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Quantitative effects of enhanced CO₂ on jasmonic acid induced plant volatiles of lima bean (*Phaseolus lunatus* L.)

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Summary

Globally rising CO₂ has been predicted to affect plant biochemistry and in consequence the interaction of plants with multiple associated organisms. While CO₂ mediated effects on nutritive compounds and direct anti-herbivore defenses appear to be well studied for many plant species, the importance of altered atmospheric CO₂ concentrations on indirect defenses is only little understood. In this study, we analyzed the effects of enhanced CO₂ on the release of volatile organic compounds (VOCs) using jasmonic acid (JA) as a natural hormone-like inductor. We cultivated lima bean plants (*Phaseolus lunatus* L.) under different CO₂ concentrations (ambient, 500, 700, and 1000 ppm) and quantified the release of VOCs using GC-MS. The total release of VOCs significantly increased in response to higher CO₂ concentrations. However, the quantitative emission of individual compounds was differently affected. Nine out of twelve VOCs were released at significantly higher levels (cis-3-hexenylacetate, cis-ocimene, linalool, cis-3-hexenyl butyrate, methyl salicylate, cis-jasmone, β-caryophyllene, trans-geranylacetone, and methyl jasmonate) at enhanced CO₂, whereas two were not affected (2-ethylhexan-1-ol, cis-3-hexenyl isovalerate) and the release of indole decreased. Shifts in VOCs release under elevated CO₂ likely affect efficacy of plants' resistance to herbivores, as a range of the affected compounds are of central importance in defense-associated plant-plant and within-plant signaling. Given the importance of plant-herbivore interactions in terrestrial ecosystems, CO₂ mediated changes in defense-associated VOCs might have implications on agricultural and natural ecosystem functions.

Introduction

During this century, atmospheric CO₂ concentration is predicted to double (HOUGHTON et al., 1996). We are only beginning to comprehend the consequences of globally rising CO₂ on plants and potentially resulting effects at the ecosystemic level (Bazzaz, 1990; MOONEY et al., 1991; EMMERSON et al., 2005; LONG et al., 2004). However, in numerous laboratory and controlled out-door studies (Free-Air CO₂ Enrichment; FACE-experiments) enhanced carbon dioxide have been demonstrated to alter plant phenotypes and to induce significant changes in phytochemical composition (reviewed by BEZEMER and JONES, 1998; DRIGO et al., 2007). Elevated levels of CO₂ commonly increase photosynthetic carbon uptake albeit plants adapt their carboxylation potential to higher CO₂ supply by reduction of their Rubisco content (AINSWORTH and LONG, 2005). Results from FACE experiments reveal that the degree of Rubisco reduction is linked to nitrogen (N) supply and carbon (C) sink capacity: plants growing at high N supply exhibit lower reduction of carboxylation potential than those grown under N limitation. Also, plants with large C sink capacities minimize the associated loss of Rubisco when grown at elevated CO₂ (LEAKEY et al., 2009). In legumes, N supply is linked to C sink strength via

the extensive allocation of photoassimilates to N-fixing symbiotic bacteria. Thus, the prominent response of legumes to elevated CO₂ (AINSWORTH and LONG, 2005) can be explained by the fact that the surplus of C is invested into higher N fixation allowing simultaneously to strengthen C sink capacity and to increase N supply which both counteract Rubisco loss at elevated CO₂ (LEAKEY et al., 2009). Nevertheless, the surplus of photosynthates under elevated CO₂ seems to exceed the requirements even of high performing root symbionts leading to an evident increase in foliar carbohydrate content (ROGERS et al., 2006). Thus, it can be expected that the increased concentration of photosynthates and their intermediates alter the homeostasis of various biosynthetic pathways resulting in modified leaf traits. Excess carbon may be directed to production of various storage carbohydrates but also to a broad range of defensive traits ranging from carbon-based secondary metabolites to special defense-associated structures such as trichomes or cell walls. While providing a mechanical defense, these structures also dilute plant nutritive compounds and, thus, additionally reduce palatability to herbivores (PENUÉLAS and ESTIARTE, 1998; BEZEMER et al., 2000; VETELI et al., 2002; HOLTON et al., 2003).

