. A positive relationship was found between the appearance of wheat leaves and the concentration of elevated CO_2 (700 µL L^{-1}) in the study of McMaster *et al*. (1999), where accelerated leaf and tiller appearance rates resulted in faster canopy development and higher plant biomass (shoot, root and spike production). Significant increases in aboveground biomass due to elevated

Table 7. Linear regression analysis between abundance of insects and concentrations of nitrogen in leaves of spring wheat (2006–08) and oilseed rape (2007–09)

For an explanation of M_1 and M_2, refer to Table 4. DAS, days after sowing; BBCH, Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie; r^2, regression coefficient; P, level of probability for linearity. Significant regressions ($P \leq 0.05$) with r^2 >0.30 are shown in bold

Insect and crop	DAS	BBCH code*	N concentration r^2	N concentration P
2006				
Spring wheat				
Rhopalosiphum padi (M_1)	62	32	0.327	0.084
2007				
Oilseed rape				
Meligethes aeneus (M_2)	63	71	0.044	0.560
Athalia rosae (M_2)	63	71	0.101	0.371
Aleyrodes proletella (M_2)	63	71	0.367	0.064
Thrips species (M_2)	63	71	0.038	0.591
M. aeneus (M_1)	63	71	0.079	0.432
Cicadella viridis (M_2)	63	71	0.006	0.825
Dasyneura brassicae (M_2)	63	71	0.059	0.500
Delia radicum (M_2)	63	71	0.069	0.463
2008				
Spring wheat				
Oulema melanopus (M_1)	70	71	0.084	0.416
R. padi (M_1)	70	71	0.153	0.264
Zabrus tenebrioides (M_1)	70	71	0.190	0.208
Thrips species (M_1)	70	71	0.380	0.058
Delia coarcata (M_2)	70	71	**0.241**	**0.015**
Chaetocnema aridula (M_2)	70	71	0.000	0.989
Sitodiplosis mosellana (M_2)	70	71	0.092	0.394
Thrips species (M_2)	70	71	0.205	0.189
C. viridis (M_2)	70	71	0.152	0.265
Cephus pigmaeus (M_2)	70	71	0.028	0.643
Phyllotreta vittula (M_2)	70	71	0.011	0.772
Agriotes sputator (M_2)	70	71	0.168	0.239
Haplodiplosis marginata (M_2)	70	71	0.001	0.948
2009				
Oilseed rape				
M. aeneus (M_2)	74	67	0.000	0.963
A. rosae (M_2)	74	67	0.143	0.281
A. proletella (M_2)	74	67	**0.441**	**0.036**
Thrips species (M_2)	74	67	0.136	0.294
M. aeneus (M_1)	74	67	**0.518**	**0.019**
C. viridis (M_2)	74	67	0.058	0.501
D. brassicae (M_2)	74	67	0.000	0.996
D. radicum (M_2)	74	67	**0.429**	**0.040**

CO_2 were observed on wheat (19%, Dijkstra *et al.* 1999), broad beans (14%, Awmack and Harrington 2000) and silver birch (*Betula pendula* Roth), black alder (*Alnus glutinosa* L.) and common beech (*Fagus sylvatica* L.) (17%, Hoosbeek *et al.* 2011), but the aboveground stem biomass of potato (*Solanum tuberosum* L. cv. Bintje) was negatively influenced by CO_2 enrichment (680 $\mu L\,L^{-1}$) at canopy maturity (Högy and Fangmeier 2009). Furthermore, the concentrations of foliar C and N were not significantly changed under elevated CO_2 in our study. In part, the lack of significant responses in the present study may be explained by differences in annual climatic conditions. Plants were supplied with sufficient water and all essential nutrients, which may explain why no effects of the CO_2 fertilisation were found on foliar C and N. In contrast, Cotrufo *et al.* (1998) reviewed that elevated CO_2 significantly altered C and N metabolism, resulting in increased concentration of C and reduced concentration of N in the leaves of C_3 plants.

Changes in plant metabolism under elevated CO_2 may have an impact on pathogen–host relationships. According to Chakraborty and Datta (2003), elevated CO_2 significantly increased the concentration of foliar carbohydrates of *Stylosanthes scabra* Vogel, which, in turn, increased the fecundity of the fungal anthracnose pathogen (*Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc). Those authors suggested that the results could also differ under different climatic conditions. In our study, however, differences in the disease infestation levels on wheat in 2006 and 2008 were not statistically significant for all treatments. The reason for delayed development and spread of powdery mildew infection, and probably also for the absence of CO_2 effects in 2006, may be due to a mild, rainy spring and a hot, dry and sunny summer (Stadtklima Stuttgart 2006). However, the incidence level of various fungal pathogens was higher in 2006 than 2008. In 2008, the development of powdery mildew was accelerated by 10 days in comparison to 2006. In contrast to our results, Hibberd *et al.* (1996) observed that elevated CO_2 (700 $\mu L\,L^{-1}$) significantly inhibited the infestation of powdery mildew on barley (*Hordeum vulgare* L.). In 2007, OSR was not infested by any pathogens during the whole vegetation period; in 2009, the development of downy mildew was especially observed on plants under elevated CO_2. Eastburn *et al.* (2010) reported the opposite effect, namely a significant reduction of disease severity by 39–66% on soybean plants. These contrasting results can be explained by differences in crop species and the crop-specific microclimate. Furthermore, higher precipitation was observed during the growing season in present study, whereas Eastburn *et al.* (2010) associated the reduction in the severity of the disease with drought conditions.

Published literature concerning the effects of CO_2 on plant–pathogen interactions reveals contrasting results. Different pathogens may respond differently under the same climatic conditions, whereas the same pathogen may respond differently to different agronomical growing conditions. Some pathogens, like powdery mildew, are more likely to infest host plants with lower moisture, whereas other diseases tend to thrive in conditions where moisture is increased and temperatures are lower. It was not clear in our study which combination of environmental factors ultimately favoured the pathogens. Therefore, the physiology of host plants and pathogens under both FACE and controlled chamber environments should be observed more detail in future studies in order to better determine the nature of plant–pathogen interactions and CO_2-induced impacts on it.

In the present study, the monitoring of the recorded pests was conducted using two different methods, which helped was to observe both crawling and flying insects. M_2 was more effective than M_1, as it resulted in a wider variety of pest species. Due to the exclusivity of the individual methods of assessment and the incompatibility of the data obtained, with M_2 being suited to monitoring flying insects and M_1 being better suited to crawling

Table 8. Frequency of infestation (FI) and severity of infestation (SI) due to plant pathogens on spring wheat (2006–08) and oilseed rape (2009) in ambient (AMB) and high CO_2 (ELE) treatments

Parameters	Days after sowing	Plant disease									
		Erysiphe graminis		*Puccinia striiformis*		*Puccinia recondita*		*Septoria tritici*		*Peronospora parasitica*	
		AMB	ELE	AMB	ELE	AMB	ELE	AMB	ELE	AMB	ELE
2006											
Spring wheat											
FI (%)	69	0	1	0	2	0	2	–	–	–	–
	76	0	1	2	6	9	7	–	–	–	–
	83	4	5	8	13	8	7	–	–	–	–
	90	17	15	33	25	62	56	–	–	–	–
	97	3	0	96	93	100	98	–	–	–	–
SI (%)	69	0	0	0	0	0.01	0.02	–	–	–	–
	76	0	0.01	0.02	0.09	0.11	0.09	–	–	–	–
	83	0.05	0.12	0.11	0.14	0.12	0.09	–	–	–	–
	90	0.21	0.31	0.46	0.32	0.99	0.94	–	–	–	–
	97	0.04	0	2.42	2.59	4.05	3.95	–	–	–	–
2008											
Spring wheat											
FI (%)	72	14	2	–	–	–	–	–	–	–	–
	79	0	6	–	–	6	2	14	12	–	–
	87	0	12	–	–	10	4	16	12	–	–
	93	–	–	–	–	18	10	36	46	–	–
	103	–	–	–	–	12	2	32	26	–	–
SI (%)	72	0.17	0.02	–	–	–	–	–	–	–	–
	79	0	0.07	–	–	0.17	0.17	0.05	0	–	–
	87	0	0.15	–	–	0.2	0.2	1.12	0.02	–	–
	93	–	–	–	–	0.65	0.67	0.25	0.15	–	–
	103	–	–	–	–	0.65	0.32	0.15	0.02	–	–
2009											
Oilseed rape											
FI (%)	89	–	–	–	–	–	–	–	–	1	2
	94	–	–	–	–	–	–	–	–	0	6
SI (%)	89	–	–	–	–	–	–	–	–	0.01	0.02
	94	–	–	–	–	–	–	–	–	0	0.07

insects, no direct comparison could be made between the two datasets.

Insect species on both crops responded differently to elevated CO_2. Species prevalent on spring wheat in 2008 were beetles, such as *C. aridula*, *O. melanopus*, *A. sputator*, *Z. tenebrioides* and *P. vittula*. These are chewing insects, damaging the plants by causing skeletonisation and mining of leaves, causing an unsightly appearance and a stressed plant, leaving it susceptible to other insects and diseases. In 2007 and 2009, dominant species on the OSR were Diptera like *A. rosae*, *D. brassica* and *D. radicum*, and Hemiptera *A. proletella*.

The Hymenoptera *A. rosae*, the larvae of which skeletonise leaves with their chewing mouthparts, and the *Delia* species as miners are considered serious specialists on cruciferous plants. *Aleyrodes proletella*, a specialist feeding on the phloem of cruciferous plants only, may reach high population densities, dependent on the nutritional quality of the phloem.

In our study, the abundance of some insects was significantly decreased on spring wheat and OSR due to elevated CO_2. The studies of Butler (1985) on the flea beetle (*Chaetocnema ectype* Stephens (Coleoptera: Chrysomelidae)) feeding on *Gossypium hirsutum* L. and of Brooks and Whittaker (1998) on the green dock beetle (*Gastrophysa viridula* De Geer (Coleoptera: Chrysomelidae)) feeding on *Rumex obtusifolius* L. showed significant reductions in populations under elevated CO_2. Vu *et al*. (1989) and Stiling and Cornelissen (2007) showed that myrtle oak (*Quercus myrtifolia* Willd), sand live oak (*Q. geminata* Small), Chapman oak (*Q. chapmanii* Sargent) and Elliott's milk pea (*Galactia elliottii* Nuthall) grown under elevated CO_2 contained higher levels of carbohydrates and decreased amounts of N, reducing the nutritive value for several herbivorous insects. However, the reduction in insect abundance in our study was not significantly correlated with the concentration of foliar C and N under elevated CO_2.

