Emission of *Plutella xylostella*-Induced Compounds from Cabbages Grown at Elevated CO_2 and Orientation Behavior of the Natural Enemies[1]

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Several plant species defend themselves indirectly from herbivores by producing herbivore-induced volatile compounds that attract the natural enemies of herbivores. Here we tested the effects of elevated atmospheric CO_2 (720 μmol mol^{-1}) concentration on this indirect defense, physiological properties, and constitutive and induced emissions of white cabbage (*Brassica oleracea* ssp. *capitata*, cvs Lennox and Rinda). We monitored the orientation behavior of the generalist predator *Podisus maculiventris* (Heteroptera: Pentatomidae) and the specialist parasitoid *Cotesia plutellae* (Hymenoptera: Braconidae) to plants damaged by *Plutella xylostella* (Lepidoptera: Plutellidae) in the Y-tube olfactometer. Elevated CO_2 levels did not affect stomatal densities but reduced specific leaf area and increased leaf thickness in cv Lennox. In addition to enhanced constitutive monoterpene emission, *P. xylostella*-damaged cabbages emitted homoterpene (*E*)-4,8-dimethyl-1,3,7-nonatriene, sesquiterpene (*E,E*)-α-farnesene, and (*Z*)-3-hexenyl acetate. Growth at elevated CO_2 had no significant effect on the emissions expressed per leaf area, while minor reduction in the emission of homoterpene (*E*)-4,8-dimethyl-1,3,7-nonatriene and (*E,E*)-α-farnesene was observed at elevated CO_2 in one of two experiments. The generalist predator *P. maculiventris* discriminated only between the odors of intact and *P. xylostella*-damaged cv Rinda plants grown at ambient CO_2 concentration, preferring the odor of the damaged plants. The specialist parasitoid *C. plutellae* preferred the odor of damaged plants of both cultivars grown at ambient CO_2 but did not detect damaged cv Lennox plants grown at elevated CO_2. The results suggest that elevated atmospheric CO_2 concentration could weaken the plant response induced by insect herbivore feeding and thereby lead to a disturbance of signaling to the third trophic level.

Several plant species express traits which can indirectly participate in controlling the herbivore population, i.e. the release of volatile compounds after herbivore damage which participate in top-down control of herbivores by attracting predators and parasitoids (e.g. Dicke et al., 1990; Turlings et al., 1991). The emission of these herbivore-induced volatile compounds are strongly affected by abiotic factors such as soil and air humidity, temperature, light, and fertilization rate (Gouinguené and Turlings, 2002) as well as biotic factors such as plant cultivar, growth stage of the leaf, and attacking herbivore species (Takabayashi et al., 1994).

The concentration of atmospheric CO_2 is expected to double by the end of this century (Houghton et al., 2001), and a simultaneous increase in the emissions from plants is predicted due to climate warming (Constable et al., 1999a, 1999b). This will affect the hydrocarbon load into the atmosphere and cause changes in ozone and aerosol formation (Andreae and Crutzen, 1997; Peñuelas and Llusià, 2001, 2003). Monoterpenes (MTs) have an antioxidative role protecting plants probably in a wide range of stress situations (Loreto et al., 2004), and these changes in MT formation may well be an important issue to be considered in climate change studies.

Changes in the atmospheric CO_2 concentration affect plant physiology: plants are larger, grow faster, have increased carbon:nitrogen ratios and a decreased specific leaf area (SLA) under elevated CO_2 (Bazzaz, 1990; Bezemer and Jones, 1998; Poorter and Navas, 2003; Sallas et al., 2003). Presumably higher trophic levels, predators, and parasitoids will also be affected through changes in the host plant quality. An elevated CO_2 concentration has evoked variable changes in the MT emission of plants: no effect (Peñuelas and Llusià, 1997; Constable et al., 1999b), MT emission has been higher at elevated CO_2 than at ambient air (Staudt et al., 2001), or CO_2 exposure has decreased total MT emission (Loreto et al., 2001; Vuorinen et al., 2004). Changes in the emitted MTs and other compounds might be dependent on the availability of photosynthetic carbon which is needed in the production of volatiles (Loreto et al., 2001). Even though an elevated CO_2 level stimulates plant growth, the density of stomata might be decreased, as detected with Arabidopsis (Woodward et al., 2002), and this could

potentially diminish the emission of volatile compounds.