With focus on chemical defenses, enhanced CO₂ frequently has been reported to increase concentration of C-based phenolics and terpenoids (e.g. LINDROTH et al., 1997; HARTLEY et al., 2000; MUMM et al., 2008), but also the production of nitrogen-based defensive compounds such as cyanogenic glycosides or alkaloids can be affected (FREHNER et al., 1997; ZISKA et al., 2005; GLEADOW et al., 2009; BALLHORN et al., 2011a). In contrast to direct defenses, effects of elevated CO₂ on variation of indirect plant defenses against herbivores have been comparably rarely studied (MUMM and HILKER, 2006). Indirect plant defenses involve a higher trophic level by attracting carnivores (generally arthropod predators or parasitoids) to the damaged plant, which then prey or parasitize herbivores and consequently reduce herbivore pressure (Fig. 1). These defenses are considered to have high importance for multiple interactions among organisms in natural and agricultural ecosystems (reviewed by HEIL, 2008).

Lima bean (Fabaceae: *Phaseolus lunatus* L.) is an important crop plant in many subtropical and tropical regions around the globe (MARTÍNEZ-CASTILLO et al., 2008) and it is a prominent model plant for studies on direct and indirect plant defenses against herbivorous arthropods (e.g., HORIUCHI et al., 2003; MITHÖFER et al., 2005; BALLHORN and LIEBEREI 2006; BALLHORN et al., 2008a, 2009, 2010a,b, 2011b). Indirect defenses of lima bean include the release of herbivore-induced volatile organic compounds (VOCs) and the secretion of extrafloral nectar (EFN) (Fig. 1). Both types of inducible defenses have been investigated extensively for the last decade under laboratory (e.g. CHOH and TAKABAYSHI 2006; CHOH et al., 2006) and field conditions (HEIL and SILVA BUENO, 2007; HEIL, 2008). In addition to attracting carnivores, VOCs of lima bean have multiple functions in defense-associated plant signaling. Herbivore induced VOCs apparently can be perceived by neighboring, yet-undamaged plant individuals (plant-plant signaling) or plant parts of the same

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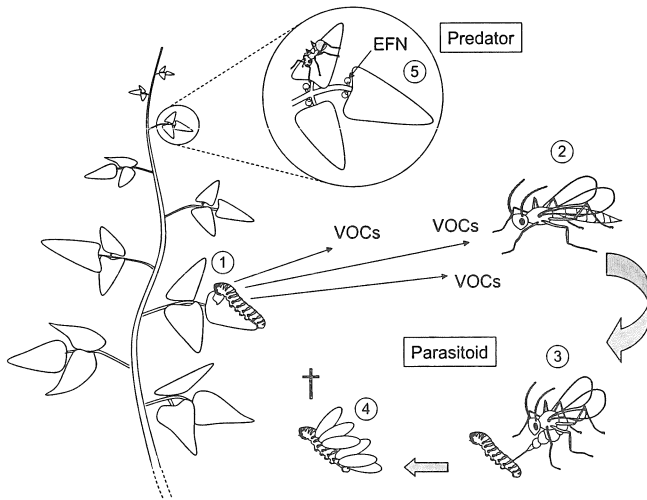


Fig. 1: Indirect defenses of lima bean

In response to herbivore damage (1) lima bean plants release a bouquet of volatile organic compounds (VOCs), which serve as location cues for parasitoid wasps (2). These wasps deposit eggs in their hosts (3), the developing larvae feed on the host's inner tissues, and in the end pupate outside the host's cuticle (4). Since the host finally is killed by wasp's larvae, the attraction of parasitoid wasps represents an indirect plant defense. In addition to VOCs, damaged lima bean plants produce extrafloral nectar (EFN), which is used as food source predominantly by ants (5). Searching for EFN, the ants patrol the plant's surface and deter or prey on arthropod herbivores. Thus, the attraction of ants by wound-induced secretion of EFN represents an additional indirect defense.

individual (within-plant signaling) leading to priming or induction of defensive responses (e.g. ARIMURA et al., 2002; HEIL and SILVA BUENO, 2007).

