

Monoterpene and herbivore-induced emissions from cabbage plants grown at elevated atmospheric CO₂ concentration

Terhi Vuorinen^{a,*}, G.V.P. Reddy^b, Anne-Marja Nerg^a, Jarmo K. Holopainen^a

^a Department of Ecology and Environmental Science, University of Kuopio, P.O.Box 1627, FIN-70211 Kuopio, Finland

^b Agricultural Experiment Station, University of Guam, Mangilao, Guam 96923, USA

Received 27 March 2003; received in revised form 25 September 2003; accepted 10 October 2003

Abstract

The warming of the lower atmosphere due to elevating CO₂ concentration may increase volatile organic compound (VOC) emissions from plants. Also, direct effects of elevated CO₂ on plant secondary metabolism are expected to lead to increased VOC emissions due to allocation of excess carbon on secondary metabolites, of which many are volatile. We investigated how growing at doubled ambient CO₂ concentration affects emissions from cabbage plants (*Brassica oleracea* subsp. *capitata*) damaged by either the leaf-chewing larvae of crucifer specialist diamondback moth (*Plutella xylostella* L.) or generalist Egyptian cotton leafworm (*Spodoptera littoralis* (Boisduval)). The emission from cabbage cv. Lennox grown in both CO₂ concentrations, consisted mainly of monoterpenes (sabinene, limonene, α -thujene, 1,8-cineole, β -pinene, myrcene, α -pinene and γ -terpinene). (*Z*)-3-Hexenyl acetate, sesquiterpene (*E,E*)- α -farnesene and homoterpene (*E*)-4, 8-dimethyl-1, 3, 7-nonatriene (DMNT) were emitted mainly from herbivore-damaged plants. Plants grown at 720 $\mu\text{mol mol}^{-1}$ of CO₂ had significantly lower total monoterpene emissions per shoot dry weight than plants grown at 360 $\mu\text{mol mol}^{-1}$ of CO₂, while damage by both herbivores significantly increased the total monoterpene emissions compared to intact plants. (*Z*)-3-Hexenyl acetate, (*E,E*)- α -farnesene and DMNT emissions per shoot dry weight were not affected by the growth at elevated CO₂. The emission of DMNT was significantly enhanced from plants damaged by the specialist *P. xylostella* compared to the plants damaged by the generalist *S. littoralis*. The relative proportions of total monoterpenes and total herbivore-induced compounds of total VOCs did not change due to the growth at elevated CO₂, while insect damage increased significantly the proportion of induced compounds. The results suggest that VOC emissions that are induced by the leaf-chewing herbivores will not be influenced by elevated CO₂ concentration.

© 2003 Elsevier Ltd. All rights reserved.

Keywords: Herbivory; Induced defense; Monoterpene; *Plutella xylostella*; *Spodoptera littoralis*; VOCs

1. Introduction

CO₂-induced warming of the lower atmosphere as well as elevated CO₂ concentration intrinsically, are predicted to increase the emissions of volatile organic compounds (VOCs) from plants (Constable et al., 1999a). Biogenic VOCs substantially contribute to the

hydrocarbon load into the atmosphere, and significantly affect ozone and aerosol formation, methane oxidation and carbon monoxide budget (Andreae and Crutzen, 1997; Peñuelas and Llusà, 2001, 2003). Nevertheless, the number of experiments to analyse VOC emission from plants grown at elevated CO₂ is still limited.

Feeding damage of leaf-chewing insect larvae on plant tissue result in emission of wide variety of VOCs including mono-, homo- and sesquiterpenes, alcohols, ketones, aldehydes, esters, nitriles, sulfides, (iso)thiocyanates, carboxylic acids, and others (Geervliet et al.,

*Corresponding author. Tel.: +358-17-163188; fax: +358-17-163230.

E-mail address: terhi.vuorinen@uku.fi (T. Vuorinen).

1997). The ratios among herbivore-induced monoterpenes (MTs) and sesquiterpenes may vary considerably between plant varieties (Gouinguene et al., 2001). The proportion of MTs attains nearly 50% of the total VOC emission spectrum in intact white cabbage plants, but remains below 25% in herbivore-damaged plants (Geervliet et al., 1997). Green leaf volatiles (GLVs), like (Z)-3-hexen-1-ol and (Z)-3-hexenyl acetate, are derived from the octadecanoid pathway and they are emitted rapidly after mechanical or herbivore damage (Turlings et al., 1998; Kessler and Baldwin, 2001), while the induced terpenes are emitted more slowly, starting to be released after 4 h and peaking within 10 h after the damage (Turlings et al., 1998).