In contrast in some instances, our study revealed significant increases in the abundance of insects on spring wheat and OSR under elevated CO_2. Moreover, the abundance of *A. proletella* and *D. radicum* in 2009 were significantly increased due to elevated CO_2 and related to the concentration of leaf N. According to Long *et al*. (2006), increases in atmospheric CO_2 by the middle of this century are predicted to increase the susceptibility of crops to invasive coleopterans. In agreement,

Hamilton *et al*. (2005) reported increases in the populations of the Japanese beetle, *Popillia japonica* Newman (Coleoptera: Scarabaeidae), on soybean under elevated CO_2.

The chemical composition of plant materials greatly influences the host plant specialisation characteristics of insects, but in our study, it was not clear whether the decreases or increases in the abundance of insects were affected by changes in the nutritional suitability or quality of the host plant. It is possible due to the limited effects of CO_2 concentrations on the C and N content in the leaves, few differences were observed in the abundance of some insects. However, relationships can be seen in the abundances of *D. coarcata* (70 DAS, M_2), *A. proletella* (74 DAS, M_2), *M. aeneus* (74 DAS, M_1) and *D. radicum* (74 DAS, M_2), which were significantly related to the N concentration. It was also suggested that the increases and decreases in the population of insects were a result of microclimatic factors, which, in turn, can be affected by CO_2 enrichment (Franzaring *et al*. 2010). Changes in the canopy climate may affect the development and geographical distribution of insects by overwintering, species-specific reactions, crop–pest synchronisation of phenology and the risk of invasion by migrant pests (Memmott *et al*. 2007). The major variable factors of microclimate are temperature and relative humidity, which influence insect activity. Temperature positively influences the oviposition of some insects (tephritid fly, *Sphenella marginata* (Diptera: Tephritidae)), whereas relative humidity has a negative impact on it (Raghu *et al*. 2004). Nevertheless, the combined effects of CO_2 enrichment and climatic conditions (humidity and temperature) could influence plant–insect interactions. In our study, higher precipitation and soil water content in May and June 2007 in comparison to 2009 resulted in the greater infestation of thrips species (63 DAS, M_2) on OSR, demonstrating that climatic conditions and their interactive effects with CO_2 enrichment deserve further attention. In addition, each individual species of insect may respond differently under different conditions (i.e. the responses are species-specific).

This study showed that elevated CO_2 concentration may have an impact on plants and insect;, however, the connection of climate change to other climate factors should not be neglected in the future.

Conclusions

Our study showed that the effects of elevated CO_2 on plant–disease–insect interactions can be studied under field conditions using Mini-FACE technology using several replicated plots. Plant characteristics (phenological development, aboveground biomass, foliar C and N) and the damage on OSR and spring wheat induced by pathogens were not significantly changed under CO_2 enrichment. In contrast, insect species on both crop species responded to elevated CO_2, a significant reduction (*Delia coarcata*, *Chaetocnema aridula*, *Haplodiplosis marginata*, *Meligethes aeneus*, *Dasyneura brassicae*) as well as a significant increase (*Phyllotreta vittula*, *Athalia rosae*, *Aleyrodes proletella*, *Delia radicum*, thrips species) in their abundance. The strong differences in responses in different years are explained by changes in CO_2 concentration, and microclimatic effects (temperature, humidity, drought) may have been involved as well. Some species of insects were favoured bythe elevated CO_2 concentrations and high humidity, whereas other insects were positively affected by drier conditions. Moreover, different species may respond differently under the same environmental conditions, indicating that the responses to climatic change and CO_2 fertilisation will be species-specific. It is therefore highly advisable to perform further experimentation on this topic in order to elucidate the differences in the effects in among and on different plant species, pathogens and insects under elevated CO_2 by setting up a long-term monitoring and modelling of insect behaviour and their population levels.

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5. Response of spring crops and associated aphids to elevated atmospheric CO_2 concentrations

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Summary

Having evolved a parasitic relation to their host plants, aphids may serve as indicators of plant responses to environmental changes. The present rise in atmospheric CO_2 concentrations is expected to alter plant leaf chemistry and may thus alter host plant – aphid relations. We involved a climate chamber system and used bird cherry-oat aphid (*Rhopalosiphum padi* L.) and green peach aphid (*Myzus persicae* S.) and their respective host plants, spring wheat (*Triticum aestivum* L. cv. "Triso") and oilseed rape (*Brassica napus* cv. "Campino"), to elucidate the effects of atmospheric CO_2 enrichment on such bitrophic systems. Spring wheat grown at elevated CO_2 (600 ppm) generally had greater above ground biomass than plants grown at ambient CO_2 (400 ppm). Bird cherry-oat aphid infestation resulted in reduced spring wheat above ground biomass compared to the non-infested control. Relative crop growth rate (RGR) was increased by elevated CO_2. In our study, the relative developmental stage (rDS) and intrinsic rate of increase (r_m) of the aphids was only slightly and non-significantly increased under elevated atmospheric CO_2 conditions. The response of aphid weight and RGR to elevated CO_2 differed, increasing by 24% and 18.2% for bird cherry-oat aphid and decreasing by 12% and 12.5% for green peach aphid, respectively. Aphids reared on spring wheat at elevated CO_2 had a shorter lifespan, whereas the opposite effect was found for aphids reared on oilseed rape. The average number of nymphs of the two pest species showed both an increase under elevated CO_2. No consistent picture emerges from these findings, and further investigation on host – aphid relations under changing atmospheric conditions such as CO_2 enrichment appear necessary.

Introduction

Atmospheric carbon dioxide (CO_2) concentration has increased from 290 ppm (parts per million) in 1850 to 375 ppm in 2007 (IPCC, 2007) and will continue to rise in the coming decades due to anthropogenic activities. According to current climate scenarios CO_2 concentration will increase up to 450-550 ppm at the middle of this century. Besides indirect impacts due to climate change CO_2 enrichment will directly affect both plants and insects (MASTERS et al., 1998; HUGHES, 2000).

Several effects of CO_2 enrichment on plants have been observed such as an increase in photosynthesis rates, leaf area, dry weight and other growth characteristics (OWENSBY et al., 1999). Many studies have shown an increase in plant growth in elevated compared to ambient CO_2 (NORBY et al., 1999; LONG et al., 2004; AINSWORTH and LONG, 2005). In earlier work when a doubling of atmospheric CO_2 was considered, CURE and ACOCK (1986) reported an increase in yield by 41% on average after assembling yield data for 10 major crops (leaf, grain, tuber and fiber). Corresponding results were obtained by AMTHOR (2001) who estimated an increase in wheat grain mass by 31% on average based on wheat yield data from 50 publications. In more recent work involving FACE technology (Free Air Carbon dioxide Enrichment) at only ca. 200 ppm above ambient instead of doubling, elevated CO_2 increased aboveground biomass by 12% and grain yield by 10-15% in wheat (KIMBALL et al., 2002a). For oilseed rape, only few data are available on yield and growth response to CO_2 enrichment. According to FRANZARING et al. (2008b), shoot biomass of summer oilseed rape tended to be 20% greater and seed output increased by approximately 17% under elevated CO_2. In this study, plant height and the dry weight of reproductive organs was also significantly increased under elevated CO_2, indicating a speeding up of plant development. The significant increase in the dry weights of senescent leaves in plant specimens from the elevated CO_2 treatment strongly suggests that plant phenology is also affected.

It was also revealed that elevated CO_2 influences the primary and secondary metabolism of plants (PENUELAS and ESTIARTE, 1998). Many studies have shown changes in foliar sugars, starch and increases in concentrations of carbon based secondary structural compounds due to elevated CO_2 (PENUELAS and ESTIARTE, 1998; STILING et al., 1999). The foliar nitrogen content in plants grown under increased CO_2 was often reported to be reduced by up to 15% (COTRUFO, 1998; HEAGLE et al., 2002).

The rise in CO_2 can thus indirectly affect herbivores by biochemically altering the nutritive value of the host plants. The increase in the carbon:nitrogen ratio in host plants generally decreases the nutritive quality for some feeding guilds of pests (e.g. phloem-feeders, leaf

miners, xylem-feeders, seed-eaters, whole-cell-feeders and leaf-chewers), leading to an increase in their food consumption rates in order to compensate for the reduced quality (SALT et al., 1995; MARKS and LINCOLN, 1996; BEZEMER and JONES, 1998). The increased siphoning from phloem-feeders in turn causes a massive reduction in host plant assimilates (WATT et al., 1995). The change in the allocation patterns of compounds and the chemical composition of plant tissues indirectly affects the food ecology of phytophagous insects (HUNTER, 2001).

RHODES et al. (1996) have shown that phloem-feeding aphids use amino acids for their protein metabolism, and carbohydrates for energy. The phloem of plants contains high amounts of carbohydrates (0.8-1.8 M), small amounts of amino acids (60-200 mM) and very few lipids (KLINGAUF, 1987; DILLWITH et al., 1993; SANDSTÖM and MORAN, 2001; WILKINSON and DOUGLAS, 2003; DOUGLAS, 2006). In order to obtain the necessary amounts of amino acids required for growth, aphids thus consume considerable amounts of carbohydrates from the phloem. Improved food quality of a host plant with respect to aphids expresses itself in a higher amino acid to carbohydrate ratio within the phloem (MITTLER and MEIKLE, 1991). Elevated CO_2 may change the concentrations of some individual amino acids in the phloem sap, thereby affecting the performance of aphids. A study of DOCHERTY et al. (1997) proved that reduction of total amino acid concentration in phloem sap was 31% at elevated CO_2.