Although there is an accumulating amount of data on herbivore induced plant volatiles and their ecological functions (DICKE and BALDWIN, 2010) until now it remains elusive which factors other than herbivores themselves contribute to variation of VOCs in a given plant species. The aim of our present study is to contribute understanding of how elevated CO₂ affects the quantitative and qualitative release of defense-associated VOCs. Results of our work are discussed in the light of indirect plant defenses against herbivores, plant-plant and within plant signaling under changing environmental conditions.

Material and methods

Plant material

Lima bean plants were grown from seeds collected in a natural population at a coastal site 10 km west of Puerto Escondido, Oaxaca, Mexico (~15°55'446'' N and 097°09'107'' W, elevation 11 m). The testa of the seeds was scratched to facilitate water absorption and to ensure homogenous germination of seeds. Plants were cultivated separately in black plant-containers with 8 cm in diameter in a 1:1 ratio of standard substrate (TKS-1-Instant, Floragard, Oldenburg, Germany) and sand (grain size 0.5-2.0 mm), and were fertilized with 50 mL of a 0.1% aqueous solution of Flory-3® (NPK-Fertilizer, EUFLOR GmbH, Munich, Germany) twice a week and watered daily. Plants were grown in climatic chambers (see below) for 4 weeks and were allowed to climb up wooden sticks of 60 cm (from substrate surface). When reaching the top of these sticks (after approx. 2 weeks), climbing shoots were wrapped around the sticks by hand to avoid burning of shoot tips at the lamps.

Experimental design

In this study, we used closed-top acryl glass cabinets positioned in an automated climate chamber. In the cabinets we applied four different CO₂ treatments: (i) ambient (= control) CO₂ (~ 380 ppm), (ii) 500 ppm CO₂, (iii) 700 ppm CO₂, and (iv) 1000 ppm CO₂. The actual CO₂ partial pressure was continuously monitored by the IRGA system BINOS 100 (Rosemount/Emerson). Pure CO₂-gas (CARBO/Bad Hönningen) was mixed with ambient air by means of float type gas flow meters (HPS, Martinsried). In each cabinet we placed 12 lima bean plants. Artificial climate was adjusted to 29:23 °C in a 13:11 hr light/dark period (light: photon flux density 450-500 μmol photons s⁻¹ m⁻² at table height, Son-T Agro 400, Philips®, Hamburg, Germany) and an air humidity of 70-80%. During the experimental period, the treatment groups of plants were rotated between the 4 cabinets at regular intervals (once a week) and the particular CO₂ atmospheres were re-adjusted respectively. This rotational procedure was conducted to avoid any effects of physical environment on plant development.

Analysis of VOCs

Plants were induced for volatile production by spraying 10 ml of a 1 mmol L⁻¹ aqueous solution of jasmonic acid (JA) per plant one hour after the beginning of the light period in the chambers (N = 8 plants per CO₂-treatment group). Jasmonic acid is a natural plant hormone centrally involved in the release of herbivore-induced plant volatiles and is frequently used for experimental induction of these volatiles (e.g., MITHÖFER et al., 2005; HEIL and SILVA BUENO, 2007; BALLHORN et al., 2008b). Outside the chamber, plants were sprayed until runoff and subsequently allowed to dry (ca. 30 min) in the chamber; the procedure was repeated once. After drying, plants were placed in a PET (poly ethylene) bag ('Bratenschlauch', Toppits®, Minden, Germany), a material that does not emit detectable amounts of volatiles even after exposure to temperatures up to 150 °C. Both ends of the bag were tied up avoiding shoot damage. Bagged plants were placed into their respective chambers. Volatiles were collected continuously over 24 h on charcoal filters (1.5 mg charcoal, CLSA-Filters, Le Ruissaeu de Montbrun, France) using air circulation in closed loop stripping as described in DONATH and BOLAND (1995). After 24 h volatiles were eluted from the carbon filter with dichloromethane (40 μl) containing 1-bromodecane (200 ng μl⁻¹) as internal standard (IS). Samples were analyzed on a GC-Trace mass spectrometer (Trace GC Ultra DSQ; Thermo Electron, Austin, TX). The program for separation [RTX5-MS column (Restek, Philadelphia, PA), 15 m x 0.25 mm; 0.25 μm coating] was 40 °C initial temperature (2 min), 10 °C min⁻¹ to 200 °C, then 30 °C min⁻¹ to 280 °C with He (constant flow 1.5 ml min⁻¹) as carrier gas. Compounds were identified by comparison to standard substances (Fluka, Seelze, Germany) and with the Nist 05 library (Xcalibur 1.4 software; Thermo Electron Corp., Austin, TX). Individual compounds (peak areas) were quantified with respect to the peak area of the internal standard (1-bromodecane), and quantities are presented as a percentage of the internal standard's area. Only compounds for which reference substances were available were included in quantitative analysis. Quantitative data on released VOCs were corrected for g plant fresh weight.