In the few studies investigating the effects of elevated CO₂ concentration on plant emissions the results are controversial. Staudt et al. (2001) studied emissions of non-terpene storing *Quercus ilex* L. seedlings, and measured on average 1.8-fold higher MT emission capacities in plants grown at elevated CO₂ than in plants grown at ambient CO₂. They did not detect any short-term effect of assay CO₂ concentration (350 and 700 $\mu\text{l l}^{-1}$) on the emission capacities in both CO₂ treatments. On the other hand, elevated CO₂ inhibited the emission of certain MTs from *Q. ilex* seedlings due to concurrent down-regulation of corresponding MT synthase activities (Loreto et al., 2001). In the case of limonene, the down-regulation of corresponding enzyme activity did not occur, leading to the enhanced emission of limonene at elevated CO₂ (Loreto et al., 2001). Constable et al. (1999b) found that in Ponderosa pine and in Douglas fir, which both store terpenes in specialized secretory organs, there was no significant effect of elevated CO₂ (700 $\mu\text{mol mol}^{-1}$) on either needle MT concentration or emission rate per unit biomass. However, they concluded that the effect of elevated CO₂ on leaf area index and the effect of climate warming on MT biosynthesis and volatilization could increase MT emissions from the canopy.

The objective of our present study was to evaluate the effects of growth at elevated CO₂ concentration on VOC emission from cabbage plants damaged by either crucifer insect specialist or generalist insect herbivore. Cabbage is an important crop plant which is cultivated in both southern and northern hemispheres. Diamond-back moth is the most serious pest of crucifers throughout the world (Talekar and Shelton, 1993). Also another lepidopteron species, polyphagous Egyptian cotton worm, was selected since plant response might be different to particular herbivore species (DeMoraes et al., 1998). Another aim was to provide information of the emission response of plants to the insect damage under stress factor of global change. This information is needed in the estimation of future biogenic VOC emissions under changing climatic conditions, as well as in the development of novel plant protection methods

based on tritrophic signalling (Dicke et al., 1990; Lewis et al., 1997; Thaler, 1999; Cortesero et al., 2000; Hunter, 2001), repellents (Ibrahim et al., 2001) and attractants (Reddy and Guerrero, 2000). As far as we know, this is the first investigation to examine the capability of plants grown at elevated CO₂ to emit VOCs after herbivore damage.

2. Materials and methods

2.1. Plant material and CO₂ exposure

Seedlings of white cabbage (*Brassica oleracea* subsp. *capitata* cv. Lennox) were individually sown in 1–1 plastic pots filled with *Sphagnum* peat and sand (3:1 v/v). Seedlings were grown 24–26 days under ambient (360 $\mu\text{mol mol}^{-1}$) or elevated (720 $\mu\text{mol mol}^{-1}$) CO₂ concentration in growth chambers (Bioklim 2600T, Kryo-Service Oy, Helsinki, Finland) constructed for air pollutant exposures (e.g. Sallas et al., 2003) at 23:18°C, 70:80% RH and 22h light:2h dark photoperiod (250 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR during light period). The CO₂ exposure was maintained 24 h day⁻¹ for the whole experimental period. The seedlings were watered daily with tap water and fertilized weekly with 0.1% of 9-Suprex (19:5:20 N:P:K, Kekkilä, Finland) at the rate of 0.05–0.11 plant⁻¹, starting 2 weeks after sowing. The CO₂ treatments and seedlings were rotated among the two chambers weekly to randomize any systematic chamber effect across the seedlings. The growth chamber conditions were constantly monitored.

2.2. Insect feeding

The larvae of leaf-chewing moths were used to cause herbivore damage on cabbage seedlings. Larvae of crucifer specialist *Plutella xylostella* L. (Lepidoptera: Yponomeutidae) were reared in acrylic polyester gauze cages (60 cm × 33 cm × 33 cm, external dimensions) at 25°C, 50% RH and 16 h light:8 h dark photoperiod. Each cage contained a 5–6 weeks-old broccoli (*B. oleracea* subsp. *italica*) seedling. Fresh plants were provided every 3–4 days for larval feeding. Larvae of generalist *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) were reared in the laboratory at 26 ± 1°C, 75 ± 10% RH and 16 h light:8 h dark photoperiod on slightly modified artificial diets from those previously reported (Poitout and Bues, 1974). Both insect cultures were maintained in the laboratory for several generations before the current experiments started.

Herbivore feeding damage was caused by 48-h feeding either by transferring eight third instar larvae of *P. xylostella* or four second or third instar larvae of *S. littoralis* on five randomly selected plants grown at

ambient or elevated CO₂. Five additional intact plants from both CO₂ levels were used as controls. To avoid any disturbance in control plants induced by VOC emissions from herbivore-damaged plants, plants with the feeding larvae were kept in a separate growth chamber in similar environmental conditions as described earlier.

