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ORIGINAL ARTICLE

Plant acclimation to elevated CO_2 affects important plant functional traits, and concomitantly reduces plant colonization rates by an herbivorous insect

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Abstract Plants growing under elevated CO_2 concentration may acclimatize to this environmental change by modification of chemical, physiological, and/or morphological traits. As a consequence, not only plant functioning but also plant–insect interactions might be altered, with important consequences particularly for agricultural systems. Whereas most studies have focused on the plant acclimation effects of elevated CO_2 with regard to crop growth and productivity, acclimation effects on the behavioral response of insects associated with these plants have been largely neglected. In this study, we used a model system comprised of Brussels sprout *Brassica oleraceae* var. *gemmifera* and a specialized herbivorous insect, the cabbage aphid *Brevicoryne brassicae*, to test for the effects of various periods of exposure to an elevated (2× ambient) CO_2 concentration on key plant functional traits and on host plant location behavior by the insect, assessed as plant colonization rates. Elevated CO_2 had no measurable effect on colonization rates or total plant volatile emissions after a 2-week exposure, but it led to 15 and 26 % reductions in plant colonization rates after 6- and 10-week exposures, respectively. This reduction in plant colonization was associated with significant decreases in leaf stomatal conductance and plant volatile emission. Terpene emission, in particular, exhibited a great reduction after the 10-week exposure to elevated CO_2. Our results provide empirical evidence that plants might acclimatize to a future increase in CO_2, and that these acclimation responses might affect host plant choice and colonization behavior by herbivorous insects, which might be advantageous from the plant's perspective.

Keywords *Brevicoryne brassicae* · Carbon dioxide · Plant volatiles · Stomatal conductance · Terpenes

Introduction

The atmospheric concentration of carbon dioxide (CO_2) has constantly increased since the pre-industrial era and is predicted to double by the end of the century (Houghton et al. 2001).

Because CO_2 is the substrate for photosynthetic carbon fixation in plants, it has been proposed that elevated CO_2 concentrations will increase rates of photosynthesis, which in turn could enhance plant growth and increase biomass accumulation and plant size (Coley et al. 2002; Asshoff et al. 2006; Stiling and Cornelissen 2007). Increased plant growth could potentially benefit agricultural crop production (Stiling and Cornelissen 2007). Furthermore, increased CO_2 concentration could affect physiological and chemical plant traits, including leaf stomatal conductance and plant volatile emissions (Yuan et al. 2009; Lammertsma et al. 2011). Such morphological, physiological and chemical changes have been attributed to plant acclimation or adaptation occurring within several weeks to several generations, respectively, of exposure to CO_2 (Coley et al.

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2002; Räisänen et al. 2008; Frenck et al. 2011). Focusing on plant acclimation to elevated CO_2, many studies have tested direct effects on growth and productivity of annual crop plants (e.g. Drewry et al. 2010; Otera et al. 2011). In contrast, the effects of plant acclimation on the behavioral responses of insect pests associated with these crops have not yet been investigated (Stiling and Cornelissen 2007; Cornelissen 2011). For instance, the potential effects of plant acclimation to CO_2 on host plant colonization by specialized herbivorous insects, which is a key step in the process of plant attack leading to potential yield losses, are largely unknown.

Host plant colonization by specialized herbivorous insects involves recognition of a host-specific composition of stimuli, especially of chemical nature (Dicke 2000). Most insects first respond to volatiles released from the plant and, after landing on a host, to compounds present on the plant surface. Behaviorally effective volatile plant chemicals that attract herbivorous insects include terpenes as a major group of secondary metabolites (Bruce et al. 2005; Vallat and Dorn 2005; Dudareva et al. 2006), and plant chemicals exploited by insects after landing include surface lipids (e.g. Eigenbrode and Espelie 1995; Piskorski et al. 2011). In addition to chemical cues, visual cues such as plant size, color and shape can also affect insect host colonization behavior (Prokopy and Owens 1983). Thus, focusing on plant traits that affect host plant–herbivorous insect interactions, we hypothesized that CO_2-mediated qualitative and/or quantitative changes of distinct chemical, physiological, and/or morphological traits contributing to plant functioning might affect plant colonization rates by herbivorous insects.

To test our hypothesis, we used a model system comprised of an annual plant of the genus *Brassica*, the Brussels sprout *Brassica oleraceae* L. var. *gemmifera* (Brassicaceae), and a specialized insect, the cabbage aphid *Brevicoryne brassicae* (L.) (Hemiptera: Aphididae). *Brassica* plants are important annual crops that are grown worldwide due to their nutritional, medicinal, bio-industrial, and bio-control properties (Singh et al. 2006; http://www.faostat.fao.org). Furthermore, *Brassica* species are used as model plants to study diverse aspects of plant–insect interactions (Gu and Dorn 2000; Gutbrodt et al. 2011). Aphids are key herbivorous insect pests that attack most crop plants globally (Blackman and Eastop 2000). The cabbage aphid is a specialist herbivore of European origin that feeds exclusively on phloem sap of brassicacean plants, causing considerable economic damage (Blackman and Eastop 2000).

Here, we investigated the degree to which changes in plant morphology and physiology induced by elevated CO_2 are the result of plant acclimation, and how such changes affect the interaction of the plant with its associated herbivorous insect. To our knowledge, this is the first study to relate the phenotypic response of plants to elevated CO_2 to the plant's interaction with its herbivore. We simultaneously tested Brussels sprout plants exposed to elevated compared to ambient CO_2 concentrations for three different periods of exposure, i.e. 2, 6 and 10 weeks, for (i) key morphological and physiological traits as well as chemical profiles and for (ii) colonization rate by cabbage aphids.

Materials and methods

Study organisms and CO_2 concentrations

Four-week-old Brussels sprout (*Brassica oleraceae* var. *gemmifera*) plants (obtained from Sativa Rheinau AG, Rheinau, Switzerland) were placed in groups of 80 into 1 of 2 walk-in climate chambers (Conviron PGV36—Controlled Environments Limited, Winnipeg, MB, Canada) that maintained two different CO_2 concentrations: (i) ambient CO_2 (corresponding to the background concentration of air entering the climate chamber facility; 400 ± 10 ppm) or (ii) elevated CO_2 (double the ambient concentration; 800 ± 10 ppm). Plants were grown in these CO_2 concentrations for 2, 6 or 10 weeks at day/night temperatures of 24/20 °C, with a 16:8 h L:D photoperiod and 250 µmol m^{-2} s^{-1} light intensity (based on Himanen et al. 2009) and 50 % relative humidity on 'Optima Einheitserde' soil (Optima-Werke, Arlesheim, Switzerland; a peat substitute with a pH value between 5.5 and 6.2, NPK: 400, 200, 370 mg L^{-1}, 2,200 mg L^{-1} lime, 32 mg L^{-1} magnesium, trace elements and chelates). Both elevated CO_2 concentration and temperature level were chosen based on the moderate climate change scenario values predicted for the year 2060 (Houghton et al. 2001). Plants within each climate chamber were randomized weekly to avoid any positional effects. During the corresponding period of exposure to the CO_2 concentrations, plants were watered twice a week and fertilized weekly (Wuxal liquid fertilizer, 5 mL added per pot, concentration 0.5 mL/L, N:P:K 10:10:7.5, Maag Syngenta Agro, Dielsdorf, Switzerland), to prevent changes in plant metabolism due to nutritional deficiency (Reddy et al. 2004). All experiments were conducted twice. Between the replications, the CO_2 concentration in each chamber was switched to the other concentration tested to control for potential chamber effects, hence excluding pseudoreplication (Andalo et al. 1998; Agrell et al. 2006; see also "Statistical analysis" below). Growth chamber conditions were monitored with a 'Telaire 7001 CO_2 and temperature monitor' (Ge Measurement & Control, Fremont, CA, USA) connected to a HOBO data logger (Onset Computer Corporation, Bourne, MA, USA) throughout the experiments.

Cabbage aphid (*Brevicoryne brassicae*) stock colonies originated from parthenogenetic females that were collected in Wädenswil (Zurich region, Switzerland). Stock colonies were kept on Brussels sprout plants that were placed inside insect rearing cages (30 × 30 × 30 cm) (BugDorm, Megaview Science CO., Ltd., Taichung, Taiwan) under controlled conditions (day/night temperatures of 24/18 °C, 16:8 h photoperiod, 50 % relative humidity). At the time of the experiments, the aphids had been reared under these conditions for 15–18 generations.