The reduction in food quality due to elevated CO_2 also impacts the behaviour and physiology of leaf miner insects (STILING and CORNELISSEN, 2007). Many species of herbivorous insects tend to show altered behaviour and characteristics under CO_2 enrichment. The consequences differ between species and include retarded growth rates, increased nymphal development times and higher mortality rates (LINDROTH et al., 1993; SMITH and JONES 1998; COVIELLA and TRUBLE, 1999; GOVERDE and ERHARDT, 2003). In contrast, some studies concluded that the development time of phloem-feeding insects may be reduced by 17%, and that adult weight, relative growth rate (RGR) and population size may actually increase due to elevated CO_2 (BEZEMER and JONES, 1998; NEWMAN et al., 2003).

In this paper, we investigated the responses of host plants to elevated CO_2 in order to observe the indirect effects on phloem feeding insects. The experiment was carried out with bird cherry-oat aphids (*Rhopalosiphum padi* L.) on spring wheat (*Triticum aestivum* L. cv. "Triso") and with green peach aphid (*Myzus persicae* S.) on oilseed rape (*Brassica napus* cv. "Campino"). Determining the effects on these sap-feeding insects is very important for agriculture. *Myzus persicae* causes both direct (leaf curling) and indirect damage of plants

(transmission of plant viruses such as lettuce mosaic virus (LMV) and cucumber virus I) (NAMBA and SYLVESTER, 1981). *Myzus persicae* can achieve very high population densities on plant tissue, retarding plant growth rate and thereby causing a perceptible reduction in yield of root and foliage crops (PETITT and SMILOWITZ, 1982). *Rhopalosiphum padi* in turn causes a significant decrease in yield on cereal crops via feeding damage, resulting in a reduction of kernel amount and mass. Kernel amount was reduced by 36-50% in winter wheat, 24-48% in rye, 41-60% in barley and 41-63% in winter oats. The reduction of thousand kernel weight was 33-65% in winter wheat, 13-26% in rye, 25-47% in winter barley and 43-75% in winter oats (KUROLI, 2009).

Other researchers have conducted experiments on the indirect effects of elevated CO_2 on *Myzus persicae* feeding on *Brassica napus* (HIMANEN et al., 2008) and on *Solanum dulcamara* (HUGHES and BAZZAZ, 2001), but the growth parameters of aphids were not taken into account. Review of literature showed that the relative growth rate of aphids may be increased under elevated CO_2. However, these observations were carried out with other species of aphid as *Aulacorthum solani* (AWMACK et al., 1997) and *Sitobion avenae* (CHEN and WU, 2006) on host plants such as *Vicia faba*, where the relative growth rate of *Sitobion avenae* was increased by 33% at 550 ppm CO_2 and by 74% at 750 ppm CO_2.

Unfortunately insect response to elevated CO_2 differs between host plants and aphid species (BEZEMER et al., 1998). It is thus necessary to observe specific species of aphids on specific host plants. For the first time, in this study the development of *R. padi* on spring wheat and development of *M. persicae* on oilseed rape from the nymph to the adult stage under elevated CO_2 was observed, making record of the relative developmental stage, population growth rate and relative growth rate of the aphids.

Materials and methods

Cultivation of plants and experimental conditions

The experiment was carried out on spring wheat (*T. aestivum* L. cv. "Triso") from 16 June to 13 August 2008 and on oilseed rape (*Brassica napus* cv. "Campino") from 27 May to 17 August 2009 at the Institute for Landscape and Plant Ecology of Hohenheim University, Germany. A pot experiment was conducted in six controlled-environment chambers (Vötsch Bioline ®) with two levels of CO_2 (ambient, 400 ppm and elevated, 600 ppm). Seeds of spring wheat and oilseed rape were sown in pots (Ø 18 cm) with a mixture of substrate (Fruhstorfer Erde Typ LD 80, Industrie-Erdenwerk Archut, Lauterbach, Germany) and sand (9:1). Germination took place at 22 ± 2°C, 80% relative humidity and 18:6 hour L: D

photoperiod. Out of the sixteen host plants in each chamber, ten were chosen for aphid infestation and six for plant analysis. Plants were grown having a photoperiod of 18 h, photosynthetic photon flux density (PPFD) of approximately 520 µmol $m^{-1}s^{-1}$, a day/night temperature of 22/12°C, irrigated daily with 50 ml water and fertilized weekly using 50 ml of 0.3% nutrient solution (Wuxal ®, Aglukon GmbH). Host plants and climate profiles were rotated weekly between chambers in order to ensure results were not chamber specific. Further chamber characteristics are given in details in FRANZARING et al. (2008a).

Biomass production and plant phenology

In order to determine the aboveground biomass of plants at ambient and elevated CO_2, spring wheat and oilseed rape were harvested at growth stages 12 and 30 (BBCH code) according to ZADOKS et al. (1974) and WEBER and BLEIHOLDER (1990), respectively, dried at 105°C to constant weight and then weighed on a balance (Sartorius analytics A 120 S). Subsequently, relative growth rate (RGR, HUNT, 1982) of the plants was calculated using equation (1). Since in any experiment start weight was similar, we did not refer to start weight as required by HUNT (1982).

(1) $RGR = (1n\ W_2 - 1n\ W_1) / t_2 - t_1$

where W_1 is the dry weight (DW) at start of the experiment (t_1), W_2 is the final DW at the end of the experiment (t_2), and $t_2 - t_1$ is the time (days) elapsed between the weighing.

Cultivation of aphids

In order to infest the experimental plants with similar aged aphids, synchronized colonies of *R. padi* and *M. persicae* were established. A synchronised long-term cultivation was carried out in greenhouse at 20 ± 1°C, relative humidity 60-70%, a lighting duration of 16 h and PPFD of approximately 22.5 µmol m^{-2} s^{-1}. Then the synchronised adult, female apterous aphids were placed on plants, grown in climate chambers under two levels of CO_2 to produce progeny. Petri dishes that had been converted into small plexiglass cages (Ø 3.5 cm) and attached with clip on the second leaf of each plant (BBCH code 12) were used for aphid rearing. After five hours, female aphids were removed and five newly born nymphs (L_1) were allowed to develop until they reach late-nymphal instars in order to determine the relative developmental stages (rDS), developmental time and preimaginal mortality. The cages nymphs were observed daily. To assess longevity of adults and reproduction, one of the five aphids per cage after adult moult was put separately in a cage on a young leaf and observed until death. Nymphs deposited per female were counted and removed with a paintbrush daily. Excess freshly born nymphs and adult pre-reproductive aphids were weighed to determine body size and relative growth rate (RGR).

Determination of aphids' growth parameters

The development of *R. padi* and *M. persicae* was observed and counted daily from start of the experiments until entering the adult stage. To depict any indirect effect of elevated CO_2 into aphid development, the relative developmental stage (rDS), implemented to show the effects if insect growth regulators (ZEBITZ, 1984), was calculated after daily counting and subsequential removal of exuviates of nymphs using equation (2):

(2) $rDS = \sum (n_t S_p \cdot F_p) / N_t S$

where $n_t S_p$ is the number of individuals per development stage at time t, F_p the multiplication factor of relevant development stage (nymphal stages 1-4, adult stage 5) and $N_t S$ the total number of individuals per cage.

The intrinsic rate of increase (r_m, WYATT and WHITE, 1977) of *R. padi* and *M. persicae* were calculated from the number of offspring per female after one generation time using the following equation:

(3) $r_m = (0.754 (\ln M_d)) / d$

where M_d is the number of offspring per generation time and d is the generation time (days).

In order to determine the relative growth rate (RGR, HOWARD and DIXON, 1995) of *R. padi* and *M. persicae*, weights of single adults were measured using a precision balance (Sartorius analytic 4504 MP8) and calculated following equation (1).

Statistical analyses

The effects of elevated CO_2 concentration on growth parameters of *R. padi* and *M. persicae* (e.g. nymphs and adult weight as well as the relative growth rate of aphids) were tested using analysis of variance (ANOVA, Visual-XSel® 9.0/ DoE & Weibull). The combined effect of CO_2 elevation and aphids on plant above ground biomass and relative growth rate were analysed by ANOVA with CO_2 treatment and aphid infestation as independent variables. Treatment means were compared by means of LSD-test. Comparison of relative development stages of aphids was done applicating the Kruskal-Wallis-Test. As the fecundity was not normally distributed, treatments were analysed using the non-parametric Mann-Whitney U-test. The suitable statistical test methods were chosen according to KÖHLER et al. (2002).

Results

Plant biomass and phenology

In 2008, the phenology of spring wheat was determined from leaf development (9 DAS, days after sowing) until stem elongation (57 DAS). The results suggest that plant development was

not significantly altered due to elevated CO_2 during these developmental stages (data not shown).

Spring wheat grown under elevated CO_2 significantly increased above ground biomass by 41% as biomass was 7.25 ± 0.24 g DW at 400 ppm and 10.19 ± 0.058 g DW at 600 ppm when the plants were not infested with aphids (Tab. 1). This CO_2-induced increase was even higher (+ 48%) in spring wheat infested with *R. padi*, above ground biomass being 6.25 ± 0.071 g DW at 400 ppm and 9.27 ± 0.259 g DW at 600 ppm**.** As expected, the infestation by *R. padi* impacted plant above ground biomass negatively, reducing it by 14% at 400 ppm CO_2 and by 9.1% at 600 ppm CO_2. However, no statistically significant interactions between CO_2 enrichment and aphid infestation on wheat above ground biomass were detected. The relative growth rate (RGR) of *T. aestivum* was significantly increased due to elevated CO_2 (on average by 19%) and significantly reduced when the plants were infested with aphids (on average by 6.1%). There was a slightly higher depression of wheat RGR due to aphid infestation at ambient compared to elevated CO_2 (7.9 vs. 4.6%), however, these CO_2 by aphid interactions were below statistical significance.

In 2009, the phenology of oilseed rape under CO_2 enrichments was determined during leaf development (from 12 until 78 DAS). Plant development was not significantly altered due to elevated CO_2 (data not shown).

Effects of CO_2 enrichment and presence of aphids on oilseed rape above ground biomass and RGR were consistently below statistical significance because of large variation between replicates. Correspondingly, no significant interactions between CO_2 enrichment and aphid treatment could be detected. Nevertheless, elevated CO_2 tended to increase rape RGR (on average by 34% across both aphid treatments) (Tab. 1).