A variety of induced compounds are released from herbivore-damaged plants (Geervliet et al., 1997; Paré and Tumlinson, 1999; Van Den Boom et al., 2004; Vuorinen et al., 2004). Feeding damage of both specialist *Plutella xylostella* and generalist *Spodoptera littoralis* larvae induced the emission of (Z)-3-hexenyl acetate and (E,E)-α-farnesene, but the homoterpene (E)-4,8-dimethyl-1,3,7-nonatriene (DMNT) emission was higher after specialist than generalist damage in cabbages (*Brassica oleracea*; Vuorinen et al., 2004). Also, larvae of *Pieris rapae* and *Pieris brassicae* induced the emission of DMNT and (Z)-3-hexenyl acetate and caused twice as high total emissions as produced by control cabbages (Geervliet et al., 1997). Potting et al. (1999) showed that *P. xylostella*-damaged *Brassica napus* plants were attractive to the parasitoid *Cotesia plutellae*. The induced compounds should contain the chemical information needed by predators and parasitoids to locate their hosts. Although, a number of studies has been conducted on volatiles that attract the natural enemies of herbivores, there has been no report yet on the volatiles emitted from plants grown at elevated CO₂ level and the subsequent consequences on the orientation behavior of the natural enemies.

The objective of this study was to evaluate the effects of an elevated CO₂ concentration on the emission of cabbage plants damaged by the crucifer specialist, the diamondback moth, *P. xylostella*, which is one of the most serious pests of cruciferous plants found throughout the world (Talekar and Shelton, 1993). The orientation behaviors of the generalist predator *Podisus maculiventris* and the specialist parasitoid *C. plutellae* were determined, since the response of specialists would be expected to differ from that of generalists. These results are important for evaluation and modeling of the effects of climate change on multitrophic signaling by plant volatiles in food chains both in natural and man-made ecosystems.

RESULTS

Plant Growth and Properties

In the first experiment, the growth of cv Lennox was more responsive to elevated CO₂ than growth of cv Rinda, cv Lennox exhibited enhanced shoot dry weight and decreased fresh/dry weight ratios in both intact

Table I. *Physiological properties (mean ± SD) of the intact control cabbages in experiments 1 and 2, and P. xylostella-damaged cabbages in experiment 2*

Plants were grown at ambient (360 μmol mol^{-1}) or elevated CO₂ (720 μmol mol^{-1}) concentration. The CO₂ effect was tested with independent samples t test.

