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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 Average numbers of individuals with s.e. | ELE Average numbers of individuals with s.e. | $CO_2$ effect *P*-values (ANOVA) |
|---|---|---|---|---|---|
| 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 ($700\,\mu L\,L^{-1}$) 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 $CO_2$ ($494\,\mu mol\,mol^{-1}$) 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 $CO_2$ ($700\,\mu L\,L^{-1}$ 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 $CO_2$ ($700\,\mu L\,L^{-1}$) 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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