Effect of elevated CO_2 on aphid performance

Elevated CO_2 concentrations resulted in several changes of growth parameters of bird cherry-oat and green peach aphids. However, the relative developmental stage (rDS) of the aphids remained almost unaffected in enhanced CO_2 environments (Tab. 2).

The comparison of average imaginal weight of *R. padi* and *M. persicae* before the nymph reproduction and RGR of aphids clearly revealed a CO_2 effect (Tab. 3). Average weight of *R. padi* imago was 570.6 ± 15.8 µg FW at ambient CO_2 and 707.2 ± 34.0 µg FW at elevated CO_2 treatment which means a significant increase by 24%. On the other hand, average weight of *M. persicae* imago decreased significantly from 416.5 ± 17.2 µg at ambient CO_2 to 366.5 ± 1.1 µg at elevated CO_2 which corresponds to a decrease by 12% due to elevated CO_2. The RGR of *R. padi* feeding on wheat achieved 0.11 ± 0.003 at ambient CO_2 and 0.13 ± 0.01 at

600 ppm CO_2. RGR of *R. padi* was higher than RGR of *M. persicae* on oilseed rape, the latter which achieved 0.08 ± 0.00 at 400 ppm CO_2 and 0.07 ± 0.00 at 600 ppm CO_2. Thus, CO_2 enrichment increased the RGR of *R. padi* by 18.2%, while it decreased the RGR of *M. persicae* by 12.5%.

R. padi lifespan was slightly shorted under elevated CO_2 concentration, although this effect was not significant. The lifespan was 39.0 days (ambient) and 39.3 days (elevated CO_2). In contrast, lifespan of *M. persicae* was slightly prolonged by 2.1 days.

Elevated CO_2 not also affected growth but also reproductive characteristics of aphids. The intrinsic rate of increase (r_m) of both aphids was slightly but not significantly higher under elevated CO_2. The average number of *R. padi* nymphs per female in plants grown under elevated CO_2 was increased by 6.0%, although this was not significant. The respective values were 69.2 ± 8.7 nymphs in ambient and 73.3 ± 12.4 nymphs under elevated CO_2. The average number of *M. persicae* nymphs per female in plants grown under elevated CO_2 was increased by 3.5%. The respective values were 59.3 ± 7.8 nymphs in ambient and 61.4 ± 9.5 nymphs under elevated CO_2. In order to establish the frequency with which the female aphids reproduced under normal and CO_2 enriched conditions, the daily appearance of nymphs was recorded. During reproduction, the number of *R. padi* nymphs increased, peaking on day nine. Afterwards, it tapered off, the last nymph produced on day 20 (Fig. 1). Significant CO_2 effects were found on days 5 to 7 and on days 13 and 14.

Regarding *M. persicae*, the number of nymphs increased during the first sixteen days, after which it declined, the last nymph produced on day 32 (Fig. 2). A significant CO_2 effect was found on day 21.

Discussion

According to the current predictions, plants and insects will be influenced due to increasing atmospheric CO_2. The responses of plants and aphids to these changes in our research corresponded partially with predictions. The experiment in controlled-environment chambers was established in order to understand the positive or negative impacts of CO_2 enrichment on agricultural crops and phloem feeding aphids such as *R. padi* and *M. persicae*.

Our observations showed that the phenology of spring wheat and oilseed rape was not significantly altered due to elevated CO_2. SLAFER and RAWSON (1997) have argued that elevated CO_2 has no effect on growth and leaf development in wheat. However, FRANZARING et al. (2008b) suggested that phenological development of oilseed rape was significantly enhanced under elevated CO_2. Slight phenology acceleration under rising CO_2 was also found

by KIMBALL et al., (2002b) on spring wheat. In our experiment, above ground biomass of spring wheat was increased by 41% due to elevated CO_2. This supports earlier findings on the fertilizing effects of CO_2 enrichment on C_3 plants (e.g. KÖRNER, 1991; TAYLOR et al., 1994) and is well in agreement with POORTER (1993) who surveyed literature (156 plant species) and found that with a doubling in atmospheric CO_2 plant biomass during vegetative growth was increased on average by 37% for C_3 crops. Correspondingly, the RGR of spring wheat was increased by 17% under elevated CO_2 in our study. Similarly, FLYNN et al. (2006) investigated potted plants (*Solanum dulcamara)* in glass-topped chambers under two conditions of atmospheric CO_2 concentration (350/ 750 ppm) and confirmed enhancement of RGR due to elevated CO_2.

Increase of CO_2 led to significant gain in plant above ground biomass, while the presence of aphids reduced the above ground biomass of spring wheat in both ambient and enriched CO_2 environments. Our results showed that infestation with *R. padi* caused significant reductions in wheat biomass of 14% and 9.1% at 400 ppm and 600 ppm, respectively. However, no significant effects were found when oilseed rape was infested with *M. persicae*. HUGHES and BAZZAZ (2001) proved that out of five aphid species (*Acyrthosiphon pisum, Aphis nerii, Aphis oenotherae, Aulacorthum solani* and *Myzus persicae*) grown on five host plants (*Vicia faba, Asclepias syriaca, Oenothera biennis, Nicotiana sylvestris* and *Solanum dulcamara*) only *Aphis nerii* had significantly negative effects on the biomass of *Asclepias syriaca* at both ambient and elevated CO_2. The interaction between CO_2 and aphid presence on above ground biomass and RGR was insignificant for spring wheat and oilseed rape in our study. However, HUGHES and BAZZAZ (2001) suggested that there was highly significant interaction between CO_2 and presence of two species of aphid (*Myzus persicae* and *Aphis nerii*) on above ground biomass of *Asclepias syriaca* and *Solanum dulcamara*.

Regarding our findings on CO_2 effects on aphids, *R. padi* showed an increase in weight of 24% and RGR of 18.2% in the high-CO_2 treatment. Similar results were obtained by BEZEMER and JONES (1998), supporting the theory that insects perform better when feeding on plants grown under CO_2 enrichment. According to AWMACK et al. (1997), the aphid *Aulacorthum solani* (Homoptera: Aphididae) reared on bean (*Vicia faba*) and tansy (*Tanacetum vulgare*) also responded to elevated CO_2 conditions with increased growth. However, FLYNN et al. (2006) adduced evidence that CO_2 did not significantly affect the weight of aphids (*Macrosiphum euphorbiae* Thomas). Other studies concluded that CO_2 enrichment can negatively affect insect weight (JOHNS and HUGHES, 2002; ROTH and LINDROTH, 1995) and RGR of leaf-miner pests, reducing RGR by 8.3% (STILING and

CORNELISSEN, 2007). In agreement, *M. persicae* showed decreased aphid weights by 12% and RGR by 12.5% under CO_2 enrichment in our study. In accordance with BALE et al. (2002) the decrease in weights reflect accelerated plant development due to global climate changes (increase of CO_2 or temperature), which decrease the amount of feeding time available to the aphids.

We observed that the rDS and r_m of aphids was only slightly and non-significantly increased under rising atmospheric CO_2 conditions. Our study showed that the fecundities of *R. padi* and *M. persicae* feeding on plants grown at elevated CO_2 increased by 6.0% and 3.5%, respectively. In contrast, TRAW et al. (1996) reported reduced fecundity of insects. Additionally, WILLIAMS et al. (2003) concluded that elevated CO_2 has no impact on fecundity of phloem feeding insects. According to LINCOLN et al. (1993), CO_2-induced alterations in phytochemical constituents important to insects can potentially alter their behaviours.

In our study, the duration of aphids' life was prolonged by an average of 2.2 days for *R. padi* and shortened by an average of 0.3 days for *M. persicae* under elevated CO_2 concentration. COVIELLA and TRUBLE (1999) concluded that aphid's lifespan is likely to be extended under elevated CO_2.

Overall, climate change will impact plants and insects. CO_2 enrichment can have dramatic consequences for plants due to acceleration of phenological development, changes in phytochemical, biochemical and biosynthetic processes, which in turn may alter future phytophagous insect populations, behaviour, performance and feeding habits. However, from the work published so far, no clear systematic rules on the mode of action and the direction of responses can be derived; rather, experimental results appear to depend on the particular organisms investigated and the experimental conditions applied in the respective studies. Thus, further studies in this area are highly recommended.

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Tab. 1 Above ground biomass [g pot^{-1}] and relative growth rate (RGR) of spring wheat and oilseed rape under ambient or elevated CO_2 concentration and with or without aphid colonization shortly before stem elongation stage. Values represent treatment average ± standard error from three replicate climate chambers, respectively.

Plant species / plant trait	ambient CO_2, without aphids	elevated CO_2, without aphids	ambient CO_2, with aphids	elevated CO_2, with aphids	Significance of treatment effects (F-test)		
					CO_2	aphids	CO_2*aphids
Wheat biomass	7.25 ± 0.24 ^{A}	10.19 ± 0.058 ^{B}	6.25 ± 0.071 ^{C}	9.27 ± 0.259 ^{D}	< 0.001	0.001	ns
Wheat RGR	1.99 ± 0.033 ^{A}	2.34 ± 0.008 ^{B}	1.84 ± 0.011 ^{C}	2.23 ± 0.024 ^{D}	< 0.001	< 0.001	ns
Rape biomass	4.66 ± 2.10 ^{A}	6.72 ± 1.35 ^{A}	5.19 ± 0.642 ^{A}	6.07 ± 0.660 ^{A}	ns	ns	ns
Rape RGR	1.32 ± 0.435 ^{A}	1.94 ± 0.136 ^{A}	1.51 ± 0.003 ^{A}	1.85 ± 0.001 ^{A}	ns	ns	ns

Different letters in superscript within one row indicate significantly different treatment means at $P < 0.05$ (LSD-test), ns is not significant

Tab. 2 Relative development stages of *R. padi* and *M. persicae* from first nymphal instar to apterous virgo. Columns 1-9 (*R. padi*) or 1-10 (*M. persicae*) refer to days after leaving five instar nymphs in the cages.