2.3. Collection of volatile compounds

After 48-h larval feeding, five intact control plants from ambient air or elevated CO₂ concentration, as well as five *P. xylostella* or *S. littoralis*-damaged plants from both CO₂ concentrations were used in VOC collections. Whole plants with carefully rinsed and slightly pruned root system in a 15 ml vial filled with tap water were individually enclosed inside 1–1 glass cuvettes, which were closed with Teflon-sealed lids with one inlet for purified air with ambient CO₂ concentration and one for sampling. The airflow was calibrated with the mini-Buck calibrator (Model M-5, A.P. Buck, Inc., Orlando, FL, USA) and flow rate was set to 0.110 l min⁻¹ for filtered and pressured air, and 0.100 l min⁻¹ for sampling. The collection was performed at 22°C and at light intensity of 250 μmol m⁻² s⁻¹ under ambient CO₂ concentration. VOCs were collected for 1 h on ca. 150 mg Tenax-TA adsorbent (Supelco, mesh 60/80) by pulling the sample through 6 mm diameter Teflon tubing with a vacuum pump (KNF Neuberger, Inc., Freiburg, Germany, Model N022AN.18). More details of the collection system are given by Turtola et al. (2002). Samples were analysed by GC-MS (Hewlett Packard GC type 6890, MSD 5973). Trapped compounds were desorbed (Perkin Elmer ATD400 Automatic Thermal Desorption System) at 250°C for 10 min, cryofocused at -30°C and injected onto HP-5 capillary column (50 m × 0.2 mm i.d. × 0.5 μm film thickness, Hewlett Packard). The carrier gas was helium. The temperature program began at 40°C for 1 min, followed by increases of 5°C min⁻¹ to 210°C and 20°C min⁻¹ to 250°C. Compounds were identified by comparison of the mass spectra with those in the Wiley library and pure standards. For quantification commercially available reference substances were used. Reference substances for α-thujene, DMNT and (*E,E*)-α-farnesene were not available; therefore, the concentration of these compounds were calculated by proposing the responses to be the same as the responses of α-pinene, (*Z*)-ocimene and (*E*)-β-farnesene, respectively. After VOC collection, the above-ground biomass of the plants was determined and emissions were calculated as ng g dry weight⁻¹ h⁻¹.

2.4. Statistical analyses

Statistical analyses were performed using SPSS 11.0 for Windows statistical package. VOC data were

analysed mainly with multivariate general linear model (GLM) procedure using CO₂ as two-level fixed variable and herbivore damage as three-level fixed variable, and Tukey and Dunnett T3 were used as post hoc tests. In the case of (*Z*)-3-hexenyl acetate and DMNT, the main effect of herbivore damage was tested with Kruskal–Wallis test. Plant properties between two CO₂ levels in each herbivore damage treatment were tested with independent samples *t*-test.

3. Results

The dry weight of control cabbage shoots and *S. littoralis*-damaged shoots was higher in plants grown at elevated CO₂ concentration than at ambient CO₂ (Table 1). The number of leaves and the fresh/dry weight ratios of shoots were almost equal in all treatments at both ambient and elevated CO₂ (Table 1). Intensity of leaf damage did not differ between CO₂ treatments, but in plants grown at ambient air *S. littoralis* caused more ($t = -2.54$, $df = 8$, $p = 0.035$) small-sized feeding holes than *P. xylostella*.

The emission rates of individual MTs from cabbage cv. Lennox grown at ambient or elevated CO₂ were in descending order: sabinene, limonene, α-thujene, 1,8-cineole, β-pinene + myrcene (compounds were not separated on HP-5 column), α-pinene and γ-terpinene. The emissions per shoot dry weight of all the MTs, except γ-terpinene, were significantly decreased when plants were grown at elevated CO₂, but increased by herbivore damage (Table 2). In general, total MT emission per shoot dry weight was approximately 27% reduced from plants grown at elevated CO₂ and this proportion was not affected by insect damage (Fig. 1a). On average, insect feeding increased total MT emission significantly (Fig. 1a), emission being significantly higher from *P. xylostella*-damaged plants than from control plants. On the other hand, total MT emission expressed per shoot fresh weight, was only slightly reduced in plants grown at elevated CO₂ ($F = 3.899$, $p = 0.060$), while herbivore damage again increased total MT emission significantly ($F = 5.092$, $p = 0.014$). Using shoot dry weight as a covariate, the total MT emission per plant did not respond to CO₂ treatment, but increased significantly due to herbivore damage ($F = 8.294$, $p = 0.002$).