To test for effects of CO₂ on the constitutive release of VOCs, four plants per CO₂ treatment were analyzed in the same way as described above but with application of water instead of of JA.

Quantification of plant growth

When VOC collection experiments were finished, PET bags were removed, plants were cut off at substrate surface, and fresh weight of above ground plant material was determined. We determined

biomass after drying the plants at 45 °C for 96 h. Dry plant material was weighed to the nearest 0.001g (MC 1 Analytic AC 210 S, Sartorius®, Göttingen, Germany).

Statistical analysis

To test for treatment effects on VOC emission, we applied a general linear model (GLM) for analysis of variance after a univariate design with 'Quantity of compound' as dependent variable and 'CO₂-Treatment' as factor variable (Tab. 1). We conducted post hoc analysis after one-way ANOVA to test for significant differences in release among the various CO₂ treatments. In these analyses, each compound was considered separately. In the same way we tested for significant differences for total release of VOCs, plant fresh weight and biomass (dry weight). All statistical analyses were carried out using SPSS 17 (SPSS for Windows; SPSS Inc., Chicago, IL, USA).

Tab. 1: Effects of CO₂ treatment on release of JA induced plant volatiles. Results of univariate ANOVA obtained using the GLM with 'Quantity of compound' as dependent variable and 'CO₂-Treatment' as factor variable.

Dependent variable	SS	F	p
cis-3-Hexenyl acetate	1565.59	10.14	< 0.001
2-Ethylhexan-1-ol	0.36	0.56	0.647
cis-β-Ocimene	11.98	3.30	< 0.05
Linalool	383.43	4.44	< 0.05
cis-3-Hexenyl butyrate	2495.43	5.86	< 0.01
Methyl salicylate	13.89	8.22	< 0.001
cis-3-Hexenyl isovalerate	17.35	1.89	0.155
Indole	451.97	5.34	< 0.01
cis-Jasmone	2284.74	3.70	< 0.05
β-Caryophyllene	29118.12	5.20	< 0.01
trans-Geranylacetone	32.12	2.33	0.096
Methyl jasmonate	2209.86	3.20	< 0.05

Results

Release of VOCs

The overall constitutive release of volatile organic compounds (VOCs) was low and showed no significant differences depending on CO₂ treatment (Fig. 2A). Among the constitutively released VOCs, β-caryophyllene was the most prominent compound and was released in quantities ranging from 3.74 and 5.11 ng g⁻¹ fw (Fig. 3). cis-3-Hexenylacetate, cis-ocimene, linalool, cis-3-hexenyl butyrate, and cis-3-hexenyl isovalerate were released in substantially lower concentrations (< 1.96 ng g⁻¹ fw), while 2-ethylhexan-1-ol, methyl salicylate, indol, cis-jasmone, trans-geranylacetone, and methyl jasmonate were below detection limit (Fig. 3).

In response to jasmonic acid (JA) treatment plants grown at different ambient CO₂ concentrations showed substantial quantitative variation in release of VOCs (Fig. 2B). The total amount of VOCs released per g plant fresh weight increased significantly corresponding to enhanced CO₂ concentration [according to post-hoc analysis (LSD; P < 0.05) after one-way ANOVA; Fig. 2B]. Total VOCs released from plants growing at 500, 700, and 1000 ppm increased by factor 1.89, 2.11 and 3.68 (mean) compared to plants grown at ambient (i.e. natural) CO₂ concentration.