CO_2 treatment	rDS of *Rhopalosiphum padi* (from L_1 to apterous virgo) [days]								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
400 ppm	1.0	1.7	2.2	2.7	3.1	3.7	4.3	4.7	5.0
600 ppm	1.0	1.8	2.1	2.6	3.1	3.7	4.4	4.8	5.0

	rDS of *Myzus persicae* (from L_1 to apterous virgo) [days]									
400 ppm	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
	1.0	1.1	1.7	2.1	2.5	2.9	3.2	3.9	4.3	5.0
600 ppm	1.0	1.2	1.8	2.1	2.5	3.0	3.4	4.0	4.4	5.0

Tab. 3 Mean imaginal weight (IW), relative growth rate (RGR), increase rate (r_m-values) and mean adult longevity of *R. padi* and *M. persicae* under ambient and enhanced CO_2 conditions.

Parameters	Ambient CO_2	Elevated CO_2	*P* values (ANOVA)
	Rhopalosiphum padi		
Imaginal weight (IW) [µg] [1]	570.6 ± 15.8	707.2 ± 34.0	0.01
Relative growth rate (RGR) [µg/µg/day] [1]	0.11 ± 0.003	0.13 ± 0.01	0.01
Increase rate r_m [d^{-1}] [2]	0.354 ± 0.01	0.358 ± 0.015	ns
Duration of life [days] [2]	39.3 ± 3.2	39.0 ± 3.5	ns
	Myzus persicae		
Imaginal weight (IW) [µg] [1]	416.5 ± 17.2	366.5 ± 1.1	0.001
Relative growth rate (RGR) [µg/µg/day] [1]	0.08 ± 0.00	0.07 ± 0.00	0.001
Increase rate r_m [d^{-1}] [2]	0.30 ± 0.01	0.31 ± 0.01	ns
Duration of life [days] [2]	39.0 ± 6.5	41.1 ± 9.2	ns

[1] n = 50, [2] n = 30, ns, not significant

Fig. 1 Daily average number of *Rhopalosiphum padi* nymphs per treatment (n = 30). Asterisks indicate significant CO_2 effects according to the Mann-Whitney U-test (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

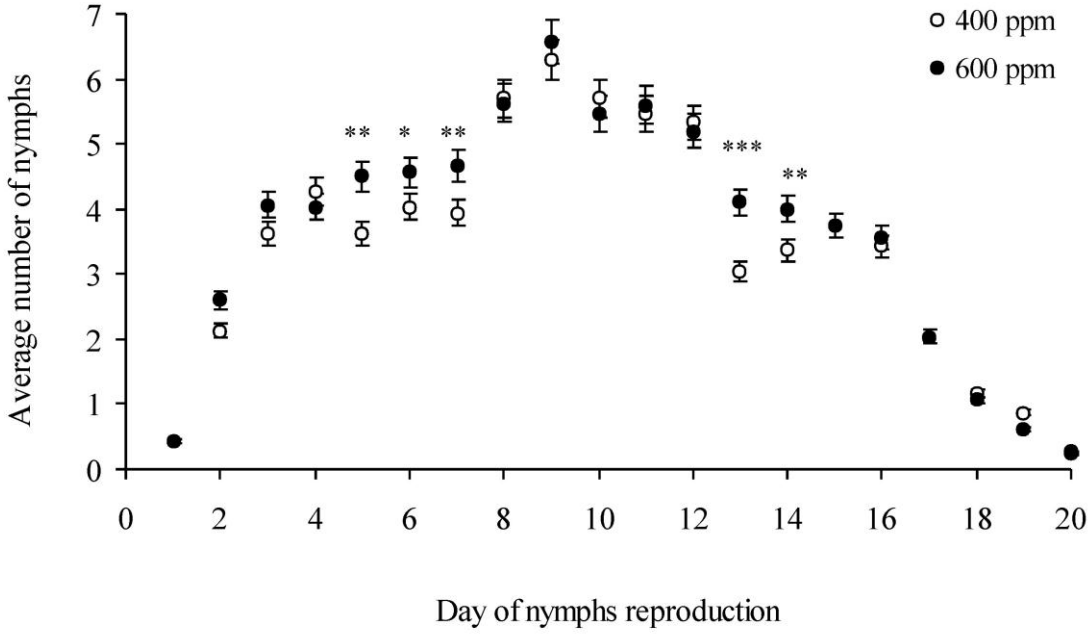
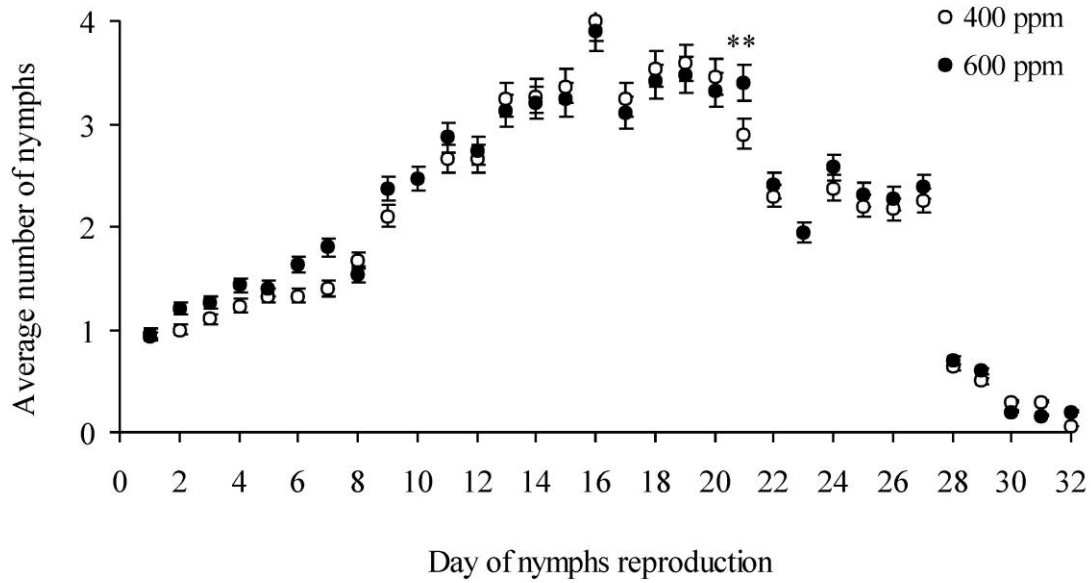


Fig. 2 Daily average number of *Myzus persicae* nymphs per treatment (n = 30). Asterisks indicate significant CO_2 effects according to the Mann-Whitney U-test (** $p < 0.01$).



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

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Abstract

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. In spring wheat, CO_2-induced significant increases were observed for the concentrations of lysine, leucine, isoleucine, phenylalanine, valine, tyrosineserine, alanine, threonine, glutamic acid and asparagine at leaf development stage (LDS) and for arginine, γ-aminobutyric acid, leucine and alanine at stem elongation stage (SES). The concentration of ornithine was significantly decreased in wheat (LDS) and in OSR (SES). Among concentrations of carbohydrates in the phloem sap, significant increases were observed for fructose and glucose in spring wheat under CO_2 enrichment, while no changes were observed in OSR. The concentrations of individual amino acids and carbohydrates in the phloem sap affected the relative growth rate (RGR) of aphids.

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

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 and Navas 2003). CO_2 enrichment has been shown to promote aboveground biomass by 12% in spring wheat (Högy et al. 2009) and by 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 et al. 2008a). Accordingly, the plant metabolism is also altered in wheat and OSR, leading to changes in the composition of generative plant parts (Högy and Fangmeier 2008; Högy et al. 2009; Högy et al. 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 and 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 and Ge 2010).

In general, the phloem sap of plants contains high concentrations of carbohydrates (800-1,800 mM) and relatively low concentrations of minerals and amino acids (60-200 mM) (Klingauf 1987; Sandström and Moran 2001; Douglas 2006). Host plants of high food quality are characterised by a rise in the ratio of amino acids to carbohydrates (Mittler and 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 in negative impacts on phloem-feeding insects due to limitations in nitrogen supply (Awmack and Leather 2002; Stiling and 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 and Lincoln 1996; Sun 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 and Douglas 2003), which determines their reproductive capability and abundance (Mittler and 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 and Bashford 1971; Sandström and Moran 1999, 2001). While *Myzus persicae* needs Met and γ-amino butyric acid (GABA), the amino acids Thr, His and alanine (Ala) are important for *Rhopalosiphum padi* (Kazemi and 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) 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 be also 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 and 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% at 800 µl l^{-1} CO_2, respectively (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 µl 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 µl l^{-1}). In both cases, it revealed a close relationship between aphid population size and nutrient availability.

The aim of this study was to analyse 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 analysed 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.

Materials and methods

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. Controlled-environment chambers (Vötsch Bioline ®) operated either at 400 µl l^{-1} CO_2 (ambient) or 600 µl l^{-1} CO_2 (elevated) with three replicates each were used. Seeds were sown in pots (Ø 18 cm, 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 levels during the 18 hours light were 1,100 µmol $m^{-2}s^{-1}$ as photosynthetically active radiation. Out of the sixteen host plants in each chamber, ten were chosen for aphid infestation and six 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 et al. (2008b).

Aphid rearing and growth parameters

In order to introduce aphids in a synchronised 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. After the synchronisation aphids were placed for five hours on spring wheat (BBCH stage 12, Zadoks et al. 1974) and OSR (BBCH stage 14, Weber and Bleiholder 1990) to produce larvae. Afterwards, adult aphids were removed and only five newly born larvae (L_1) remained in the cages. 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).

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 (BBCH12, 14) and 44 and 48 days after infestation (BBCH 30), respectively. 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 three hours, plants were removed from the vials and the phloem sap fraction was frozen at -25^{o}C until chemical analyses (Hellwald 1989).

Carbohydrate analysis

In the phloem sap, the concentrations of sucrose, glucose and fructose were analysed by high–performance liquid chromatography (HPLC) using a Perkin Elmer Pump on a Shodex Asahipak NH_2 P-50 column (5 µm, 250 x 4.6 mm) at 30^{o}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^{o}C.

Amino acid analysis

In order to define the composition of amino acids, exudates were analysed by HPLC using a fluorescence detector (Jasco FP-1520.S). The lyophilised 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 derivatisation 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 x 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}.

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 analysed 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.

Results

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.