The total VOC emission per shoot dry weight was also significantly increased by damage of both insect species compared to control; *P. xylostella*-damage causing higher increase in emission than *S. littoralis*-damage (Fig. 1b). When total VOC emission was expressed per shoot fresh weight, corresponding significant increase in emission by both herbivores was found ($F = 18.923$, $p < 0.001$). Total VOC emission expressed per shoot dry weight or shoot fresh weight was not affected by the growth CO₂ concentration. Using plant dry weight as a

Table 1

Plant dry weight, the number of leaves per plant, fresh/dry weigh ratio, number of small feeding holes ($\varnothing < 2$ mm) and number of big feeding holes ($\varnothing > 2$ mm) per leaf (mean \pm SE) in intact control cabbage plants and in cabbage plants damaged by *P. xylostella* or *S. littoralis* grown under ambient ($360 \mu\text{mol mol}^{-1}$) or elevated ($720 \mu\text{mol mol}^{-1}$) CO_2 ($n = 5$)

Treatment	Ambient CO_2	Elevated CO_2	<i>t</i>	<i>p</i> -value
<i>Control</i>				
Plant dry weight (g)	0.89 \pm 0.03	1.31 \pm 0.13	-3.038	0.016
Leaves per plant	5.80 \pm 0.20	5.80 \pm 0.20	0.000	1.000
Fresh/dry weigh ratio	7.51 \pm 0.57	6.21 \pm 0.41	1.851	0.101
<i>P. xylostella</i>				
Plant dry weight (g)	1.02 \pm 0.09	1.15 \pm 0.09	-0.984	0.354
Leaves per plant	5.40 \pm 0.40	5.20 \pm 0.20	0.447	0.667
Fresh/dry weigh ratio	7.18 \pm 0.28	6.53 \pm 0.53	1.080	0.312
$\varnothing < 2$ mm feeding holes per leaf	0.90 \pm 0.48	2.09 \pm 0.59	-1.569	0.155
$\varnothing > 2$ mm feeding holes per leaf	2.40 \pm 0.35	3.27 \pm 0.54	-1.341	0.217
<i>S. littoralis</i>				
Plant dry weight (g)	0.84 \pm 0.06	1.12 \pm 0.10	-2.422	0.042
Leaves per plant	5.20 \pm 0.20	5.40 \pm 0.24	-0.632	0.545
Fresh/dry weigh ratio	6.89 \pm 0.16	6.84 \pm 0.39	0.120	0.909
$\varnothing < 2$ mm feeding holes per leaf	2.35 \pm 0.36	3.60 \pm 0.79	-1.442	0.187
$\varnothing > 2$ mm feeding holes per leaf	2.95 \pm 0.71	2.19 \pm 0.66	0.781	0.457

CO_2 effect was tested with independent samples *t*-test.

Table 2

The significance (*p*-values from GLM analysis) of CO_2 and herbivore damage effects on the emission of individual monoterpenes

Compound	CO_2	Insect damage	$\text{CO}_2 \times$ insect damage
α -Thujene	0.042	0.019	0.997
α -Pinene	0.027	0.034	0.992
Sabinene	0.032	0.031	0.988
β -Pinene + myrcene	0.030	0.022	0.966
Limonene	0.047	0.003	0.961
1,8-Cineole	0.029	0.021	0.818
γ -Terpinene	0.069 ^a	0.241	—

^a Tested with independent samples *t*-test.

covariate for total VOC emission from whole plant, CO_2 treatment did not have any effect, while herbivore damage increased significantly the total VOC emission ($F = 25.487$, $p < 0.001$). Again, *P. xylostella*-damaged plants had higher emission than control or *S. littoralis*-damaged plants. Nonetheless, statistical analysis did not indicate significant interactive effects of CO_2 and herbivore treatments.

(*Z*)-3-Hexenyl acetate (Fig. 2a) and sesquiterpene (*E,E*)- α -farnesene (Fig. 2b) were emitted mainly from *P. xylostella* and *S. littoralis*-damaged plants, while homoterpene (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT) (Fig. 2c) was emitted only from *P. xylostella*- and

S. littoralis-damaged plants. The emission of DMNT was significantly higher from plants damaged by *P. xylostella* than from plants damaged by *S. littoralis* (Fig. 2c). In contrast to MTs, growth of cabbage at elevated CO_2 did not affect the emissions per shoot dry weight of these herbivore-inducible compounds (Fig. 2a–c). Furthermore, few herbivore-damaged plants emitted small amounts of linalyl acetate, (*Z*)-3-hexenol and (*E*)-2-hexenal.

Relative proportions of total MTs, total induced compounds (DMNT, (*E,E*)- α -farnesene and (*Z*)-3-hexenyl acetate) and total other compounds (nonanal, decanal and hexanal) of the total VOC emission did not change due to growth at elevated CO_2 concentration. However, insect damage altered the proportions of these compound groups by increasing the proportion of induced compounds, and thereby decreasing the proportions of MTs and other compounds (Table 3).