While the total release of VOCs showed a significant overall increase, emissions of individual compounds were differently affected

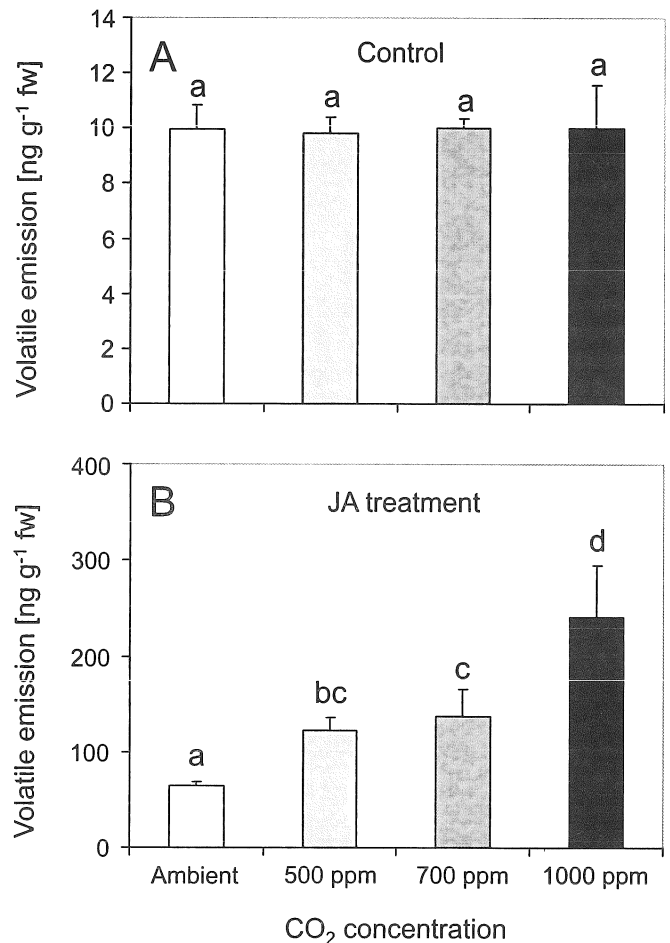


Fig. 2: Constitutive and induced release of VOCs at different CO₂ concentrations.

The total amount of volatiles released from non-induced (A) and jasmonic acid induced (B) lima bean plants grown under different CO₂ concentrations was measured over a period of 24 h. Values represent means ± SE; N = 8 (A) and N = 4 (B) plants per treatment. Different letters at the columns indicate significant differences in release of VOCs among the various CO₂ treatments [according *post-hoc* analysis (Tukey HSD, P < 0.05) after one-way ANOVA].

by CO₂ treatments (according to GLM; Tab. 1). Nine of twelve compounds analyzed in this study were released at significantly higher rates from plants grown at elevated CO₂ (cis-3-hexenyl acetate, cis-β-ocimene, linalool, cis-3-hexenyl butyrate, methyl salicylate, cis-jasmone, β-caryophyllen, trans-geranylacetone, methyl jasmonate), two were not significantly affected by CO₂ treatments (2-ethylhexan-1-ol and cis-3-hexenyl isovalerate) or even decreased (indole) (Fig. 4). This quantitative variation resulted in substantial differences in volatile bouquet composition released from lima bean plants exposed to different CO₂ regimes. Indole was the major compound released from induced plants at ambient CO₂ followed by β-caryophyllene, and cis-3-hexenyl acetate, whereas plants treated with slightly enhanced CO₂ (500 ppm) released β-caryophyllene at highest rates followed by methyl jasmonate. The release of indole, from plants treated with 500 ppm CO₂, however, showed a significant decrease (Fig. 4). While this pattern was similar for plants treated with 700 ppm CO₂, the VOC composition of plants growing under enhanced CO₂ at highest concentration (1000 ppm) was dominated by the five compounds β-caryophyllene, cis-3-hexenyl butyrate, methyl jasmonate, cis-jasmone, and cis-3-hexenyl acetate (Fig. 4).