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).**

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).** 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 concentrations were non-significantly increased under elevated CO_2 (data not shown). 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.

Correlation between RGR of aphids and carbohydrates and total amino acids

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 1).** 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.

Correlation of aphid RGR with 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 2).** 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 3).**

Discussion

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 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* L. Merr.) and broccoli (*Brassica oleracea* var. "Italica") by 8.4% and about 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*) showed a total decrease in soluble carbohydrates by 52%, of which a proportion of 49% derived from sucrose.

CO_2 enrichment indirectly affects the performance of aphids (development time, RGR, survival, fecundity) through direct effects on chemical composition of host plants. In the present study, high-CO_2 treatment significantly decreased the RGR of *M. persicae*. Nevertheless, no relationship was found between the aphid RGR and the concentration of sucrose in the phloem sap of OSR under elevated CO_2. On the contrary, Douglas et al. (2006) observed that the RGR of pea aphid (*Acyrthosiphon pisum*) was significantly related to sucrose concentration in the diet.

In spring wheat, the RGR of *R. padi* was significantly increased under elevated CO_2. A significant relationship was also found between the aphid RGR and the fructose concentration (BBCH 12) under elevated CO_2. On the contrary, Watt et al. (1995) argued that the herbivorous insect responded to increased levels of CO_2 by reducing their growth rates.

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* L. Merr. cv. Clark) 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% to 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. 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 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 µl 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 and Pettersson 1994). According to Karley et al. (2002), the correlation between RGR of two aphid species (*Myzus persicae* and *Macrosiphum euphorbiae*) 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 and 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 above.

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 *A. pseudoplantanus* 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* 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 it was confirmed that elevated CO_2 may alter the concentration of carbohydrates and amino acids in the phloem sap of spring wheat and OSR. In general, the concentration of carbohydrates (sucrose), as well as the concentrations of total and the individual amino acids, except for Orn, in OSR, were not significantly changed under high-CO_2 conditions. In spring wheat, however, carbohydrates (glucose, fructose) and several individual amino acids showed significant increases. The changes in the phloem of host plants affected the nutritional quality for phloem-feeding herbivores, resulting in an increase in the RGR of *R. padi* and a decrease in the RGR of *M. persicae*.

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Table 1 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.

CO_2 treatment	Development stage of plants	Carbohydrates			Total amino acids
		Sucrose	Fructose	Glucose	
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 2 Correlations of RGR (*R. padi*) and individual amino acid concentrations in spring wheat (essential amino acids (Dadd, 1985) are given in bold characters).

Amino acids	Ambient CO_2				Elevated CO_2			
	BBCH 12		BBCH 30		BBCH 12		BBCH 30	
	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 3 Correlations of RGR of *M. persicae* and individual amino acid concentrations in oilseed rape (essential amino acids (Dadd, 1985) are given in bold characters).

Amino acids	Ambient CO_2				Elevated CO_2			
	BBCH 14		BBCH 30		BBCH 14		BBCH 30	
	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.

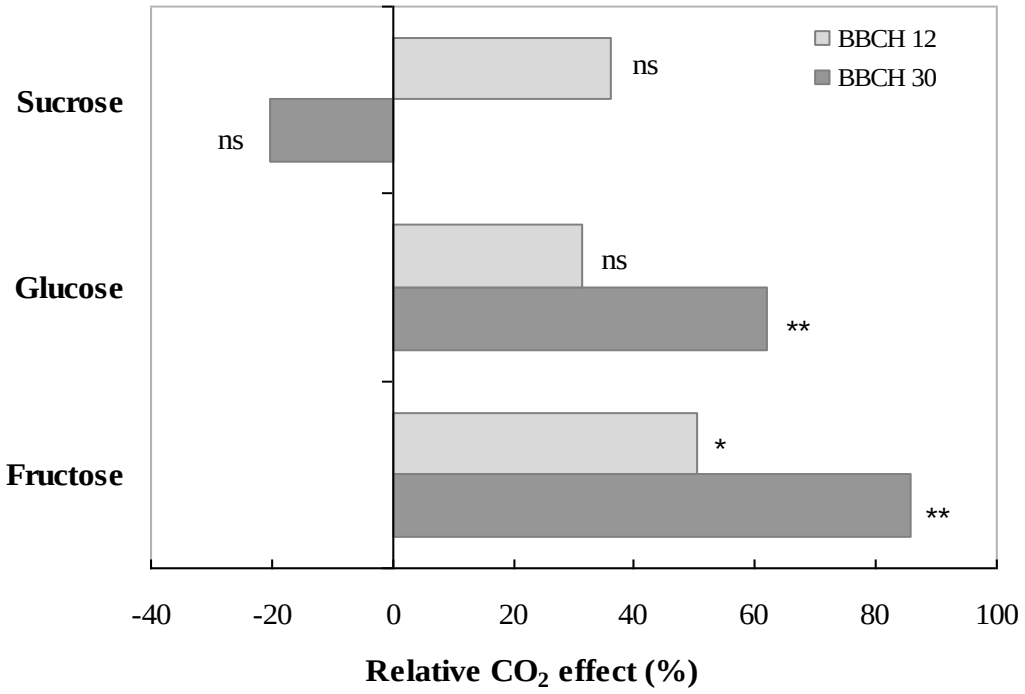

Figure 1 **CO_2-induced changes (ambient = 100) of sucrose, glucose and fructose concentrations in phloem sap of spring wheat at leaf development (BBCH 12) and stem elongation (BBCH 30) stages in 2008. The results of the ANOVA are denoted by asterisks (^{ns} $p > 0.05$, * $p < 0.05$, ** $p < 0.01$).**

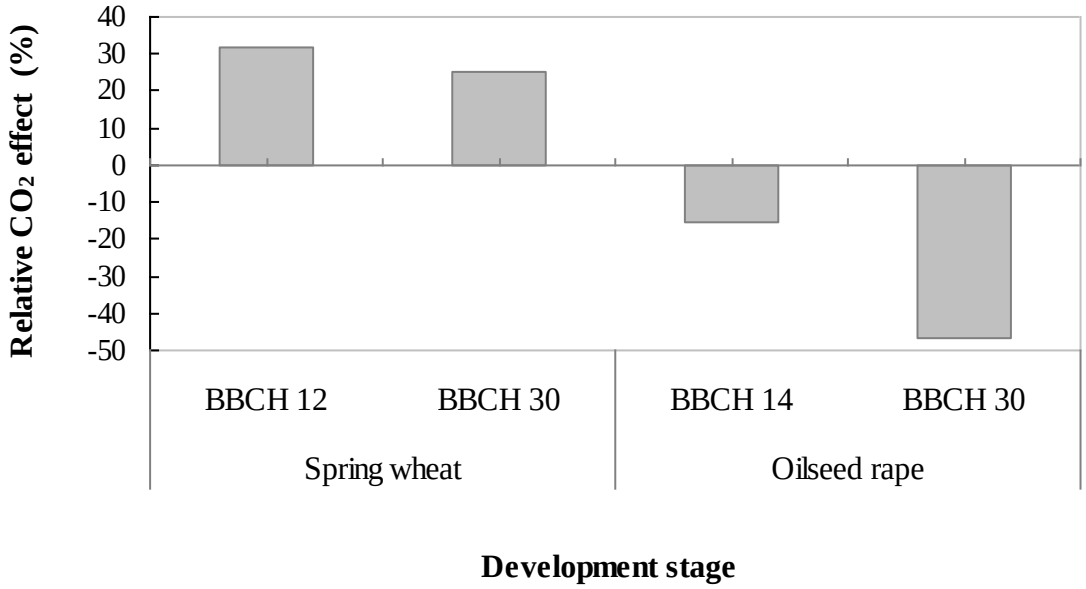

Figure 2 Relative CO_2 effects (%; elevated *versus* ambient; ambient = 100) on the concentration of total amino acids in the phloem sap of spring wheat and oilseed rape at leaf development and stem elongation stages.