4. Discussion and conclusions

Our results indicate that intact cabbage plants and herbivore-damaged plants grown at elevated CO_2 concentration had reduced MT emissions per shoot dry weight, while such reduction did not take place in case of emission of herbivore-induced compounds per shoot dry weight. The dry mass of plants grown at elevated CO_2 was increased, which is in agreement with the earlier studies showing that thicker and heavier

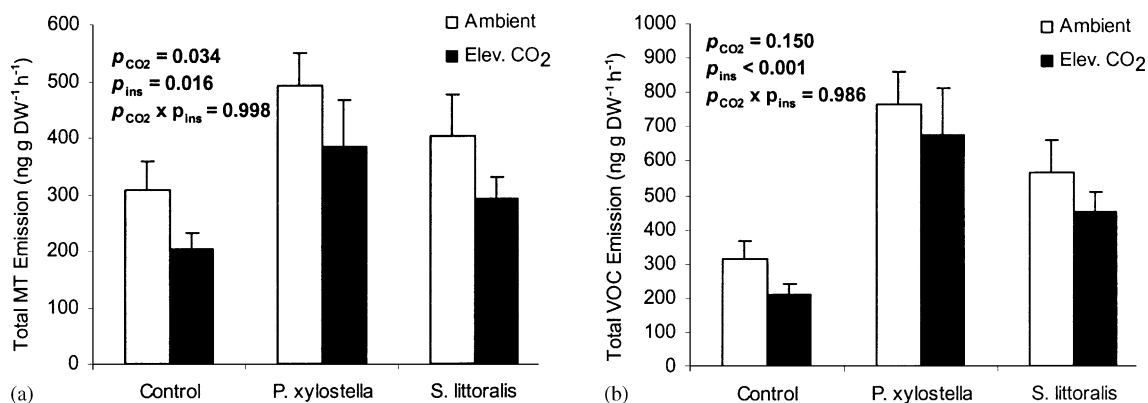


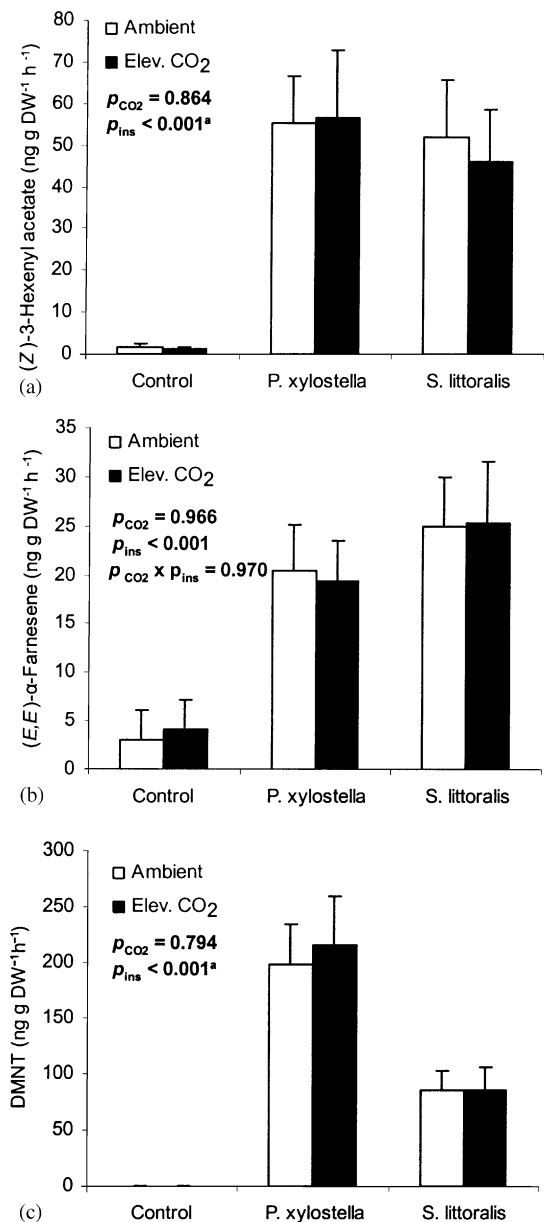
Fig. 1. (a) Total MT emission ng g D W⁻¹ h⁻¹ (α -thujene, α -pinene, β -pinene, 1,8-cineole, sabinene, myrcene, limonene and γ -terpinene) (mean +SE) from cabbage plants ($n = 5$) grown at ambient (360 $\mu\text{mol mol}^{-1}$) or elevated (720 $\mu\text{mol mol}^{-1}$) CO₂ concentration or damaged by the larvae of *Plutella xylostella* or *Spodoptera littoralis*. (b) Emissions of total VOCs (total MT, (Z)-3-hexenyl acetate, (E,E)- α -farnesene and DMNT) from the same plants. *P*-values are from multivariate GLM procedure using CO₂ as two-level and insect damage as three-level fixed factors.

leaves are a typical response to elevated CO₂ (Pritchard et al., 1999; Staudt et al., 2001). Studies with excised leaves may exaggerate emission rates induced by mechanical damage and oral secretions of moth larvae showing even eight-fold greater emissions (Schmelz et al., 2001). Expressing emissions on dry weight basis might distort the CO₂ effect on emissions to some extent, but quantification of emissions per dry weight has been frequently used (e.g. Peñuelas and Llusà, 1997; Constable et al., 1999b; Staudt et al., 2001).