Plant morphological and physiological traits after different periods of exposure to the CO_2 concentrations

We quantified potential effects of different periods of exposure to elevated CO_2 on plant morphological and physiological traits, i.e. plant height, leaf number, fresh and dry weight, leaf area, chlorophyll fluorescence, stomatal density and leaf stomatal conductance. For this purpose, ten Brussels sprout plants from each combination of CO_2 concentration and period of exposure were randomly chosen. For each plant, the aboveground plant height (measured from soil level to apex) and the number of leaves were recorded. The fresh weight of all aboveground tissues per plant, including the stem and the leaves, was measured with a laboratory scale (AB204-S/FACT Analytical Lab Balance Scale, Mettler Toledo, Switzerland, accuracy: 0.1 mg). Total plant dry weight was determined after drying all aboveground parts at 60 °C until weight constancy. The leaf area of all fully expanded leaves per plant was determined with a leaf area meter (Model LI-3100, LI-COR Bioscience, Lincoln, NE, USA). Chlorophyll α fluorescence, taken as the quantum yield of photosystem II (PSII) and calculated as $(F_m' - F_t)/F_m' = \Delta F/F_m'$ (F_m' = maximal fluorescence in light-adapted leaves; F_t = fluorescence steady state in light-adapted leaves), was quantified on the 6th youngest, fully expanded leaf per plant with a portable pulse amplitude modeling fluorometer (PAM-2000, Heinz Walz GmbH, Effeltrich, Germany). The $\Delta F/F_m'$ ratio measures changes in the proportion of the light absorbed by chlorophyll associated with the PSII that is used in photochemistry and thus is considered a sensitive indicator of plant photosynthetic rate and performance (Maxwell and Johnson 2000). Stomatal density was determined following the method described by Beerling et al. (1992). A leaf was flooded with acetone and then a film of cellulose acetate was applied on the abaxial surface. The acetone was allowed to evaporate for 10 min. The resulting leaf surface replica, i.e. a negative version of the leaf epidermal surface, was mounted with a drop of water on a glass slide and stomata were counted at a ×40 magnification under a microscope (Olympus BX50, Olympus Deutschland GmbH, Hamburg, Germany) for a fixed area of 300 × 300 µm. Stomatal density was expressed as number of stomata per square millimeter. For a parallel set of ten plants, randomly chosen from each CO_2 concentration, leaf stomatal conductance was determined on the 6th youngest, fully expanded leaf per plant with a leaf porometer (SC-1, Decagon Devices Inc., Pullman, WA, USA). This same group of plants was also used at each period of exposure tested for volatile collection as described below.

Chemical profiles of the host plant after different periods of exposure to CO_2 concentrations

Plant volatile emissions

To test the effects of different periods of exposure to elevated CO_2 on plant volatile emission, we collected headspace volatiles from Brussels sprout plants that were grown under either of the two CO_2 concentrations for 2, 6, or 10 weeks. Ten plants (the same as used for leaf stomatal conductance measurements) per CO_2 concentration were randomly selected at the beginning of the growing period to be sampled for plant volatile emissions at each period of exposure tested. Prior to each volatile collection, the leaf stomatal conductance of each plant was also measured, as alterations in this plant parameter have been linked to changes in volatile emissions (Niinemets et al. 2004; Yuan et al. 2009).

Headspace volatiles were collected inside the climate chambers from 10 a.m. ± 1 h to 4 p.m. under the plants' respective CO_2 concentration. Plant volatile collection and analysis followed the method described by Piskorski and Dorn (2010) with some modifications as detailed below. Headspace volatiles were collected using a radial diffusive sampling system (*Radiello* model 120-2, Supelco, Buchs, Switzerland). In general, passive volatile sampling systems are advantageous for sampling volatiles at low abundance (Tholl et al. 2006), what applies in particular to the Radiello system used here. This system has low detection limits (Clément et al. 2000; Vallat and Dorn 2005), provides results that are as accurate as active sampling (Gallego et al. 2011), allows for efficient volatile collection without the need of heavy and encumbering pumping systems (Thammakhet et al. 2006), and minimizes plant manipulation and thus potential mechanical damage. Prior to sampling, sorbent cartridges (60 mm long, 4.8 mm diameter) containing *Tenax TA* (250 ± 10 mg; particle size 20–35) were conditioned at 320 °C with a helium (5.0) flow of 60 mL min^{-1} for 7 h. For storage, the cartridges were enclosed in stainless steel tubes that were sealed with brass *Swagelok* caps (Arbor Ventil & Fitting AG, Niederrohrdorf, Switzerland). Volatile samples were taken from the aboveground part of an entire plant that was, together

with a sorbent cartridge inserted in a diffusive body, enclosed in a preconditioned polyester bag (Toppits Brat-Schlauch, Melitta GmbH, Egerkingen, Switzerland). Samples of volatiles from an empty polyester bag and from the air surrounding and entering the climate chambers were collected as controls at each period of exposure tested.

Gas chromatography–mass spectrometry (GC–MS) was used to analyze the qualitative and quantitative composition of the headspace volatiles. The volatiles were desorbed from the sorbent tubes with a *Unity/Ultra* thermal desorption (TD) system (Markes Int. Ltd., Llantrisant, UK) interfaced with a Hewlett-Packard 6890 GC-5976 MS (Hewlett-Packard Co., Palo Alto, CA, USA). The TD flow path was purged for 7 min prior to tube desorption. The tube was then desorbed for 12 min at 280 °C and refocused on a cold trap (Tenax TA/Carbograph 1TD) at −10 °C. The trap was desorbed for 7 min at 300 °C [carrier gas: helium (10 psi); transfer line temperature: 160 °C]. The GC was equipped with a retention gap (uncoated; 5 m × 0.25 mm; Hewlett-Packard) and a *DB-5 ms* column (30 m × 0.25 mm; 0.25 µm film thickness; J&W Scientific, Folsom, CA, USA). The GC oven temperature was held for 5 min at 40 °C, then increased to 100 °C at 3 °C/min, up to 320 °C at 5 °C/min, and held at 320 °C for 4 min (transfer line temperature: 280 °C; ionization energy: 70 eV; ion source temperature: 230 °C; quadrupole temperature: 150 °C; mass range: 30–500 amu). ChemStation software (MSD Productivity Chem Station software, Agilent Technologies Inc., Santa Clara, CA, USA) was used for volatile identification and quantification. Identification was initially performed via comparison of recorded spectra to spectra published in the NIST library and in Adams (1995). Linear retention indices (RIs) were calculated using a mixture of *n*-alkanes (C_7–C_{40}) (Supelco, Bellefonte, PA, USA) as standards and were compared with those in Adams (1995).

For semiquantification, cyclohexane in the gas phase was injected onto the sorbent cartridge as internal standard prior to GC–MS analysis. The internal standard was prepared as in Piskorski and Dorn (2010), based on the method described in Dewulf et al. (1999), and the *Henry's* law coefficient for calculation was used from therein. In principle, cyclohexane (0.235 mmol; 25.5 µL) (≥99.8 %, Sigma-Aldrich, Buchs, Switzerland) was first dissolved in methanol (100 mL). Aliquots of the cyclohexane/methanol solution (50 µL) were then dissolved in Milli-Q water (4.0 mL) in a series of 24.5 mL amber glass vials which were tightly closed with Mininert valves (Sigma-Aldrich, Buchs, Switzerland) and finally incubated in a thermostatic chamber (Heraeus BK 6160 testing chamber, Thermo Fisher Scientific, Schwerte, Germany) at 25.0 ± 0.1 °C for 24 h. Prior to desorption, aliquots of the gas phase (2.0 mL) that were taken from consecutive vials were injected into the sorbent tubes with a gastight syringe with a He flow of 60 mL min^{-1}.

Morphological plant traits, except leaf area, differed significantly between the two CO_2 concentrations, especially after longer exposure periods. As these parameters might influence the amount of volatiles emitted by plants (Guenther et al. 1995), quantities of volatiles detected per plant were standardized per its biomass (dry weight) and expressed in ng/dry weight (based on Owen et al. 2001). Relating volatile amounts emitted to plant biomass is a well-established procedure (Possell et al. 2005; Himanen et al. 2008). Since measurements of dry weight are destructive and thus could not have been taken directly from the same plants that were used for sequential volatile collection, the ng/dry weight per plant was calculated from the dry weight mean values recorded for the ten plants per CO_2 concentration that were used for plant morphological trait measurements at each period of exposure tested.

Leaf surface lipids

To quantify potential effects of elevated CO_2 on leaf surface lipids of Brussels sprout plants, the 4th and 5th youngest fully developed leaves from each of the same 10 plants that were used for plant morphological trait measurements per CO_2 concentration and per period of exposure were combined and used for surface extraction. After weighing, the two leaves (that together yielded 3–7 g fresh weight) were dipped for 7–10 s into dichloromethane (40 mL), as described in Griffiths et al. (2001). The dichloromethane extract was then filtered and evaporated to dryness and afterward re-dissolved in 1.5 mL of dichloromethane. As an internal standard, 0.5 mL decanal (0.64 mM) (≥95.0 %, Fluka, Buchs, Switzerland) dissolved in dichloromethane was added to an equal fraction of each sample. The resulting extracts were derived from the equivalent of 1–2.3 g of fresh plant material per mL of dichloromethane. Extracts were stored at −80 °C for further analysis. For lipid analysis the GC–MS instrument described above was used, with a split/splitless injector operating in splitless mode. The GC oven temperature was held at 50 °C for 5 min, then increased to 320 °C at 5 °C/min and held isothermal at 320 °C for 5 min (ionization energy: 70 eV; ion source temperature: 230 °C; quadrupole temperature: 150 °C; mass range, 30–500 amu). Identification of lipids was based on characteristic mass spectra (Sharkey et al. 1956; Netting and Macey 1971; Kolattukudy et al. 1973) and chromatographic behavior of the compounds. Secondary alcohols were identified by examination of their characteristic α-fragment ions in the spectra of their trimethylsilyl (TMSi) ethers (Sharkey et al. 1957). To obtain TMSi derivates, three selected extracts were pooled, concentrated to a volume of 1 mL, treated with *N*-methyl-*N*-(trimethylsilisyl)-

trifluoracetamide (0.25 mL) at 60 °C for 1 h, and directly analyzed by GC–MS (Piskorski et al. 2011). Amounts of lipids were calculated in ng/g of leaf fresh weight.