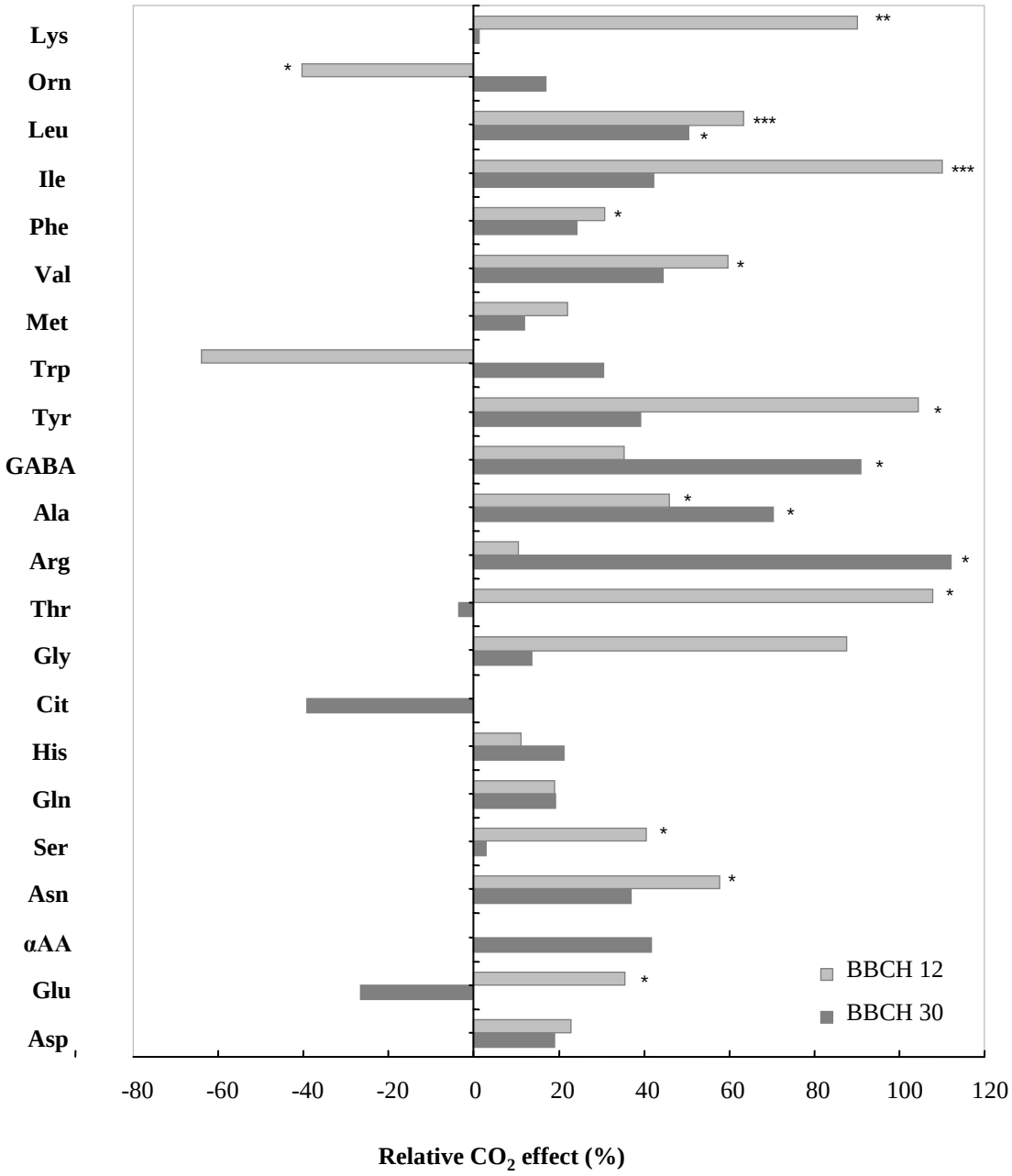

Figure 3 Changes in amino acid concentrations of phloem sap of spring wheat at leaf development (BBCH 12) and stem elongation (BBCH 30) stages under elevated CO_2 (ambient = 100). Given are the mean value and the standard deviation of three replicates. The results of the ANOVA are denoted by asterisks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

7. General Discussion

Current predictions say that plants, herbivore insects and pathogens will be affected by rising atmospheric CO_2, causing drastic changes in the biosphere (Mitchell *et al.* 1993; Caulfield & Bunce 1994). Such increases in CO_2 concentrations are likely to affect plants directly, due to alterations in growth, allocation and composition of chemicals within tissues, and indirectly by the altered climate (Penuelas & Estiarte 1998). Insofar herbivores will also be affected by rising CO_2, altering their physiology and behaviour (Stiling & Cornelissen 2007), caused by changes in the leaf chemistry and nutritional quality of host plants (Masters *et al.* 1998). Thus it is imperative to explore the effects of elevated CO_2 exposure on plants and the resultant effects on insects in order to establish what potential risks exist with regard to future agriculture (Coviella & Truble 1999).

7.1 Changes in the phenology of plants induced by elevated CO_2.

In our study, development of spring wheat and OSR was observed under elevated CO_2 in controlled-environment chambers and in a Mini-FACE system. The results suggested that the phenological development of spring wheat and OSR in controlled-environment chambers was not significantly altered due to elevated CO_2. In the Mini-FACE system, the phenology of spring wheat in 2008 was prolonged by seven days at booting stage and shortened by seven days at dough development stage with CO_2 enrichment, while in OSR, the ripening stage was prolonged by six days (2007), however, both these cases were not statistically significant. In contrast, Atwell *et al.* (1999) proved that CO_2 enrichment (700 µl l^{-1}) hastened the development of wheat (*Triticum aestivum* L.) and rice (*Oryza sativa* L.), significantly accelerating the appearance of successive leaves and shortening the flowering time. Significant enhancement of phenological development under elevated CO_2 was also observed for OSR (Franzaring *et al.* 2008**)** and maize (Leakey 2009). According to Garbutt *et al.* (1990), *Amaranthus retroflexus* flowered significantly earlier under elevated CO_2 (700 µl l^{-1}

vs. 350), while *Setaria faberii* significantly later. A positive relationship was found between the appearance of wheat leaves and the concentration of elevated CO_2 (700 µl l^{-1}) in the study of McMaster *et al.* (1999), where accelerated leaf and tiller appearance rates resulted in quicker canopy development and greater plant biomass (shoot, root and spike production). Significant increases in above ground biomass due to elevated CO_2 were observed on wheat (30%, Atwell *et al.* 1999), broad beans (14%, Awmack & Harrington 2000), upland cotton (85%, Derner *et al.* 2003) and silver birch, black alder and common beech (17%, Hoosbeek *et al.* 2011), while aboveground stem biomass of potato (*Solanum tuberosum* L. cv. Bintje) was negatively impacted by CO_2 enrichment (680 µmol mol^{-1}) at canopy maturity (Högy & Fangmeier 2009). The above ground biomass of sorghum plants (*Sorghum bicolor* L.) also showed a significant decrease by 2% (Derner *et al.* 2003).

In controlled-environment chambers, above ground biomass of non-infested spring wheat was significantly increased due to elevated CO_2, while the above the ground biomass of aphid infested plants showed significant reduction. In accordance, Poorter (1993) observed significant increase (37%) in the biomass of non-infested C_3 plants in the high-CO_2 treatment. In our study, the relative growth rate (RGR) of non-infested spring wheat was significantly increased due to elevated CO_2, while the RGR of aphid infested spring wheat showed significant reduction. Under elevated CO_2 (720 µl l^{-1} vs. 360), the RGR of C_3 plants was between 11.2 and 24.2 mg g^{-1} day^{-1} (Poorter 1993). Although elevated CO_2 led to increase (34%) the RGR of OSR in our experiment, both the effects of CO_2 enrichment and the presence of aphids were consistently below statistical significance. Correspondingly, the interaction between CO_2 and aphid presence on above ground biomass and RGR was insignificant for both spring wheat and OSR. The opposite results were found by Awmack & Harrington (2000), where significant effects by aphids were shown on beans. The pea aphid (*Acyrthosiphon pisum* Harris) decreased the shoot weight of broad beans (*Vicia faba* L.) by 27% under elevated CO_2 (700 µl l^{-1}) and by 20% under ambient CO_2 (350 µl l^{-1}). The potato

aphid (*Aulacorthum solani* Kaltenbach) decreased the shoot biomass of *V. faba* by 20% under elevated CO_2, while no effect was found on plant growth in ambient conditions.

7.2 The consequences of elevated CO_2 on phloem-feeding insects in controlled-chamber system.

Biomass production of major plants responded to elevated CO_2 concentrations with an increase, whereas the food quality for most herbivorous insects was reduced due to decreased protein concentrations (Neumeister 2010). This reduction in the nutritional quality naturally influences the behaviour, performance and feeding habits of insects (Fajer 1989; Asshoff 2005; Zvereva & Koslov 2006). These changes in the leaf chemistry as well as related changes in insect behaviour due to elevated CO_2 occur by degrees (Asshoff 2005). In our study, high-CO_2 treatment significantly increased the weight and RGR of *R. padi*, whereas significant decreases in weight and RGR were established for *M. persicae*. Supporting our results, Lincoln *et al.* (1986) and Fajer (1989) have shown that insect herbivores consistently respond to changes in plant nutritional quality induced by elevated CO_2 with reduced growth, while Goverde & Erhardt (2003) proved increased development of *Coenonympha pamphilus* (Lepidoptera, Satyridae) on four native grassland grass species (*Agrostis stolonifera, Anthoxanthum odoratum, Festuca rubra, Poa pratensis*). Contrary to these results, several researchers established no CO_2 effects on aphid performance (Salt *et al.* 1996; Diaz *et al.* 1998). Mondor *et al.* (2010) showed that the RGRs of green and pink pea aphids were not impacted due to elevated CO_2. The impact on the average weight of aphids (*Macrosiphum euphorbiae*) per plant (*Solanum dulcamara*) under elevated CO_2 (750 µl l^{-1} vs. 350, Flynn *et al.* 2006) was also non-significant. No clear explanations exist for the differing responses of the same insect RGR on different host plants. Insomuch, Haettenschwiler & Schafellner (2004) proved that RGR of gypsy moth (*Lymantria dispar*) was reduced (30%) on *Quercus*

petraea, while significant increase (29%) on *Carpinus betulus* and non-significant trend on *Fagus sylvatica* were observed under elevated CO_2 (530 µl l^{-1} vs. 370).

Non-significant to slight increases due to elevated atmospheric CO_2 conditions were observed for the relative developmental stage (rDS) of *R. padi* and *M. persicae* in our experiment. Accordingly, Mondor *et al.* (2010) argued that elevated CO_2 had no impact on development times of green and pink pea aphids. However, Coviella & Truble (1999) and Asshoff (2005) suggested that lowered nutritional quality induced by elevated CO_2 lengthened the larval developmental times for many species of herbivorous insects.

Changes in nutritional quality of host plants affected the survival and population dynamics of insects (Coviella & Truble 1999). In our study, intrinsic rates of increase of *R. padi* and *M. persicae* were not significantly increased due to elevated CO_2. These results being in concurrence with Flynn *et al.* (2006), who suggested that elevated CO_2 did not significantly change the population of aphid (*Macrosiphum euphorbiae*) on *Solanum dulcamara*, however, were in contrast to Dermody *et al.* (2008), who observed significant increases in aphid numbers (*Aphis glycines*) on soybean plants (*Glycine max* L.).

7.3 CO_2-induced changes in plant chemistry, grown within controlled environment conditions.

The phloem sap from spring wheat and OSR grown within controlled environmental conditions was analysed in order to clarify CO_2-impacts on the insect performance.

The reduction in food quality induced by elevated CO_2 concentrations has clearly been revealed by an increase in the carbon:nitrogen ratio within host plants (Bezemer & Jones 1998). In our study, analysis of phloem sap showed significant increases in fructose (BBCH 12, BBCH 30) and glucose (BBCH 30) within spring wheat under elevated CO_2. Similarly, significant increases in the concentration of glucose were found in the foliage of rice (Shimono *et al*. 2010) and broccoli (Krumbein *et al.* 2010) in high-CO_2 treatments, although

the concentration remained unchanged in the leaves of maize (Leakey *et al.* 2006). In our study, the concentration of fructose in spring wheat was not statistically significant related to RGR of *R. padi* under ambient (BBCH 30) and elevated CO_2 (BBCH 12). On the contrary, Douglas *et al.* (2006) argued a significant relationship between the concentration of sucrose and the RGR of pea aphid (*Acyrthosiphon pisum*) via their diet.