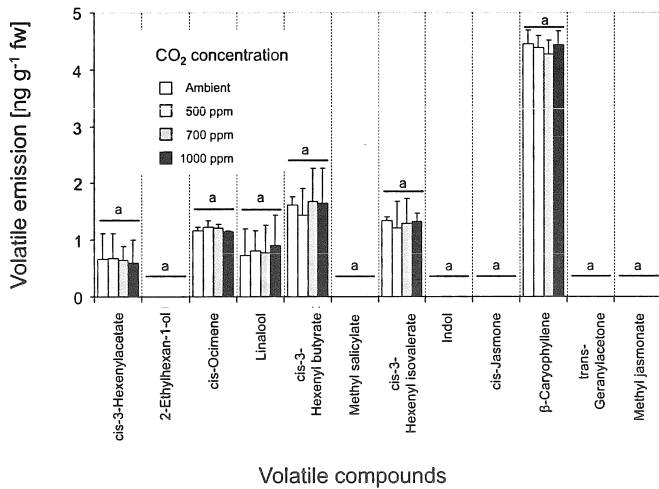


Fig. 3: Variation of constitutively released plant volatiles at different CO₂ concentrations.

Lima bean plants were exposed to various CO₂ concentrations and the amount of constitutively emitted volatile compounds (VOCs) was measured over a period of 24 h. Different letters at the columns indicate significant differences in release of VOCs among the various CO₂ treatments [according to *post-hoc* analysis (LSD, $P < 0.05$) after one-way ANOVA]. Analyses were conducted for each compound separately. Values are means \pm SE; N = 8 plants per treatment.

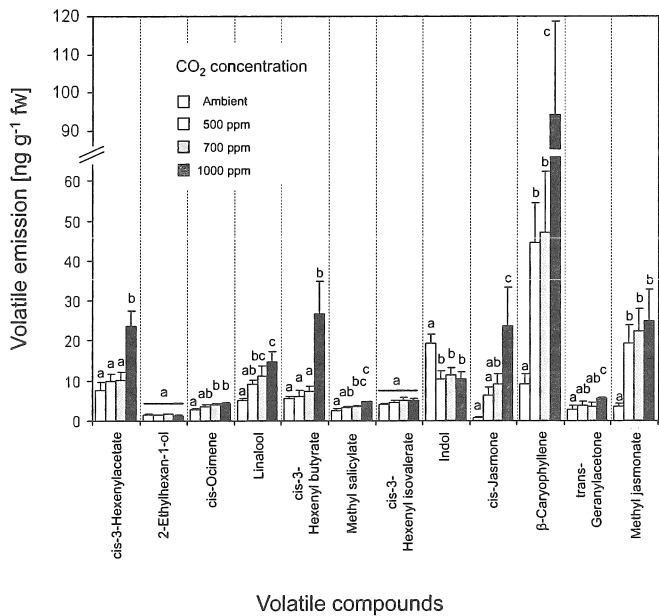


Fig. 4: Variation of induced plant volatiles at different CO₂ concentrations. Lima bean plants were exposed to various CO₂ concentrations and the amount of volatile compounds (VOCs) released in response to jasmonic acid treatment was measured over an experimental period of 24 h. Different letters at the columns indicate significant differences in release of VOCs among the various CO₂ treatments [according to *post-hoc* analysis (LSD, $P < 0.05$) after one-way ANOVA]. Analyses were conducted for each compound separately. Values are means \pm SE; N = 8 plants per treatment.

Plant biomass accumulation

Over the experimental period, lima bean plants exposed to elevated CO₂ accumulated significantly more biomass than the control plants

(Fig. 5). Differences in biomass accumulation among CO₂ treated plants corresponded quantitatively to the experimentally applied CO₂ concentration. Biomass of plants grown at 500 ppm was increased by factor 1.22 compared to the control plants, while plants grown at 700 and 1000 ppm CO₂ showed an increase in biomass of factor 1.36 and 1.40 (mean) respectively. The dry matter - fresh weight ratio was not significantly different between the four CO₂-treatments (data not shown).