Host plant colonization by aphids after different periods of plant exposure to the CO_2 concentrations

To determine whether different periods of plant exposure to the CO_2 concentrations have an effect on plant colonization by winged aphids, defined as the proportion of aphids landing and settling on a plant 24 h after release, we conducted dual-choice wind tunnel bioassays. Bioassays were carried out inside walk-in climate chambers (Conviron PGV36, Controlled Environments Limited) that maintained either of the two CO_2 concentrations tested. Young winged adult aphids in post-teneral stage, i.e. behaviorally inclined to settle on a plant, were obtained from aphid colonies that were kept in walk-in climate chambers (Conviron PGV36) under one of the two CO_2 concentrations for 20 days (approx. 2–3 generations). Thus, aphids used in bioassays were already acclimatized to their respective CO_2 concentration. Our approach involved the use of winged aphids as these morphs have the crucial role of locating, recognizing and accepting suitable hosts for new colony establishment in all aphid species (Blackman and Eastop 1990; Dixon 1998). Winged aphids that took off and alighted on the sides and roof of the cages were captured with a suction tube and transferred into small plastic Petri dishes (height 20 mm, diameter 30 mm) in groups of ten. Prior to bioassays, the aphids were deprived of food for 1 h in order to enhance their responses to plants and to standardize their physiological status (Caillaud 1999; Najar-Rodriguez et al. 2009).

For each bioassay, a group of 30 winged aphids was released from a plastic stand (13 cm height) positioned in the center of a wind tunnel, and aphids were offered the choice between a Brussels sprout plant (exposed to either of the CO_2 concentrations for 2, 6, or 10 weeks) and a non-host plant (common sorrel *Rumex acetosa*) that had been grown under the ambient CO_2 concentration for 3 weeks. Common sorrel is a weed that is widespread in vegetable agroecosystems across central Europe, and it has leaf reflectance patterns similar to those of Brussels sprout plants (see Supplementary material, Fig. S1). The wind tunnel consisted of a Plexiglas chamber (35 × 35 × 150 cm) that was provided with a continuous air flow of 5 ± 2 cm s^{-1}. To avoid visual stimuli from outside the wind tunnel, white curtains were placed all around the tunnel. For each CO_2 concentration and period of exposure tested, ten replications were conducted. Bioassays always started at 8 a.m. ±1 h, i.e. about 2 h after onset of daylight conditions, as aphids are diurnal insects. For each replicate, the number of winged aphids present on each plant was recorded after 24 h. Furthermore, a new set of plants was used and the position of the plants inside the tunnel was exchanged to avoid positional bias.

Statistical analysis

Potential differences between climate chambers were examined by including 'chamber' as a random effect in all analyses. As no significant chamber effects were detected ($P \geq 0.05$ in all cases), this variable was excluded from the analyses described below and data from the two successive experiments were pooled. The effects of CO_2 on each plant morphological and physiological trait measured were analyzed with a separate general linear model (GLM), with CO_2 concentration and period of exposure included in the analysis as fixed factors (R Foundation for Statistical Computing, Vienna, Austria, http://www.R-project.org). Interpretation of the GLM was carried out by first examining interactions and then examining main effects. When not significant, interactions were excluded from further analysis. Whenever any effects were detected, means were subsequently compared by the Tukey's HSD test. Prior to analysis, if necessary, data were $\log_{10}(X + 1)$ transformed to meet the assumptions of normality and heteroscedasticity for parametric tests. The effects of CO_2 on headspace volatiles and leaf surface lipids were analyzed with one-way ANOVAs for each volatile and lipid compound at each period of exposure (R Foundation for Statistical Computing). Prior to analysis, volatile data were $\log_{10}(X + 1)$ transformed. Principal component analysis (PCA) was applied to yield a 2D display of the multivariable set of data in order to search for similarities/differences in samples of headspace volatiles (JMP 8.0, SAS Institute Inc., Cary, NC, USA).

Results from the wind tunnel bioassays were analyzed for colonization preference (taken as the percentage of winged aphids that were present on either plant offered 24 h after release) and insect responsiveness (taken as the proportion of winged aphids that made a choice). Differences in colonization preference were tested with a χ^2-test against the null hypothesis of no preference, and insect responsiveness was analyzed with unpaired sample *t* tests (R Foundation for Statistical Computing). The alpha value for each comparison was adjusted downward using the Benjamini and Hochberg procedure to correct for false discovery rates (type I errors) (Verhoeven et al. 2005).

Results

Plant response to different periods of exposure to the CO_2 concentrations

Plant height, leaf number, and fresh/dry weight were all significantly affected by the elevated CO_2 concentration

and by period of CO_2 exposure (Table 1). Plants grown under elevated CO_2 showed significantly higher values than plants grown under ambient CO_2, particularly after a long exposure period of 10 weeks (two-way ANOVAs, plant height: $F_{2,66} = 15.706$, $P < 0.001$; leaf number: $F_{2,66} = 8.649$, $P < 0.001$; fresh weight: $F_{2,66} = 15.050$, $P < 0.001$; dry weight: $F_{2,66} = 21.075$, $P < 0.001$). Mean values for total leaf area were also higher under elevated CO_2 throughout the experiment, but differences were not significant (two-way ANOVA, $F_{2,66} = 3.359$, $P = 0.071$) (Table 1). Chlorophyll α fluorescence yield (i.e. maximum efficiency of PSII photochemistry) remained unaffected by CO_2 throughout the study (two-way ANOVA, $F_{2,60} = 1.355$, $P = 0.248$) (Table 1).

Remarkably, stomatal conductance of plants exposed to elevated CO_2 was lower than of those exposed to ambient CO_2 (Table 1), independent of period of exposure. However, differences were statistically significant only after an exposure of 10 weeks (one-way ANOVA, $F_{1,24} = 5.839$, $P = 0.026$). Over this time period, doubling the ambient CO_2 concentration reduced leaf stomatal conductance by approximately 50 % (Table 1). In contrast, stomatal densities remained unaffected by CO_2 regardless of period of exposure (two-way ANOVA, $F_{2,54} = 0.134$, $P = 0.716$) (Table 1).

Chemical profiles of the host plant after different periods of exposure to the CO_2 concentrations

Plant volatile emissions

Elevated CO_2 affected volatile emissions from Brussels sprout plants qualitatively and quantitatively, particularly after long periods of exposure. Total numbers of compounds detected in the headspace of these plants amounted to 15 after 2 weeks of exposure to either CO_2 concentration, to 19 and 15 after 6 weeks of exposure to the ambient and elevated CO_2 concentration, respectively, and to 18 and 19 after 10 weeks of exposure to the ambient and elevated CO_2 concentration, respectively (Table 2). Among the detected compounds, the terpenes (particularly monoterpenes) were most common at each period of exposure, with up to 12 compounds being detected. Further groups of compounds identified included fatty acids derivatives and a disulfide. Undecanol is reported here for the first time in the headspace volatiles of a Brussels sprout plant.

The qualitative composition of the volatile blends that were collected at each period of exposure tested from plants subjected to either of the two CO_2 concentrations overlapped to a large degree, but differed for a few compounds for each concentration (Table 2). Quantitatively, total volatile concentration was significantly lower for plants grown under the elevated compared to the ambient CO_2 concentration after 6 and 10 weeks (one-way

Table 1 Plant parameters (mean ± SE) measured on *Brassica* plants grown under an ambient (400 ± 10 ppm) or an elevated (800 ± 10 ppm) CO_2 concentration for 2, 6, and 10 weeks

Plant parameter	Period of exposure to CO_2									
	2 weeks			6 weeks			10 weeks			
	Ambient CO_2	Elevated CO_2	P	Ambient CO_2	Elevated CO_2	P	Ambient CO_2	Elevated CO_2	P
Plant height (mm)	199.3 ± 8.5	220.6 ± 8.6	ns	269.9 ± 11.9	328.1 ± 12.5	*	369.0 ± 13.2	422.0 ± 21.5	*
Leaf number	12.30 ± 0.58	14.00 ± 0.26	*	18.23 ± 1.08	18.85 ± 0.80	ns	21.92 ± 1.21	26.23 ± 0.98	*
Leaf area (cm^2)	1232.44 ± 142.22	1552.97 ± 131.37	ns	1902.18 ± 206.14	2025.20 ± 167.90	ns	1876.77 ± 72.61	2116.65 ± 110.54	ns
Fresh weight (g)	85.72 ± 9.51	118.36 ± 10.03	*	153.54 ± 13.82	188.83 ± 13.87	ns	164.30 ± 6.72	198.25 ± 6.95	*
Dry weight (g)	5.42 ± 0.71	8.37 ± 1.08	*	19.81 ± 2.94	30.33 ± 3.61	*	37.27 ± 2.25	52.77 ± 2.73	*
Chlorophyll α fluorescence	0.68 ± 0.02	0.72 ± 0.01	ns	0.71 ± 0.03	0.72 ± 0.03	ns	0.71 ± 0.02	0.71 ± 0.02	ns
Stomatal conductance (mmol m^{-2} s^{-1})	118.14 ± 12.50	103.80 ± 15.73	ns	115.06 ± 5.68	91.79 ± 14.07	ns	83.75 ± 11.63	47.57 ± 9.43	*
Stomatal density (numbers of stomata per mm^2)	116.66 ± 14.46	130.00 ± 15.54	ns	154.44 ± 12.11	152.22 ± 14.25	ns	218.88 ± 9.37	195.55 ± 15.11	ns