It is unclear whether *Brassica* species has specialized storage ducts or glands for terpenes, from where the emission could occur. In *Q. ilex*, which does not store terpenes, the incorporation of photosynthetic carbon to emitted MTs may take place within 20 min (Loreto et al., 1996). In the case of conifers, volatile terpenes are emitted to the surrounding environment from storage structures (Langenheim, 1994). Our results of reduced MT emissions from cabbage are consistent with the results of Loreto et al. (2001) who demonstrated reduced MT emission from *Q. ilex* leaves at elevated CO₂. However, their results were expressed as emission per leaf area, and sampling was conducted at elevated CO₂, whereas we presented VOC emission per shoot dry weight and sampled VOCs at ambient air. During early ontogeny stem tissues may significantly contribute to photosynthesis (Sack et al., 2002), and thereby probably to VOC emissions. Loreto et al. (2001) showed that decrease in total MT emission resulted from decrease in α -pinene, β -pinene, and sabinene emissions, similarly as in our experiment. However, limonene emission was increased from *Q. ilex* (Loreto et al., 2001) while we detected significant reduction in limonene emission from cabbage plants grown at elevated CO₂. The explanation for reduced MT emissions from cabbage

grown at elevated CO₂ could be the reduced MT synthesis due to depressed photosynthesis at ambient CO₂ and consequent lack of carbon for their synthesis as shown by Loreto et al. (2001). On the other hand, Staudt et al. (2001) found that *Q. ilex* leaves grown at elevated CO₂ have higher MT emission per protected leaf area and per leaf dry mass than leaves grown at ambient CO₂ regardless of the assay CO₂ concentration. However, we conducted VOC collections under ambient CO₂ concentration for both CO₂ treatments.

The studies with terpene storing plant species have indicated a non-significant effect of elevated CO₂ on MT emissions per dry weight (Peñuelas and Llusà, 1997; Constable et al., 1999b). However, MT concentration in storage tissues may either decrease (Litvak et al., 2002; Sallas et al., 2003), increase (Sallas et al., 2001) or be unaltered (Kainulainen et al., 1998) in conifers grown under elevated CO₂. Litvak et al. (2002) showed that wounded Douglas fir needles had lower total MT, α -pinene and β -pinene pool sizes than intact needles at both ambient and elevated CO₂. In our study the emission of MTs from insect-damaged plants was higher than the emission of MTs from intact plants regardless of the CO₂ concentration during growing. Also, insect-damage clearly triggered the emission of three inducible compounds indicating that cabbage leans on induced defence as suggested earlier by Geervliet et al. (1997). Litvak et al. (2002) concluded that elevated CO₂ might induce the reduction in the rate of MT accumulation in relation to other constituents, like starch, in needles. They also suggested that Douglas fir depends more on constitutive defence than on induced defence, since there was no notable induction of MT cyclase activity in wounded needles.



^aMain effect tested with Kruskal-Wallis test.

Fig. 2. Emission (mean ± SE) of (a) (Z)-3-hexenyl acetate, (b) (E,E)-α-farnesene and (c) DMNT from control plants, *P. xylostella*- and *S. littoralis*-damaged cabbage plants ($n = 5$) grown at ambient ($360 \mu\text{mol mol}^{-1}$) or elevated ($720 \mu\text{mol mol}^{-1}$) CO₂ concentration. *P*-values are from multivariate GLM procedure using CO₂ as two-level and insect damage as three-level fixed factors.

On the present study, excess carbon might be allocated to the formation of other secondary metabolite groups leading to reduced MT emissions on dry weight

Table 3

Relative proportions (mean ± SD) of emissions of total MTs, total induced compounds (DMNT, (E,E)-α-farnesene, (Z)-3-hexenyl acetate and (Z)-3-hexenol) and total other compounds (nonanal, decanal and hexanal) from control and insect-damaged cabbage plants ($n = 10$)

	Control	<i>P. xylostella</i>	<i>S. littoralis</i>
Total MTs	93.7 ± 3.2a	60 ± 9.6b	65.4 ± 9.1b
Total induced	1.7 ± 2.1a	38.3 ± 8.8b	30.6 ± 9.4b
Total others	4.6 ± 2.7a	1.8 ± 1.2b	3.9 ± 1.8a

Different letters indicate a significant difference ($p < 0.05$) in between treatments in Tukey HDS- or Dunnett T3-tests.

basis and possible down-regulation of MT synthesis. It is often demonstrated that plants grown at elevated CO₂ have higher concentration of phenolic compounds (e.g. Agrell et al., 2000; Sallas et al., 2001; Coviella et al., 2002). In cotton plants elevated CO₂ may increase amounts of phenolic compounds, but terpenes are not affected as shown by Coviella et al. (2002). In cabbage seedlings (cv. Lennox) elevated CO₂ slightly increased the concentration of total phenolics, and one of the aromatic glucosinolates in foliage (Reddy et al., unpublished data). This trade-off between different biosynthetic pathways under high CO₂ could be species specific or dependent on the developmental stage of plants.