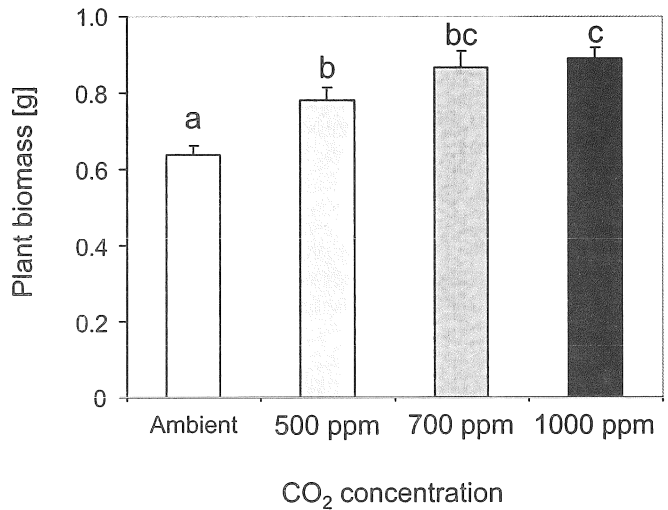


Fig. 5: Biomass accumulation of lima bean plants.

Biomass of lima bean plants grown at different CO₂ concentrations was determined. Different letters at the columns indicate significant differences among the various CO₂ treatments [according to *post-hoc* analysis (Tukey HSD, $P < 0.05$) after one-way ANOVA]. Values represent means \pm SE; N = 8 plants per treatment.

Discussion

In the present study we show that elevated CO₂ did not significantly affect the constitutive release of VOCs from lima bean plants but resulted in substantial quantitative variation once release of defense-associated VOCs is induced by application of jasmonic acid (JA) (Fig. 2A, B; Fig. 3). In response to JA treatment, various compounds were released at significantly higher rates from plants growing at elevated CO₂ than compared to the controls (Fig. 4). This observed increase in VOC emission under enhanced CO₂ concentrations could not be explained by the mere increase in VOC releasing foliage, since quantitative data on VOCs were corrected for leaf fresh weight. Furthermore, although plant biomass and, proportionally, fresh weight of foliage increased at elevated CO₂, the amounts of some volatiles showed little or no variation (2-ethylhexan-1-ol and cis-3-hexenyl isovalerate) or were significantly decreased at experimentally enhanced CO₂ levels (indole) (Fig. 3). Interestingly, indole is the only N containing compound of the volatiles detected in this study. The fact that exclusively indole release declined supports the hypothesis that even in legumes the surplus of carbohydrates under elevated CO₂ modifies the N homeostasis to the debit of N containing secondary compounds.

What could be the ecosystemic consequences of such shifts in quantitative release of individual volatile compounds? Changes in herbivore-induced VOCs may directly affect the interaction between (attacked) plants and herbivores. Chrysomelid herbivores of wild lima bean for example have been reported to be efficiently repelled by their host plants' volatiles in nature (HEIL, 2004). In addition to direct effects on herbivores, herbivore-induced VOCs play a major

role in indirect plant defense attracting natural enemies to the attacked plant (HEIL, 2008) (Fig. 1). However, parasitoids involved in indirect plant defense generally show substantial ability to adapt to signals transmitted by wound-induced plant volatiles and thus, are likely to adjust by reinterpreting altered VOC compositions (VET et al., 1995; DE BOER et al., 2005). This associative learning allows for example the hymenopteran parasitoids *Microplitis croceipes* and *Leptopilina heterotoma* to focus on the most reliable cues (VET et al., 1995; MEINERS et al., 2003) which is essential for their foraging success as natural odors encountered by the parasitoids in their natural environment are both complex and variable mixtures.