$n = 20$ plants/CO_2 concentration/period of exposure to CO_2

P values (P) based on one-way ANOVAs: * $P < 0.05$, ns $P \geq 0.05$

Table 2 Comparison of headspace volatiles emitted by *Brassica* plants grown under an ambient (400 ± 10 ppm) or an elevated (800 ± 10 ppm) CO_2 concentration for 2, 6 or 10 weeks

Compound	Period of exposure to CO_2									
	2 weeks				6 weeks			10 weeks		
	RI	Ambient CO_2	Elevated CO_2	*P*	Ambient CO_2	Elevated CO_2	*P*	Ambient CO_2	Elevated CO_2	*P*
Terpenes										
α-Thujene[a]	930	1595.75 ± 429.47	58.24 ± 10.98	*	96.13 ± 11.55	98.82 ± 5.24	ns	140.24 ± 19.46	76.46 ± 7.63	*
α-Pinene[c]	932	56.78 ± 9.62	31.54 ± 6.24	ns	54.94 ± 6.41	52.97 ± 2.95	ns	67.47 ± 9.32	38.09 ± 3.66	*
Sabinene[b]	969	299.93 ± 39.65	1817.78 ± 242.91	*	251.16 ± 25.17	248.46 ± 15.32	ns	327.08 ± 41.30	172.51 ± 16.66	*
β-Pinene[b]	974	43.41 ± 4.97	271.46 ± 39.43	*	61.38 ± 24.58	23.87 ± 3.22	*	37.23 ± 4.21	21.3 ± 1.61	*
β-Myrcene[c]	988	nd	nd	–	78.72 ± 7.98	91.88 ± 5.25	ns	114.48 ± 13.84	60.89 ± 5.68	*
3-Carene[b]	1011	nd	nd	–	nd	4.11 ± 1.20	–	nd	nd	–
Limonene[c]	1024	nd	nd	–	120.55 ± 16.60	125.15 ± 19.08	ns	232.71 ± 24.17	126.11 ± 10.88	*
Eucalyptol[c]	1026	112.95 ± 21.08	39.25 ± 7.46	*	90.97 ± 8.59	70.64 ± 8.01	ns	88.31 ± 9.81	46.99 ± 3.71	*
β-Ocimene[c]	1044	nd	nd	–	nd	1.70 ± 0.11	–	1.56 ± 0.16	0.90 ± 0.06	*
γ-Terpinene[c]	1062	nd	2.94 ± 0.71	–	5.19 ± 1.57	6.61 ± 1.59	ns	6.66 ± 1.73	2.80 ± 0.83	ns
cis-Sabinene hydrate[c]	1066	9.14 ± 0.76	5.18 ± 0.34	*	4.67 ± 0.45	3.69 ± 0.44	ns	4.86 ± 0.25	2.66 ± 0.26	*
UI 1 (a monoterpene hydrocarbon)	1069	nd	nd	–	6.27 ± 0.42	nd	–	nd	nd	–
UI 2 (a monoterpene alcohol)	1079	nd	nd	–	5.34 ± 0.51	5.86 ± 1.11	ns	nd	nd	–
α-Terpinolene[c]	1084	nd	nd	–	nd	nd	–	4.88 ± 0.98	1.90 ± 0.57	*
Aldehydes										
Nonanal[c]	1098	nd	nd	–	nd	nd	–	30.85 ± 3.64	18.49 ± 0.46	ns
Decanal[c]	1204	148.63 ± 5.62	134.82 ± 18.08	ns	nd	nd	–	32.92 ± 3.68	nd	–
Undecanal[b]	1305	91.72 ± 7.46	66.92 ± 3.19	*	nd	nd	–	19.26 ± 4.26	nd	–
Dodecanal[b]	1407	31.65 ± 1.61	23.35 ± 0.88	*	nd	nd	–	2.35 ± 0.21	4.81 ± 0.31	*
Alcohols										
1-Penten-3-ol[c]	684	nd	nd	–	8.41 ± 0.88	5.09 ± 0.30	*	nd	nd	–
3-Hexen-1-ol[c]	850	5.31 ± 2.42	2.34 ± 0.77	ns	nd	nd	–	nd	nd	–
Undecanol[c]	1370	nd	nd	–	nd	nd	–	44.88 ± 5.83	32.36 ± 2.48	ns
Ester										
(*Z*)-3-Hexenyl acetate[b]	1004	21.03 ± 7.62	8.64 ± 2.26	ns	15.57 ± 7.41	5.42 ± 1.17	ns	nd	3.27 ± 0.66	–
Hydrocarbons										
Undecene	1075	21.72 ± 1.55	16.15 ± 1.55	*	nd	nd	–	nd	nd	–
Undecane[d]	1100	nd	nd	–	66.90 ± 5.72	nd	–	nd	nd	–
Dodecane[d]	1200	191.38 ± 18.72	nd	–	nd	nd	–	nd	12.78 ± 0.48	–
Tridecane[d]	1300	nd	nd	–	nd	nd	–	nd	11.11 ± 0.49	–
Tetradecane[d]	1400	nd	270.57 ± 16.60	–	nd	nd	–	88.98 ± 2.90	56.03 ± 2.19	*
Heptacosane[d]	2700	nd	nd	–	12.11 ± 0.78	nd	–	nd	nd	–

Table 2 continued

Compound		Period of exposure to CO_2							
		2 weeks				6 weeks			
	RI	Ambient CO_2	Elevated CO_2	*P*	Ambient CO_2	Elevated CO_2	*P*	Ambient CO_2	Elevated CO_2	*P*
Octacosane[d]	2800	nd	nd	–	21.59 ± 13.47	nd	–	nd	nd	–
Nonacosane[d]	2900	nd	nd	–	11.88 ± 0.63	nd	–	nd	nd	–
Disulfide										
Dimethyl disulfide[b]	731	16.53 ± 4.52	11.76 ± 3.30	ns	6.62 ± 1.16	6.89 ± 0.92	ns	7.85 ± 0.90	4.02 ± 0.66	*
Unidentified										
UI 3 (an aliphatic hydrocarbon)	1374	132.67 ± 8.13	nd	–	nd	nd	–	nd	nd	–
Total		2778.62 ± 470.18	2760.95 ± 291.03	ns	918.42 ± 61.02	755.98 ± 47.26	*	1252.58 ± 122.39	693.55 ± 50.52	*

Concentration/dry weight (ng/g) ± SE is shown. $n = 10$ plants/CO_2 concentration/period of exposure to CO_2

P values (*P*) based on one-way ANOVAs: * $P < 0.05$, [ns] $P \geq 0.05$, – no pairwise comparison conducted

nd Not detected in more than 60 % of the samples, *RI* linear retention index on the *DB-5 ms* column, *UI* unidentified

Identification of all compounds was confirmed by GC/MS co-injection of synthetic compounds: source of standards: [a] Indofine, [b] Sigma-Aldrich, [c] Fluka, [d] Supelco

ANOVAs', $F_{1,17} = 5.264$, $P = 0.036$ at 6 weeks, and $F_{1,17} = 18.514$, $P < 0.001$ at 10 weeks) (Table 2). Indeed, pairwise comparisons of concentrations of single compounds revealed, in many cases, significant differences between volatiles detected from plants grown under the two contrasting CO_2 concentrations (Table 2). However, no consistent patterns of increase or decrease in volatile concentration were observed among CO_2 concentrations across periods of exposure tested, with the exception of three terpenes (i.e. α-pinene, eucalyptol, *cis*-sabinene-hydrate), which were consistently emitted at lower mean concentrations (and frequently significantly so) under elevated CO_2 (Table 2).

After 2 weeks of exposure, the headspace of plants grown under either of the two CO_2 concentrations contained the same number of volatile compounds. However, two compounds, γ-terpinene and tetradecane, were detected only in plants grown under elevated CO_2 whereas dodecane and an unidentified aliphatic hydrocarbon were detected only in plants grown under ambient CO_2 (Table 2). Principal component analysis (PCA) of the volatiles detected in the plants grown under the two CO_2 concentrations yielded two separated clusters, one per each concentration (Fig. 1a). Mean quantities of volatile compounds detected in plants grown under elevated CO_2 were significantly higher than those detected in plants grown under ambient CO_2 for 2 of the 6 terpenes, namely sabinene and β-pinene (one-way ANOVAs', $F_{1,17} = 65.040$, $P < 0.001$ and $F_{1,17} = 69.776$, $P < 0.001$, respectively). The opposite was true for α-thujene, eucalyptol and *cis*-sabinene-hydrate (one-way ANOVAs', $F_{1,17} = 77.631$, $P < 0.001$; $F_{1,17} = 15.368$, $P < 0.001$ and $F_{1,17} = 22.159$, $P < 0.001$, respectively) (Table 2).