So far, majority of the publications suggest that elevated CO₂ concentration will not substantially increase MT emissions from most of the plant species, but indirectly CO₂-induced increase of temperature may increase MT emission from several plant species. We quantified VOC emissions from cabbages plants and detected that there was a reduction in MT emission but not in emission of induced compounds on dry weight basis in plants grown at elevated CO₂. On whole plant level there was CO₂ effect neither in MT nor in induced compounds emission, but herbivore-damage increased these emissions significantly. Based on this study, the effect of insect herbivores overcomes the effect of elevated CO₂ on the quantity and quality of the cabbage emission. However, further evaluation of direct effects of CO₂ on VOC emission rates is needed, to define the current models of vegetation-based VOC emissions expecting 12% increase in VOC emissions by doubled CO₂ concentration (Constable et al., 1999a).

Acknowledgements

The work was supported by Research Council for Biosciences and Environment, the Academy of Finland (projects no. 48605 and 51656).

References

- Agrell, J., McDonald, E.P., Lindroth, R.L., 2000. Effects of CO₂ and light on tree phytochemistry and insect performance. *Oikos* 88, 259–272.
- Andreae, M.O., Crutzen, P.J., 1997. Atmospheric aerosols: biogeochemical sources and role in atmospheric chemistry. *Science* 276, 1052–1058.
- Constable, J.V.H., Guenther, A.B., Schimel, D.S., Monson, R.K., 1999a. Modelling changes in VOC emission in response to climate change in the continental United States. *Global Change Biology* 5, 791–806.
- Constable, J.V.H., Litvak, M.E., Greenberg, J.P., Monson, R.K., 1999b. Monoterpene emission from coniferous trees in response to elevated CO₂ concentration and climate warming. *Global Change Biology* 5, 255–267.
- Cortesero, A.M., Stapel, J.O., Lewis, W.J., 2000. Understanding and manipulating plant attributes to enhance biological control. *Biological Control* 17, 35–49.
- Coviella, C.E., Stipanovic, R.D., Trumble, J.T., 2002. Plant allocation to defensive compounds: interactions between elevated CO₂ and nitrogen in transgenic cotton plants. *Journal of Experimental Botany* 53, 323–331.
- De Moraes, C.M., Lewis, W.J., Paré, P.W., Alborn, H.T., Tumlinson, J.H., 1998. Herbivore-infested plants selectively attract parasitoids. *Nature* 393, 570–573.
- Dicke, M., Sabelis, M.W., Takabayashi, J., Bruin, J., Posthumus, M.A., 1990. Plant strategies of manipulating predator–prey interactions through allelochemical: prospects for application in pest control. *Journal of Chemical Ecology* 16, 3091–3118.
- Geervliet, J.B.F., Posthumus, M.A., Vet, L.E.M., Dicke, M., 1997. Comparative analysis of headspace volatiles from different caterpillar-infested or uninfested food plants of *Pieris* species. *Journal of Chemical Ecology* 23, 2935–2954.
- Gouinguene, S., Degen, T., Turlings, T.C.J., 2001. Variability in herbivore-induced odour emissions among maize cultivars and their wild ancestors (teosinte). *Chemoecology* 11, 9–16.
- Hunter, M.D., 2001. Effects of elevated atmospheric carbon dioxide on insect–plant interactions. *Agricultural and Forest Entomology* 3, 153–159.
- Ibrahim, M.A., Kainulainen, P., Aflatuni, A., Tiilikkala, K., Holopainen, J.K., 2001. Insecticidal, repellent, antimicrobial activity and phytotoxicity of essential oils: with special reference to limonene and its suitability for control of insect pests. *Agricultural and Food Science in Finland* 10, 243–259.
- Kainulainen, P., Holopainen, J.K., Holopainen, T., 1998. The influence of elevated CO₂ and O₃ concentrations on Scots pine needles: changes in starch and secondary metabolites over three exposure years. *Oecologia* 114, 455–460.
- Kessler, A., Baldwin, I.T., 2001. Defensive function of herbivore-induced plant volatile emissions in nature. *Science* 291, 2141–2144.
- Langenheim, J., 1994. Higher plant terpenoids: a phytocentric overview of their ecological roles. *Journal of Chemical Ecology* 20, 1223–1280.