While parasitoids show high plasticity in their responses to specific plant volatiles and VOC bouquets, plant responses to herbivore induced VOCs are more conservative. Thus, variation of herbivore-induced VOCs in response to changing environmental conditions as we report here may crucially affect defense-associated plant-plant and within-plant signaling. At level of intra-specific plant-plant signaling, wound-induced volatiles can be used by other plant individuals to adjust their defensive phenotype accordingly (RHOADES, 1983; BRUIN et al., 1992; DOLCH and TSCHARNTKE, 2000). In this line, for lima bean it has been shown that extrafloral nectar secretion by undamaged plants increased in response to volatiles from herbivore-damaged plants (CHOH et al., 2006; HEIL, 2008). Most importantly, under natural field conditions it was demonstrated that increased EFN secretion by plants exposed to volatiles from beetle-damaged conspecifics showed reduced herbivory rates (HEIL and SILVA BUENO, 2007). In addition to intraspecific plant-plant signaling, similar phenomena have been reported on the interspecies level. For example, KARBAN et al. (2000, 2003, 2006) showed that clipped sagebrush can induce polyphenol oxidase in wild tobacco plants. However, rather than showing immediate defense reactions, many plants are primed by VOCs, that is, plants do not show detectable expression of resistance traits, but they respond more strongly once they are attacked or infected (ZIMMERLI et al., 2000; ENGELBERTH et al., 2004; CONRATH et al., 2006; TON et al., 2007). As observed for induction of defenses, priming is involved in both the signaling between conspecifics and plants of different species (reviewed by DICKE and BALDWIN, 2010).

In addition to plant-plant signaling VOCs also serve as plant-internal signals and, for instance, mediate signaling among different parts of the same plant individual, particularly in cases where direct vascular connections are restricted (FARMER, 2001; ORIANS, 2005; KARBAN et al., 2006; HEIL, 2008; RODRIGUEZ-SAONA et al., 2009). In poplar, as well as in lima bean, VOCs released by damaged leaves primed and induced EFN secretion by neighboring leaves of the same individual plant (FROST et al., 2007; HEIL and SILVA BUENO, 2007). Volatile organic compounds can thus have a hormone-like function, mediating systemic induction in response to local damage. However, the efficiency of volatiles in plant-plant and within-plant signaling appears to be restricted to specific compounds. Volatile organic compounds identified to cause priming or induction of defense in undamaged plants include cis-3-hexenylacetate (HEIL et al., 2008) and several structurally related C6-volatiles (ENGELBERTH et al., 2004; FARAG et al., 2005; RUTHER and KLEIER, 2005). In addition to cis-3-hexenylacetate, methyl jasmonate and methyl salicylate show distinct biological activity. Methyl jasmonate has been demonstrated to induce the synthesis of proteinase-inhibitors in undamaged plants representing an efficient defense against herbivores (FARMER and RYAN, 1990) while methyl salicylate represents an elicitor of resistance responses directed towards both pathogens and herbivores (SHULAEV et al., 1997; SCHLENK et al., 2000).

With focus on lima bean, only recently cis-3-hexenylacetate was demonstrated to cause priming or induction of extrafloral nectar and reduce herbivore damage in nature (HEIL and SILVA BUENO, 2007; HEIL et al., 2008). Furthermore, laboratory and field studies

on lima bean showed that the inducible volatile nonanal significantly enhanced expression of pathogenesis-related protein 2 (PR-2) in undamaged plants exposed to the headspace of pathogen-infested individuals. Expression of PR-2 resulted in increased resistance to the plant-pathogenic bacterium *Pseudomonas syringae* pv. *syringae* (YI et al., 2009).

In our study we used jasmonic acid (JA) instead of herbivores to elicit the release of defense-associated VOCs, because this chemical induction has the advantage to provide controlled and comparable intensities of induction among experimental plants. Jasmonic acid is a natural plant hormone centrally involved in plant responses to herbivory. However, the question may arise whether patterns of VOCs released in response to JA treatment correspond to volatile bouquets released following herbivore damage. Studies by MITHÖFER et al., (2005) and KOCH et al., (1999) revealed that VOCs released from lima bean following JA treatment, mechanical wounding and herbivory are similar. Thus, findings on CO₂ mediated effects on VOC emission following JA treatment likely can be transferred to plant-herbivore systems.

In the present study we show that amongst other VOCs of lima bean the biologically active compounds cis-3-hexenylacetate, methyl jasmonate, and methyl salicylate underlay substantial variation in response to different CO₂ regimes. Our findings suggest that globally rising CO₂ levels can quantitatively affect defense-associated plant-plant and within-plant signaling.

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