After 6 weeks of exposure to the CO_2 concentrations, three compounds, 3-carene, β-ocimene and undecene, were detected only in plants grown under elevated CO_2, whereas an unidentified monoterpene hydrocarbon and several aliphatic hydrocarbons were detected only in samples from plants grown under ambient CO_2 (Table 2). PCA of the volatiles detected in the plants grown under the two CO_2 concentrations again yielded two separated clusters, one per each concentration (Fig. 1b). Mean quantities of volatile compounds detected in plants grown under elevated CO_2 were lower than those detected in plants grown under ambient CO_2 in 5 of the 10 terpenes detected. However, these differences were only significant for β-pinene (one-way ANOVA, $F_{1,17} = 5.020$, $P = 0.041$) (Table 2).

After 10 weeks of exposure to the CO_2 concentrations (*Z*)-3-hexenyl acetate, dodecane and tridecane were detected only in plants grown under elevated CO_2, whereas decanal and undecanal were detected only in samples from plants grown under ambient CO_2 (Table 2). Again, the PCA of the volatiles from plants grown under the two CO_2

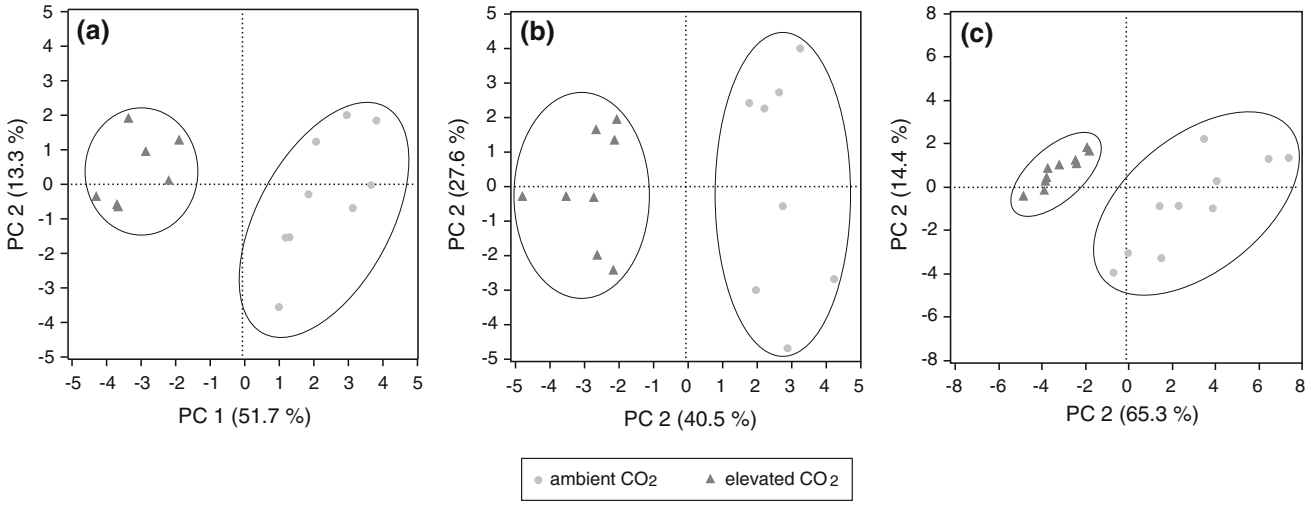

Fig. 1 Principal component analysis representation of headspace volatiles detected in *Brassica* plants grown under either an ambient (400 ± 10 ppm) or an elevated (800 ± 10 ppm) CO_2 concentration for 2 (**a**), 6 (**b**), or 10 (**c**) weeks ($n = 10$ plants/CO_2 concentration). Factor coordinates of samples are shown. *Circles* enclose plants grown under each of two CO_2 concentrations tested

concentrations yielded two clearly separated clusters, one for each group of plants (Fig. 1c). Mean quantities of volatile compounds from plants grown under elevated CO_2 were lower than those from plants grown under ambient CO_2 in all of the 11 terpenes detected, with differences being significant for 10 compounds, namely, α-thujene, α-pinene, sabinene, β-pinene, β-myrcene, limonene, eucalyptol, β-ocimene, *cis*-sabinene hydrate, and α-terpinolene (one-way ANOVAs', $F_{1,17} = 6.983$, $P = 0.016$; $F_{1,17} = 6.165, P = 0.023; F_{1,17} = 10.444, P = 0.004; F_{1,17} = 11.421$, $P = 0.003$; $F_{1,17} = 12.046$, $P = 0.002$; $F_{1,17} = 15.368$, $P = 0.001$; $F_{1,17} = 21.006$, $P < 0.001$; $F_{1,17} = 15.892$, $P < 0.001$; $F_{1,17} = 32.392$, $P < 0.001$ and $F_{1,17} = 7.254$, $P = 0.014$, respectively). In addition, mean quantity of dimethyl disulfide from plants grown under elevated CO_2 was reduced by nearly 50 % (one-way ANOVA, $F_{1,17} = 12.098$, $P = 0.002$) (Table 2).

Leaf surface lipids

The CO_2 concentration or the period of exposure did not significantly affect the quantitative or qualitative content of the detected leaf surface lipids of Brussels sprout plants. The qualitative composition of the surface lipids remained unchanged, irrespective of the CO_2 concentration and the exposure period. Among the detected lipids, six ketones, one hydroxyketone, two aldehydes, five secondary alcohols, two triterpenes and nine alkanes were identified (see Supplementary material, Table S2). Total mean quantities of detected surface lipids were lower among plants exposed to elevated compared to those exposed to ambient CO_2 for 2 and 6 weeks, whereas those quantities were higher for plants exposed to elevated compared to ambient CO_2 for 10 weeks. These differences, however, were in all cases not statistically significant (see Supplementary material, Table S2).

Host plant colonization by aphids after different periods of plant exposure to the CO_2 concentrations

Doubling the ambient CO_2 concentration had a marked effect on plant colonization by winged aphids particularly when plants were exposed to CO_2 for longer periods (Fig. 2). Brussels sprout plants exposed to the elevated CO_2 concentration for either 6 or 10 weeks were colonized significantly less than plants grown under the ambient CO_2 concentration (unpaired sample t tests, $t = -2.315$, $df = 14$, $P = 0.036$ at 6 weeks and $t = -2.845$, $df = 13$, $P = 0.014$, at 10 weeks). In contrast, a short plant exposure period of only 2 weeks did not result in a significant difference in plant colonization (Fig. 2). Under the elevated CO_2 concentration, the proportion of winged aphids that colonized our Brussels sprout plants after 2, 6 or 10 weeks of exposure was 90, 75 or 65 %, respectively, whereas under the ambient CO_2 concentration, the proportion was always around 90 %, regardless of the period of exposure (Fig. 2). However, irrespective of the CO_2 concentration tested, significantly more winged aphids colonized Brussels sprout plants than the non-host control plants (χ^2 test, $df = 19$, $P < 0.010$) (Fig. 2), but they never laid nymphs on the latter plants in contrast to the former, which always contained nymphs after 24 h (see Supplementary material, Table S1). Aphid responsiveness, i.e. the proportion of winged aphids that made a choice, was not influenced by the CO_2 concentration (unpaired sample t test, $P > 0.1$). Responsiveness, however, decreased

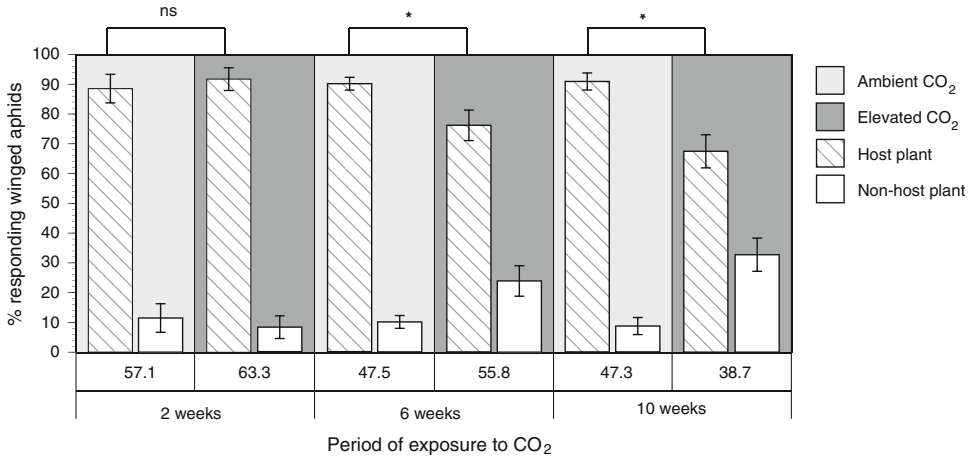

Fig. 2 Mean percentage (±SE) of winged cabbage aphids (*Brevicoryne brassicae*) colonizing either the host plant (Brussels sprout *Brassica oleraceae* var. *gemmifera*) or a non-host plant (common sorrel *Rumex acetosa*) in a dual-choice wind tunnel bioassay. Host plants were grown under an ambient (400 ± 10 ppm) or an elevated (800 ± 10 ppm) CO_2 concentration for 2, 6, or 10 weeks prior to the bioassay. Non-host plants were always grown under the ambient CO_2 concentration for 3 weeks. A group of winged aphids consisting of 30 individuals was released per replicate. Ten replicates per CO_2 concentration were conducted per tested exposure period. *Numbers below the bars* indicate the percentage of winged aphids that made a choice. *P* values shown for comparisons among CO_2 concentrations: $*P \leq 0.05$, *ns* $P > 0.05$; *P* values indicating a significant preference for host plant versus non-host plant not shown

significantly with increasing period of exposure (unpaired sample *t* tests, ambient CO_2: 6 vs. 2 weeks $t = 1.443$, $df = 17$, $P = 0.170$; 10 vs. 6 weeks $t = 2.471$, $df = 17$, $P = 0.024$; elevated CO_2: 6 vs. 2 weeks $t = 3.870$, $df = 17$, $P = 0.001$; 10 vs. 6 weeks $t = 2.415$, $df = 17$, $P = 0.027$) (Fig. 2).