- Lewis, W.J., van Lenteren, J.C., Phatak, S.C., Tumlinson, J.H. III, 1997. A total system approach to sustainable pest management. *Proceedings of National Academy of Sciences USA* 94, 12243–12248.
- Litvak, M.E., Constable, J.V.H., Monson, R.K., 2002. Supply and demand processes as controls over needle monoterpene synthesis and concentration in Douglas fir (*Pseudotsuga menziesii* (mirb.) Franco). *Oecologia* 132, 382–391.
- Loreto, F., Ciccioli, P., Brancaleoni, E., Cecinato, A., Frattoni, M., Sharkey, T.D., 1996. Different sources of reduced carbon contribute to form three classes of terpenoid emitted by *Quercus ilex* L. leaves. *Proceedings of the National Academy of Sciences USA* 93, 9966–9969.
- Loreto, F., Fischbach, R.J., Schnitzler, J.P., Ciccioli, P., Brancaleoni, E., Calfapietra, C., Seufert, G., 2001. Monoterpene emission and monoterpene synthase activities in the Mediterranean evergreen oak *Quercus ilex* L. grown at elevated CO₂ concentrations. *Global Change Biology* 7, 709–717.
- Peñuelas, J., Llusà, J., 1997. Effects of carbon dioxide, water supply, and seasonality on terpene content and emission by *Rosmarinus officinalis*. *Journal of Chemical Ecology* 23, 979–993.
- Peñuelas, J., Llusà, J., 2001. The complexity of factors driving volatile organic compound emissions by plants. *Biologia Plantarum* 44, 481–487.
- Peñuelas, J., Llusà, J., 2003. BVOCs: plant defense against climate warming? *Trends in Plant Science* 8, 105–109.
- Poitout, S., Bues, R., 1974. Élevage des chenilles de vingt-huit espèces de Lépidoptères Noctuidae at de deux espèces d'Arctiidae sur milieu artificiel simple. Particularités de l'élevage selon les espèces. *Annales de Zoologie et Ecologie Animale* 6, 431–441.
- Pritchard, S.G., Rogers, H.H., Prior, S.A., Peterson, C.M., 1999. Elevated CO₂ and plant structure: a review. *Global Change Biology* 5, 807–837.
- Reddy, G.V.P., Guerrero, A., 2000. Behavioral responses of the Diamondback moth, *Plutella xylostella*, to green leaf volatiles of *Brassica oleracea* subsp. *capitata*. *Journal of Agricultural and Food Chemistry* 48, 6025–6029.
- Sack, L., Marañón, T., Grubb, P.J., Enquist, B.J., Niklas, K.J., 2002. Global allocation rules for patterns of biomass partitioning. *Science* 296, 1923.
- Sallas, L., Kainulainen, P., Utriainen, J., Holopainen, T., Holopainen, J.K., 2001. The influence of elevated O₃ and CO₂ concentrations on secondary metabolites of Scots pine (*Pinus sylvestris* L.) seedlings. *Global Change Biology* 7, 303–311.
- Sallas, L., Luomala, E.-M., Utriainen, J., Kainulainen, P., Holopainen, J.K., 2003. Contrasting effects of carbon dioxide (CO₂) enrichment and elevated temperature on Rubisco activity, chlorophyll fluorescence, needle ultrastructure and secondary metabolites in conifer seedlings. *Tree Physiology* 23, 97–108.
- Schmelz, E.A., Alborn, H.T., Tumlinson, J.H., 2001. The influence of intact-plant and excised-leaf bioassay designs on volicitin- and jasmonic acid-induced sesquiterpene volatile release in *Zea mays*. *Planta* 214, 171–179.
- Staudt, M., Joffre, R., Rambal, S., Kesselmeier, J., 2001. Effect of elevated CO₂ on monoterpene emission of young *Quercus*

- ilex* trees and its relation to structural and ecophysiological parameters. *Tree Physiology* 21, 437–445.
- Talekar, N.S., Shelton, A.M., 1993. Biology, ecology and management of diamondback moth. *Annual Review of Entomology* 38, 275–301.
- Thaler, J.S., 1999. Jasmonate-inducible plant defence cause increased parasitism of herbivores. *Nature* 399, 686–688.
- Turlings, T.C.J., Lengwiler, U.B., Bernasconi, M.L., Wechsler, D., 1998. Timing of induced volatile emissions in maize seedlings. *Planta* 207, 146–152.
- Turtola, S., Manninen, A.-M., Holopainen, J.K., Levula, T., Raitio, H., Kainulainen, P., 2002. Secondary metabolite concentrations and terpene emissions of Scots pine xylem after long-term forest fertilization. *Journal of Environmental Quality* 31, 1694–1701.