Concerning amino acids in our study, the concentrations of total amino acids in the phloem sap of both host plants were not significantly changed due to elevated CO_2. Interestingly enough, although the relationship between RGR of *M. persicae* and total amino acids in the phloem sap of OSR in the high-CO_2 treatment was not statistically significant, the RGR of *R. padi* was significantly related to their concentration (BBCH 12). Sicher (2008) suggested that total soluble amino acids in the barley leaves (*Hordeum vulgare* L. cv. Brant) were 59% lower under elevated CO_2 (17 DAS, 100 Pa vs. 36 Pa). Supporting our results with *M. persicae*, several studies proved non-significant relationships between the aphid RGRs and the concentrations of total amino acids in the leaves of *A. pseudoplantanus* (Docherty *et al*. 1997), *Prunus padus* and *Hordeum vulgare* (Sandström 2000).

In the phloem of spring wheat, significant increases due to elevated CO_2 were observed for the concentrations of Lys, Leu, Ile, Phe, Val, Tyr, Thr, Ala, Ser, Glu and Asn (BBCH 12) and for Ala, Arg, Leu and GABA (BBCH 30), while the concentration of Orn was significantly decreased in the phloem sap of both plants. Weibull & Melin (1990) established a significant decrease in the concentrations of such individual amino acids as Gly and Met in *Brassica* plants.

In wheat, significant regressions were limited to the RGR of *R. padi* and the concentration of Gly (BBCH 12) and Gln and Phe (BBCH 30) under ambient CO_2, while in OSR, significant relations were found for the RGR of *M. persicae* and Tyr and Lys (BBCH 14). In the high-CO_2 treatment, significant relationships were observed between RGR of *M. persicae* and αAA, Tyr, Trp, Phe and Leu (BBCH 30). Only a few studies exist, in which the relationship

between the RGR of insects and the concentration of free amino acids in the plants have been observed. According to Emden & Bashford (1971), RGR of *M. persicae* was significantly related only to the concentrations of Met and GABA.

7.4 The consequences of elevated CO_2 on the abundance of insects in a Mini FACE system.

In a Mini FACE system, the effects of elevated CO_2 concentration were observed on the population dynamics of detrimental insects from different feeding guilds. Results showed that in 2008, an abundance of thrips species, cereal leaf and ground beetles, click beetles, shield bugs and bird cherry-oat aphids were observed by examination of insect numbers using direct counts (method M_1) and barley leaf beetles, green cicadas, wheat bulb flies, orange wheat blossom and saddle gall midges by examination of insect numbers using yellow sticky traps (method M_2) on spring wheat. Significant increases due to elevated CO_2 were observed for the abundance of *O. melanopus* (BBCH 59) and thrips species (BBCH 83) using method 1 and for *P. vittula* (BBCH 41) using method 2, while significant decreases were shown in *D. coarctata* (BBCH 22, BBCH 23), *C. aridula* (BBCH 31) and *H. marginata* (BBCH 83) using method 2. Supporting our results with *O. melanopus*, *P. vittula* and thrips species, Hunter (2001) suggested that herbivore insects respond to elevated CO_2 with increases in population size. In support, Hughes & Bazzaz (2001) observed a significant increase of 120% in the population of *M. persicae* on *Solanum dulcamara* under elevated CO_2 (700 µl l^{-1} vs. 350). Whittaker (1999), although revealing an increase in the population densities of phloem feeders under elevated CO_2, also showed a decrease in chewers. Changes in the population dynamics of affected insect species may influence their interactions with other insects as well as plants. Thus, insect species that may not directly be affected by the elevated CO_2 may be affected by the changes in other insect species (Coviella & Truble 1999). Although the populations of shoot aphid (*Aphis fabae*) and root-feeding aphid (*Pemphigus populitransversus*) on *Cardamine pratensis* were higher at elevated CO_2 (600 µl l^{-1}) than in

ambient conditions (350 µl l^{-1}), this increase was statistically insignificant (Salt *et al*. 1996). Again, no significant effects from elevated CO_2 (700 µl l^{-1}) were observed on the populations of *Aphis nerii* on *Asclepias syriaca*, *Aphis oenotherae* on *Oenothera biennis* and *Aulacorthum solani* on *Nicotiana sylvestris* (Hughes & Bazzaz 2001).

In 2007/2009, the abundance of thrips species, turnip sawflies, green cicadas, pollen beetles, spring cabbage flies, cabbage whiteflies, green peach aphids and brassica pod midges were observed on OSR. In 2007, significant increases were found for the abundances of thrips species (BBCH 71) and *M. aeneus* (BBCH 77) in the high-CO_2 treatment, while significant decrease was revealed in the population of cicada (BBCH 81).

In 2009, significant decreases under elevated CO_2 were observed in the abundance of *M. aeneus* (BBCH 55, BBCH 67) using method 1 and *D. brassicae* (BBCH 55, BBCH 61, BBCH 67, BBCH 80, BBCH 81) and *M. aeneus* (BBCH 80) using method 2, while significant increases were shown in the abundance of *A. rosae* (BBCH 55, BBCH 62), *D. radicum* (BBCH 55, BBCH 62, BBCH 66, BBCH 67, BBCH 80), *A. proletella* (BBCH 67) and thrips species (BBCH 55) using method 2. Supporting the *M. aeneus* and *D. brassicae* decrease, Hughes & Bazzaz (2001) argued that the population of *Acyrthosiphon pisum* on *Vicia faba* was reduced by 60% due to elevated CO_2 (700 µl l^{-1} vs. 350).

7.5 The consequences of elevated CO_2 on parasitic organisms in a Mini FACE system.

Barley powdery mildew, yellow and brown rust, septoria leaf blotch were observed on spring wheat (2006/2008 years), while OSR (2009 year) was damaged by downy mildew. Plant leaves were only slightly and not significantly damaged by these diseases in all treatments. Supporting our results, no CO_2 effects on *P. striiformis* (Luck *et al.* 2010) and *P. recondita* (Oijen & Ewert 1999) on spring wheat were observed in a FACE experiment and open-top chamber system, respectively. On the contrary, Manning & von Tiedemann (1995) argued that elevated CO_2 inhibited growth of fungi pathogens such as rust and mildew.

In our study, the frequency (FI) and severity (SI) of disease infestation on OSR and wheat were not significantly impacted by elevated CO_2. Opposing this, Eastburn *et al.* (2010) proved that SI of downy mildew (*Peronospora manshurica*) on soybeans was significantly decreased (39%) under elevated CO_2, while a significant, though small in magnitude, increase was observed for brown spot disease (*Septoria glycines*). After summarising information from different literature sources, Kobayashi *et al.* (2006) established differing responses of blast SI (*Magnaporthe oryzae*, anamorph: *Pyricularia oryzae*) to elevated CO_2 (≈ 590 to 670 µl l^{-1}). More specifically, SI of *P. oryzae* on rice plants was significantly enhanced on leaves and unaltered on panicles in a FACE experiment.

8. Conclusions

This thesis describes the effects of elevated CO_2 concentrations on plant-pathogen-insect interactions in a four year field experiment and on host plant-aphid relations in a climate chamber system in 2007 and 2009.

The field experiment aimed to show the effects of CO_2-elevation on the population dynamics of herbivorous insects and the abundance and epidemiology of plant diseases as well as the pressure they may exert on spring wheat and oilseed rape. A Mini-FACE system was involved to perform the CO_2 enrichment experiments without disturbing the natural climatic conditions.

The results showed that there were no effects on the frequency and severity of infestation of diseases due to elevated CO_2. However, insect species responded differently to the elevated CO_2 on both crops, showing significant reductions as well as significant increases in their abundance density dependent on species and host plants. These different responses are explained by changes in plant metabolism. More specifically, the decrease in nitrogen concentration in the leaves of host plants due to elevated CO_2 impacted the nutritional quality for insects. In order to meet their nitrogen requirements, insects increase the ingestion of assimilates from the plants, corresponding in greater crop damage and in some cases leading to more extensive insect outbreaks.

The experiment in the climate chamber system was directed towards the effects of elevated CO_2 on the composition of phloem sap of plants, which thus caused the changes in the behaviour, performance (i.e. the weight, relative growth rate, relative developmental stage, intrinsic rates) and feeding habits of insects (i.e. aphids). Two model biotrophic system, one consisting of bird cherry-oat aphid (*Rhopalosiphum padi* L.) feeding on wheat, the other one of green peach aphid (*Myzus persicae* S.) feeding on oilseed rape, were involved in the experiment to test whether general response patterns to atmospheric CO_2 enrichment could be detected.

The results showed that elevated CO_2 significantly impacted the weight and RGR of aphids on both host plants, whereas the relative developmental stage and intrinsic rates of increase were not significantly changed. However, CO_2 effects on weight and RGR were opposite between the two biotrophic systems, with an increase for *R. padi* and a decrease for *M. persicae*. The analysis of phloem sap showed that carbohydrate and amino acid levels of the host plants were significantly affected due to elevated CO_2, resulting in performance changes of the aphids. However, from the experiments with the two biotrophic systems under controlled conditions no general conclusions can be derived since the two systems responded in a different direction 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.

The FACE experiment has proven to be useful in determining the effects of elevated CO_2 on plant development and may assist future research in establishing the effects on other crops in order to curb the risks of pest and disease outbreaks in a CO_2–rich world. However, in spite of this sophisticated technology, even in a Mini FACE system, no perfect simulation of real world outside conditions is possible. Therefore it is also recommended to expand the conduction of experiments to other crops, and also to direct researches to include other abiotic factors in their studies such as light, temperature and moisture.

There is little knowledge on how readily plants and insects adapt to quick changes in atmospheric CO_2 concentrations, and the answer to this question is not fully experimentally accessible as yet. It is clear however that elevated CO_2 will affect any biological system. It is therefore highly advisable to perform further experimentation in this area in order to elucidate the differences in the effects in between and on different plant species, pathogens and insects under elevated CO_2.

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Publication list

Conference contributions

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