Discussion

Elevated CO_2 (approx. 800 ppm) can significantly affect interactions between the *Brassica* species studied and an associated specialist herbivore. Elevated CO_2 led to a respective 15 and 26 % reduction of colonization rates by the cabbage aphid *Brevicoryne brassicae* on Brussels sprout plants after 6 and 10 weeks of plant exposure. No difference in colonization rate was noted after an exposure of only 2 weeks. A trend indicating a similar reduction in colonization rate has been observed for the leafminer *Phyllonorycter tremuloidiella* (Kopper and Lindroth 2003). Herbivory by this insect on the trembling aspen *Populus tremuloides* was measured in a free-air CO_2 enrichment experiment, but differences between insect attack in the CO_2-enriched versus the standard system were not significant. Thus, the present study carried out under controlled conditions is, to our knowledge, the first to demonstrate a reduced colonization rate by an herbivorous insect due to elevated CO_2. Concomitantly with the marked behavioral effect of elevated CO_2 on the insects, leaf stomatal conductance and plant volatile emissions were also reduced under elevated CO_2, and these differences were significant after 10 weeks of plant exposure. In addition to lower total volatiles emitted, plant morphology was also altered after both 6 and 10 weeks of exposure, potentially leading to a cumulative effect accounting for the reduced plant colonization rates observed.

Stomatal conductance, a measure of the rate of passage of either water vapor or CO_2 through the stomata, and composition of emitted volatiles were significantly affected by elevated CO_2. Brussels sprout plants grown for 10 weeks under elevated CO_2 exhibited a reduction of nearly 50 % in stomatal conductance, as well as a qualitative and quantitative reduction in volatile emission. Such combined effects of CO_2 on stomatal conductance and volatile emission have, to our knowledge, not been reported before on a non-woody plant species. CO_2 concentrations above current atmospheric levels (i.e. 500–900 ppm) have been shown to reduce stomatal conductance in many C3 land plants (Long et al. 2004; Velikova et al. 2009). A reduction in stomatal conductance has been interpreted as an acclimation response of plants growing under elevated CO_2 concentrations (e.g. Velikova et al. 2009). However, the exact physiological mechanism underlying this plant response is still unclear (Brodribb et al. 2009; de Boer et al. 2011). Recent evidence suggests that plants could achieve reduced stomatal conductance mechanically by regulating stomatal aperture (Ainsworth and Rogers 2007; de Boer et al. 2011), or structurally by growing leaves with lower stomatal density (de Boer et al. 2011; Riikonen et al. 2010; Lammertsma et al. 2011). However, changes in stomatal density, which involve epidermal structural alterations, seem to occur over longer periods of time compared to

those involved in short-term acclimation by stomatal closure (de Boer et al. 2011; Lammertsma et al. 2011). Thus, a reduction in stomatal conductance has not always been associated with a parallel change in stomatal density (Ainsworth and Rogers 2007). We did not find any significant reduction in stomatal density when comparing plants grown under elevated CO_2 with those grown under ambient CO_2, regardless of period of exposure. This finding indicates that the significant reduction in stomatal conductance detected in this study after 10 weeks of exposure was not due to a change in stomatal density. Mechanical effects on stomatal functioning such as stomatal aperture could underlie the reduction in stomatal conductance documented here.

Alterations in stomatal conductance have been linked to a reduction in volatile organic compound emission, particularly of terpenes (Niinemets et al. 2004; Yuan et al. 2009). Thus, the quantitative changes in volatile emission of Brussels sprout plants under elevated CO_2 concentrations found in our study could have been partially due to the detected decrease in stomatal conductance. Plant volatile emissions, particularly those of terpenes, are predicted to be affected by increased CO_2 concentrations (Peñuelas and Llusia 1997; Peñuelas and Staudt 2010). However, contrasting evidence has been reported for different plant species. For instance, emission of mono- and sesquiterpenes by peppermint *Mentha piperita* (Lincoln and Couvet 1989) and ponderosa pine *Pinus ponderosa* (Constable et al. 1999) did not differ between plants grown under ambient and elevated CO_2 concentrations. Such emissions were, by contrast, reduced in eastern cottonwood *Populus deltoides* trees (Rosenstiel et al. 2003) and increased in rosemary *Rosmarinus officinalis* (Peñuelas and Llusia 1997). Among *Brassica* species, contradictory results have also been reported, with either a decrease (white cabbage *Brassica oleracea* ssp. *capitata,* Vuorinen et al. 2004), or an increase (oilseed rape *Brassica napus* ssp. *oleifera*, Himanen et al. 2009) in the quantity of volatiles released under elevated CO_2. Among the identified volatile compounds detected in Brussels sprout plants in our study, the terpenes prevailed in numbers at any period of exposure, and quantities of distinct compounds were significantly lower under elevated CO_2 after the 10-week exposure. Besides altering stomatal conductance, elevated CO_2 concentrations could have also affected the metabolic pathways leading to monoterpene biosynthesis in the cell plastids (Arimura et al. 2009), as was suggested for the trembling aspen *Populus tremuloides*, the blue gum *Eucalyptus globulus* (Wilkinson et al. 2009), and the eastern cottonwood *P. deltoides* (Rosenstiel et al. 2003). The activity of several monoterpene synthases, a large family of enzymes required for the formation of diverse cyclic and acyclic monoterpenes, can be altered under elevated CO_2, leading for instance to a decreased emission of α- and β-pinene in the holm oak *Quercus ilex* (Loreto et al. 2001). A reduction in isoprene synthase activity under elevated CO_2 has also been documented for the common reed *Phragmites australis* (Scholefield et al. 2004), the giant cane *Arundo donax* and the velvet bean *Mucuna pruriens* (Possell et al. 2005), suggesting that a similar effect might underly the reduced emissions of as many as 10 terpenes after the 10-week plant exposure in our study.

Plant volatiles are important cues mediating attraction of insects and other arthropods to host plants in the process of plant location (Bruce et al. 2005; Dudareva et al. 2006). Thus, the lower aphid colonization of Brussels sprout plants under the elevated CO_2 concentration found in our study could be related to the CO_2-induced decrease in total volatile emissions measured after 6 and 10 weeks. Indeed, changes in plant volatile emissions have been suggested to account for a lack of olfactory attraction of the black bean aphid *Aphis fabae* to its host plant spindle *Euonymus europaeus* (Nottingham et al. 1991), as well as for a change in the response of the corn leaf aphid *Rhopalosiphum maidis* to herbivore-induced versus uninfested corn *Zea mays* plants (Bernasconi et al. 1998). Recent evidence indicates that soybean aphids *Aphis glycines* were not able to discriminate between odors released by soybean *Glycine max* plants grown under ambient compared to elevated CO_2 in olfactometer tests (O'Neill et al. 2010). This lack of olfactory discrimination was attributed to the phenological plant stage used, and it was suggested that at a later stage, plants might emit detectable amounts of the attractive volatiles.

The response to elevated CO_2 of additional plant traits beyond volatile emission might have also contributed to the reduced colonization rates of aphids after the 6- and 10-week exposure periods that were recorded in our study. After a plant exposure period of 6 weeks, the total volatiles emitted were reduced by only 18 %, compared to 45 % after 10 weeks of exposure. However, the trend in reduced stomatal conductance was not significant after 6 weeks. A significant increase in plant size and biomass, measured as height and dry weight, was already noted after 6 weeks. Increased plant size and biomass are common plant growth responses to elevated CO_2 (Asshoff et al. 2006; Frenck et al. 2011) that might lead to reduced host plant choice in some insect species (Forsberg 1987; Hattendorf et al. 2006). Thus, the CO_2-induced morphological alterations of our Brussels sprout plants could have also been involved, to some extent, in the reduced behavioral response of the aphids. Our findings, however, do not indicate any general major effect of elevated CO_2 on the aphids themselves. Prior to all bioassays, aphid colonies were always exposed to their respective CO_2 concentration for the same period of time (20 days). Thus, any potential effects of exposure

to CO_2 on aphid behavior *per se* can be ruled out. However, during aphid development under elevated CO_2 conditions, some insect biochemical and/or physiological functions could have been impaired as shown for the grain aphid *Sitobion avenae*. Elevated CO_2 reduced the response of this insect to its alarm pheromone, the terpene (*E*)-*β*-farnesene (Sun et al. 2010). The lower activity of acetylcholinesterase, a key enzyme in insect neurotransmission, was postulated to be involved in the reduced response of this aphid to the pheromone.

After aphids land on a potential host plant, leaf surface chemicals, especially cuticular hydrocarbons, can act as arresting cues (Pickett et al. 1992; Eigenbrode and Espelie 1995). For instance, pea aphids *Acyrthosiphon pisum* do not distinguish between hosts and non-hosts at a distance but determine whether to colonize a plant only after landing (Caillaud 1999). Increase in atmospheric ozone concentrations, which led to a significant reduction in colonization rates of trembling aspen trees by the aspen blotch leafminer, were assumed to be related to changes in leaf surface chemicals (Kopper and Lindroth 2003). However, we found no differences in surface lipid profiles of Brussels sprout plants grown under elevated compared to ambient CO_2; this is in line with recent results reported for paper birch *Betula papyrifera* trees (Riikonen et al. 2010). Thus, it is unlikely that in our study leaf surface chemicals are the basis upon which aphids colonized significantly less plants grown under elevated compared to ambient CO_2 concentrations. CO_2-mediated changes in secondary metabolites involved in direct plant defense of *Brassica* species, particularly glucosinolates, which could also influence host acceptance by aphids (Newton et al. 2010), might be considered in future studies.

In agroecosystems, reduced crop plant colonization by an herbivorous pest insect under elevated CO_2 might be advantageous. However, this effect on herbivores could be counterbalanced by an adverse effect on their natural antagonists. Altered volatile blends emitted by the herbivore-infested plants might affect the host location behavior of natural antagonists, which rely on such plant-derived olfactory cues for herbivore finding (Scascighini et al. 2005; Yuan et al. 2009). Thus, we cannot exclude that the effects of elevated CO_2 on plant volatile emissions cascade to higher trophic levels. This could impair host location efficiency of the natural antagonists of aphids, leading to a reduced efficacy of this indirect plant defense mechanism. Plant volatile blends are also involved in a range of plant-related ecological functions, including pollinator attraction, plant–plant communication, plant–pathogen interactions, and thermotolerance (Niinemets et al. 2004; Dudareva and Pichersky 2008; Yuan et al. 2009). Thus, the CO_2-mediated changes in volatile emission of Brussels sprout plants documented here could also have important ecological effects on the plants themselves.

This study shows that extended periods of exposure to elevated CO_2 can lead to a decrease in leaf stomatal conductance and to reductions in emissions of terpenes and other plant volatiles, and that changes in these traits, which are relevant for plant functioning and defense, may contribute to a significant reduction in plant colonization by herbivorous insects. Our results highlight the importance of understanding how plants might acclimatize to increased CO_2 concentrations and the effects that these acclimation responses might have on plant–insect interactions.

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References

Adams R (1995) Identification of essential oil components by gas chromatography/mass spectrometry. Allured Publishing Corporation, IL

Agrell J, Anderson P, Oleszek W, Stochmal A, Agrell C (2006) Elevated CO_2 levels and herbivore damage alter host plant preferences. Oikos 112:63–72

Ainsworth EA, Rogers A (2007) The response of photosynthesis and stomatal conductance to rising [CO_2]: mechanisms and environmental interactions. Plant Cell Environ 30:258–270

Andalo C, Raquin C, Machon N, Godelle B, Mousseau M (1998) Direct and maternal effects of elevated CO_2 on early root growth of germinating *Arabidopsis thaliana* seedlings. Ann Bot 81:405–411

Arimura G, Matsui K, Takabayashi J (2009) Chemical and molecular ecology of herbivore-induced plant volatiles: proximate factors and their ultimate functions. Plant Cell Physiol 50:911–923

Asshoff R, Zotz G, Körner C (2006) Growth and phenology of mature temperate forest trees in elevated CO_2. Glob Change Biol 12:848–861

Beerling DJ, Chaloner WG, Huntley B, Pearson JA, Tooley MJ, Woodward FI (1992) Variations in the stomatal density of *Salix herbacea* L. under the changing atmospheric CO_2 concentrations of late- and postglacial time. Phil Trans R Soc Lond B 336:215–224

Bernasconi ML, Turlings TCJ, Ambrosetti L, Bassetti P, Dorn S (1998) Herbivore-induced emissions of maize volatiles repel the corn leaf aphid, *Rhopalosiphum maidis*. Entomol Exp Appl 87:133–142

Blackman RL, Eastop VF (1990) Specificity in aphid/plant genetic interactions, with particular attention to the role of the alate colonizer. In: Campbell RK, Eikenbary RD (eds) Aphid–plant genotype interactions. Elsevier, Amsterdam, pp 251–274

Blackman RL, Eastop VF (2000) Aphids on the world's crops, an identification and information guide. The Natural History Museum, London

Brodribb TJ, McAdam SAM, Jordan GJ, Feild TS (2009) Evolution of stomatal responsiveness to CO_2 and optimization of water-use efficiency among land plants. New Phytol 183:839–847

Bruce TJA, Wadhams LJ, Woodcock CM (2005) Insect host location: a volatile situation. Trends Plant Sci 10:269–274

Caillaud MC (1999) Behavioural correlates of genetic divergence due to host specialization in the pea aphid, *Acyrthosiphon pisum*. Entomol Exp Appl 91:227–232

Clément M, Arzel S, Le Bot B, Seux R, Millet M (2000) Adsorption/thermal desorption-GC/MS for the analysis of pesticides in the atmosphere. Chemosphere 40:49–56

Coley PD, Massa M, Lovelock CE, Winter K (2002) Effects of elevated CO_2 on foliar chemistry of saplings of nine species of tropical tree. Oecologia 133:62–69

Constable JVH, Litvak ME, Greenberg JP, Monson RK (1999) Monoterpene emission from coniferous trees in response to elevated CO_2 concentration and climate warming. Glob Change Biol 5:255–267

Cornelissen T (2011) Climate change and its effects on terrestrial insects and herbivory patterns. Neotropical Entomol 40:155–163

de Boer HJ, Lammertsma EI, Wagner-Cremer F, Dilcher DL, Wassen MJ, Dekker SC (2011) Climate forcing due to optimization of maximal leaf conductance in subtropical vegetation under rising CO_2. Proc Natl Acad Sci USA 108:4041–4046

Dewulf J, Van Langenhove H, Everaert P (1999) Determination of Henry's law coefficients by combination of the equilibrium partitioning in closed systems and solid-phase microextraction techniques. J Chromatogr A 830:353–363

Dicke M (2000) Chemical ecology of host-plant selection by herbivorous arthropods: a multitrophic perspective. Biochem System Ecol 28:601–617

Dixon AFG (1998) Aphid ecology. Chapman and Hall, London

Drewry DT, Kumar P, Long S, Bernacchi C, Liang XZ, Sivapalan M (2010) Ecohydrological responses of dense canopies to environmental variability: 2. Role of acclimation under elevated CO_2. J Geophys Res Biogeosci 115:G04023

Dudareva N, Pichersky E (2008) Metabolic engineering of plant volatiles. Curr Opin Biotechnol 19:181–189

Dudareva N, Negre F, Nagegowda DA, Orlova I (2006) Plant volatiles: recent advances and future perspectives. Crit Rev Plant Sci 25:417–440

Eigenbrode SD, Espelie KE (1995) Effects of plant epicuticular lipids on insect herbivores. Annu Rev Entomol 40:171–194

Forsberg J (1987) Size discrimination among conspecific hostplants in two pierid butterflies—*Pieris napi* L. and *Pontia daplidice* L. Oecologia 72:52–57

Frenck G, van der Linden L, Mikkelsen TN, Brix H, Jørgensen RB (2011) Increased [CO_2] does not compensate for negative effects on yield caused by higher temperature and [O_3] in *Brassica napus* L. Eur J Agron 35:127–134

Gallego E, Roca FJ, Perales JF, Guardino X (2011) Comparative study of the adsorption performance of an active multi-sorbent bed tube (Carbotrap, Carbopack X, Carboxen 569) and a Radiello (R) diffusive sampler for the analysis of VOCs. Talanta 85:662–672

Griffiths DW, Deighton N, Birch ANE, Patrian B, Baur R, Stadler E (2001) Identification of glucosinolates on the leaf surface of plants from the Cruciferae and other closely related species. Phytochemistry 57:693–700

Gu H, Dorn S (2000) Genetic variation in behavioral response to herbivore-infested plants in the parasitic wasp, *Cotesia glomerata* (L.) (Hymenoptera: Braconidae). J Insect Behav 13:141–156

Guenther A, Hewitt CN, Erickson D et al (1995) A global-model of natural volatile organic-compound emissions. J Geophys Res Atm 100:8873–8892

Gutbrodt B, Mody K, Dorn S (2011) Drought changes plant chemistry and causes contrasting responses in lepidopteran herbivores. Oikos 120:1732–1740

Hattendorf J, Hansen SO, Reznik SY, Nentwig W (2006) Herbivore impact versus host size preference: endophagous insects on *Heracleum mantegazzianum* in its native range. Environ Entomol 35:1013–1020

Himanen SJ, Nissinen A, Dong WX, Nerg AM, Stewart CN, Poppy GM, Holopainen JK (2008) Interactions of elevated carbon dioxide and temperature with aphid feeding on transgenic oilseed rape: Are *Bacillus thuringiensis* (Bt) plants more susceptible to nontarget herbivores in future climate? Glob Change Biol 14:1437–1454

Himanen SJ, Nerg AM, Nissinen A, Pinto DM, Stewart CN, Poppy GM, Holopainen JK (2009) Effects of elevated carbon dioxide and ozone on volatile terpenoid emissions and multitrophic communication of transgenic insecticidal oilseed rape (*Brassica napus*). New Phytol 181:174–186

Houghton J, Ding Y, Griggs D et al (2001) Climate change 2001: the scientific basis. Cambridge University Press, Cambridge

Kolattukudy PE, Buckner JS, Liu TYJ (1973) Biosynthesis of secondary alcohols and ketones from alkanes. Arch Biochem Biophys 156:613–620

Kopper BJ, Lindroth RL (2003) Responses of trembling aspen (*Populus tremuloides*) phytochemistry and aspen blotch leafminer (*Phyllonorycter tremuloidiella*) performance to elevated levels of atmospheric CO_2 and O_3. Agric For Entomol 5:17–26

Lammertsma EI, de Boer HJ, Dekker SC, Dilcher DL, Lotter AF, Wagner-Cremer F (2011) Global CO_2 rise leads to reduced maximum stomatal conductance in Florida vegetation. Proc Natl Acad Sci USA 108:4035–4040

Lincoln DE, Couvet D (1989) The effect of carbon supply on allocation to allelochemicals and caterpillar consumption of peppermint. Oecologia 78:112–114

Long SP, Ainsworth EA, Rogers A, Ort DR (2004) Rising atmospheric carbon dioxide: plants face the future. Annu Rev Plant Biol 55:591–628

Loreto F, Fischbach RJ, Schnitzler JP et al (2001) Monoterpene emission and monoterpene synthase activities in the Mediterranean evergreen oak *Quercus ilex* L. grown at elevated CO_2 concentrations. Glob Change Biol 7:709–717

Maxwell K, Johnson GN (2000) Chlorophyll fluorescence—a practical guide. J Exp Bot 51:659–668

Najar-Rodriguez AJ, McGraw EA, Hull CD, Mensah RK, Walter GH (2009) The ecological differentiation of asexual lineages of cotton aphids: alate behaviour, sensory physiology, and differential host associations. Biol J Linn Soc 97:503–519

Netting AG, Macey MJK (1971) Composition of ketones and secondary alcohols from *Brassica oleracea* waxes. Phytochemistry 10:1917–1920

Newton E, Bullock JM, Hodgson D (2010) Temporal consistency in herbivore responses to glucosinolate polymorphism in populations of wild cabbage (*Brassica oleracea*). Oecologia 164:689–699

Niinemets U, Loreto F, Reichstein M (2004) Physiological and physicochemical controls on foliar volatile organic compound emissions. Trends Plant Sci 9:180–186

Nottingham SF, Hardie J, Dawson GW et al (1991) Behavioral and electrophysiological responses of aphids to host and nonhost plant volatiles. J Chem Ecol 17:1231–1242

O'Neill BF, Delucia EH, Zangerl AB, Berenbaum MR (2010) Olfactory preferences of *Popillia japonica*, *Vanessa cardui*, and *Aphis glycines* for *Glycine max* grown under elevated CO_2. Environ Entomol 39:1291–1301

Otera M, Kokubun M, Tabei H, Matsunami T, Maekawa T, Okada M (2011) Is yield enhancement by CO_2 enrichment greater in genotypes with a higher capacity for nitrogen fixation? Agric For Meteorol 151:1385–1393

Owen SM, Boissard C, Hewitt CN (2001) Volatile organic compounds (VOCs) emitted from 40 Mediterranean plant species: VOC speciation and extrapolation to habitat scale. Atmos Environ 35:5393–5409

Peñuelas J, Llusia J (1997) Effects of carbon dioxide, water supply, and seasonality on terpene content and emission by *Rosmarinus officinalis*. J Chem Ecol 23:979–993

Peñuelas J, Staudt M (2010) BVOCs and global change. Trends Plant Sci 15:133–144

Pickett JA, Wadhams LJ, Woodcock CM, Hardie J (1992) The chemical ecology of aphids. Annu Rev Entomol 37:67–90

Piskorski R, Dorn S (2010) Early-season headspace volatiles from apple and their effect on the apple blossom weevil *Anthonomus pomorum*. Chem Biodivers 7:2254–2260

Piskorski R, Kroder S, Dorn S (2011) Can pollen headspace volatiles and pollenkitt lipids serve as reliable chemical cues for bee pollinators? Chem Biodivers 8:577–586

Possell M, Hewitt CN, Beerling DJ (2005) The effects of glacial atmospheric CO_2 concentrations and climate on isoprene emissions by vascular plants. Glob Change Biol 11:60–69

Prokopy RJ, Owens ED (1983) Visual detection of plants by herbivorous insects. Annu Rev Entomol 28:337–364

Räisänen T, Ryyppö A, Kellomäki S (2008) Effects of elevated CO_2 and temperature on monoterpene emission of Scots pine (*Pinus sylvestris* L.). Atmos Environ 42:4160–4171

Reddy GVP, Tossavainen P, Nerg AM, Holopainen JK (2004) Elevated atmospheric CO_2 affects the chemical quality of *Brassica* plants and the growth rate of the specialist, *Plutella xylostella*, but not the generalist, *Spodoptera littoralis*. J Agric Food Chem 52:4185–4191

Riikonen J, Percy KE, Kivimaenpaa M, Kubiske ME, Nelson ND, Vapaavuori E, Karnosky DF (2010) Leaf size and surface characteristics of *Betula papyrifera* exposed to elevated CO_2 and O_3. Environ Pollut 158:1029–1035

Rosenstiel TN, Potosnak MJ, Griffin KL, Fall R, Monson RK (2003) Increased CO_2 uncouples growth from isoprene emission in an agriforest ecosystem. Nature 421:256–259

Scascighini N, Mattiacci L, D'Alessandro M, Hern A, Rott AS, Dorn S (2005) New insights in analysing parasitoid attracting synomones: early volatile emission and use of stir bar sorptive extraction. Chemoecology 15:97–104

Scholefield PA, Doick KJ, Herbert BMJ et al (2004) Impact of rising CO_2 on emissions of volatile organic compounds: isoprene emission from *Phragmites australis* growing at elevated CO_2 in a natural carbon dioxide spring. Plant Cell Environ 27:393–401

Sharkey AG, Shultz JL, Friedel RA (1956) Mass spectra of ketones. Anal Chem 28:934–940

Sharkey AG, Friedel RA, Langer SH (1957) Mass spectra of trimethylsilyl derivatives. Anal Chem 29:770–776

Singh J, Upadhyay AK, Bahadur A, Singh B, Singh KP, Rai M (2006) Antioxidant phytochemicals in cabbage (*Brassica oleracea* L. var. *capitata*). Sci Hortic 108:233–237

Stiling P, Cornelissen T (2007) How does elevated carbon dioxide (CO_2) affect plant-herbivore interactions? A field experiment and meta-analysis of CO_2-mediated changes on plant chemistry and herbivore performance. Glob Change Biol 13:1823–1842

Sun YC, Su JW, Ge F (2010) Elevated CO_2 reduces the response of *Sitobion avenae* (Homoptera: Aphididae) to alarm pheromone. Agric Ecosyst Environ 135:140–147

Thammakhet C, Muneesawang V, Thavarungkul P, Kanatharana P (2006) Cost effective passive sampling device for volatile organic compounds monitoring. Atmos Environ 40:4589–4596

Tholl D, Boland W, Hansel A, Loreto F, Röse USR, Schnitzler JP (2006) Practical approaches to plant volatile analysis. Plant J 45:540–560

Vallat A, Dorn S (2005) Changes in volatile emissions from apple trees and associated response of adult female codling moths over the fruit-growing season. J Agric Food Chem 53:4083–4090

Velikova V, Tsonev T, Barta C et al (2009) BVOC emissions, photosynthetic characteristics and changes in chloroplast ultrastructure of *Platanus orientalis* L. exposed to elevated CO_2 and high temperature. Environ Pollut 157:2629–2637

Verhoeven KJF, Simonsen KL, McIntyre LM (2005) Implementing false discovery rate control: increasing your power. Oikos 108:643–647

Vuorinen T, Reddy GVP, Nerg AM, Holopainen JK (2004) Monoterpene and herbivore-induced emissions from cabbage plants grown at elevated atmospheric CO_2 concentration. Atmos Environ 38:675–682

Wilkinson MJ, Monson RK, Trahan N et al (2009) Leaf isoprene emission rate as a function of atmospheric CO_2 concentration. Glob Change Biol 15:1189–1200

Yuan JS, Himanen SJ, Holopainen JK, Chen F, Stewart CN (2009) Smelling global climate change: mitigation of function for plant volatile organic compounds. Trends Ecol Evol 24:323–331