	N	Rinda			Lennox		
		Ambient CO₂	Elevated CO₂	P	Ambient CO₂	Elevated CO₂	P
Experiment 1							
Control							
SLA (cm² g dw⁻¹)	6	227.1 ± 40.0	190.5 ± 31.7	0.110	139.5 ± 14.8	110.2 ± 9.0	0.002
Leaf thickness (μm)	12	293.8 ± 40.9	293.1 ± 47.9	0.967	307 ± 33.8	350 ± 41.0	0.012
Stomatal density (n mm⁻²)							
3rd youngest leaf							
Adaxial	6	146.0 ± 24.1	128.2 ± 16.7	0.153	207.7 ± 51.2	239.1 ± 103.0	0.518
Abaxial	6	203.2 ± 26.8	202.0 ± 36.9	0.950	254.4 ± 60.1	246.4 ± 21.6	0.775
5th youngest leaf							
Adaxia	6	232.0 ± 20.7	213.2 ± 42.0	0.347	288.2 ± 25.4	291.4 ± 33.4	0.860
Abaxial	6	318.9 ± 32.2	286.8 ± 77.1	0.370	345.8 ± 91.3	326.3 ± 57.4	0.667
Experiment 2							
Control							
Leaf area (cm²)	5	272.7 ± 70.0	269.8 ± 27.8	0.934	218.9 ± 24.3	228.8 ± 13.9	0.451
SLA (cm² g dw⁻¹)	5	199.1 ± 49.3	143.3 ± 24.6	0.053	168.5 ± 33.8	149.8 ± 30.0	0.381
Shoot fresh weight (g)	5	15.8 ± 4.9	16.8 ± 2.4	0.707	12.9 ± 1.4	14.2 ± 1.3	0.163
Shoot dry weight (g)	5	1.7 ± 0.6	2.2 ± 0.4	0.136	2.0 ± 1.1	1.8 ± 0.3	0.710
Fresh/dry weight ratio	5	9.6 ± 1.4	7.6 ± 1.0	0.028	7.3 ± 2.7	7.8 ± 0.9	0.717
P. xylostella							
Leaf area (cm²)	5	254.8 ± 27.9	227.94 ± 29.2	0.174	189.0 ± 30.6	200.0 ± 5.1	0.469
SLA (cm² g⁻¹)	5	172.5 ± 30.4	158.2 ± 28.5	0.466	158.2 ± 34.6	105.0 ± 7.0	0.025
Shoot fresh weight (g)	5	15.4 ± 2.4	14.23 ± 2.5	0.470	10.8 ± 2.1	12.8 ± 0.6	0.100
Shoot dry weight (g)	5	1.8 ± 0.4	1.78 ± 0.4	0.962	1.5 ± 0.5	2.2 ± 0.2	0.026
Fresh/dry weight ratio	5	8.7 ± 1.1	8.17 ± 1.1	0.458	7.4 ± 1.2	5.8 ± 0.3	0.037
∅ < 2 mm holes per leaf	5	2.1 ± 1.6	2.6 ± 3.3	0.777	0.8 ± 0.4	1.6 ± 0.5	0.020
∅ > 2 mm holes per leaf	5	7.3 ± 4.2	5.7 ± 2.4	0.496	3.1 ± 1.1	5.3 ± 1.0	0.009

and *P. xylostella*-damaged plants and enhanced shoot fresh weight in intact plants (data not shown). Leaf thickness was increased and SLA was decreased in cv Lennox at elevated CO_2, but stomatal density was not affected in either of the cultivars (Table I). In the second experiment, leaf area, SLA, shoot fresh weight, and shoot dry weight in intact Rinda and Lennox cvs

did not differ between ambient and elevated CO_2 concentrations, although SLA of *P. xylostella*-damaged plants of cv Lennox was decreased. Even though *P. xylostella* caused more feeding holes in cv Lennox grown at elevated CO_2, the shoot dry weight of those plants remained higher at elevated CO_2 than at ambient CO_2 (Table I).

Figure 1. Emission of intact control and *P. xylostella*-damaged cabbages (cvs Rinda and Lennox) grown at ambient or elevated CO_2 concentration. A and B, Emissions of total MTs (sabinene, limonene, β-pinene + myrcene, 1,8-cineol, α-thujene, and α-pinene; mean + SE). C and D, DMNT. E and F, (*E,E*)-α-farnesene. G and H, (*Z*)-3-hexenyl acetate (ng cm^{-2} h^{-1}) from intact control and *P. xylostella*-damaged cabbage plants ($n = 5$) grown at ambient (360 μmol mol^{-1}) or elevated (720 μmol mol^{-1}) CO_2 concentration in experiment 1. *P*-values indicate the main effect tested by Kruskal-Wallis-test. Different letters above the bars indicate significant differences between the treatments by Tukey or Dunnett T3 post hoc tests.
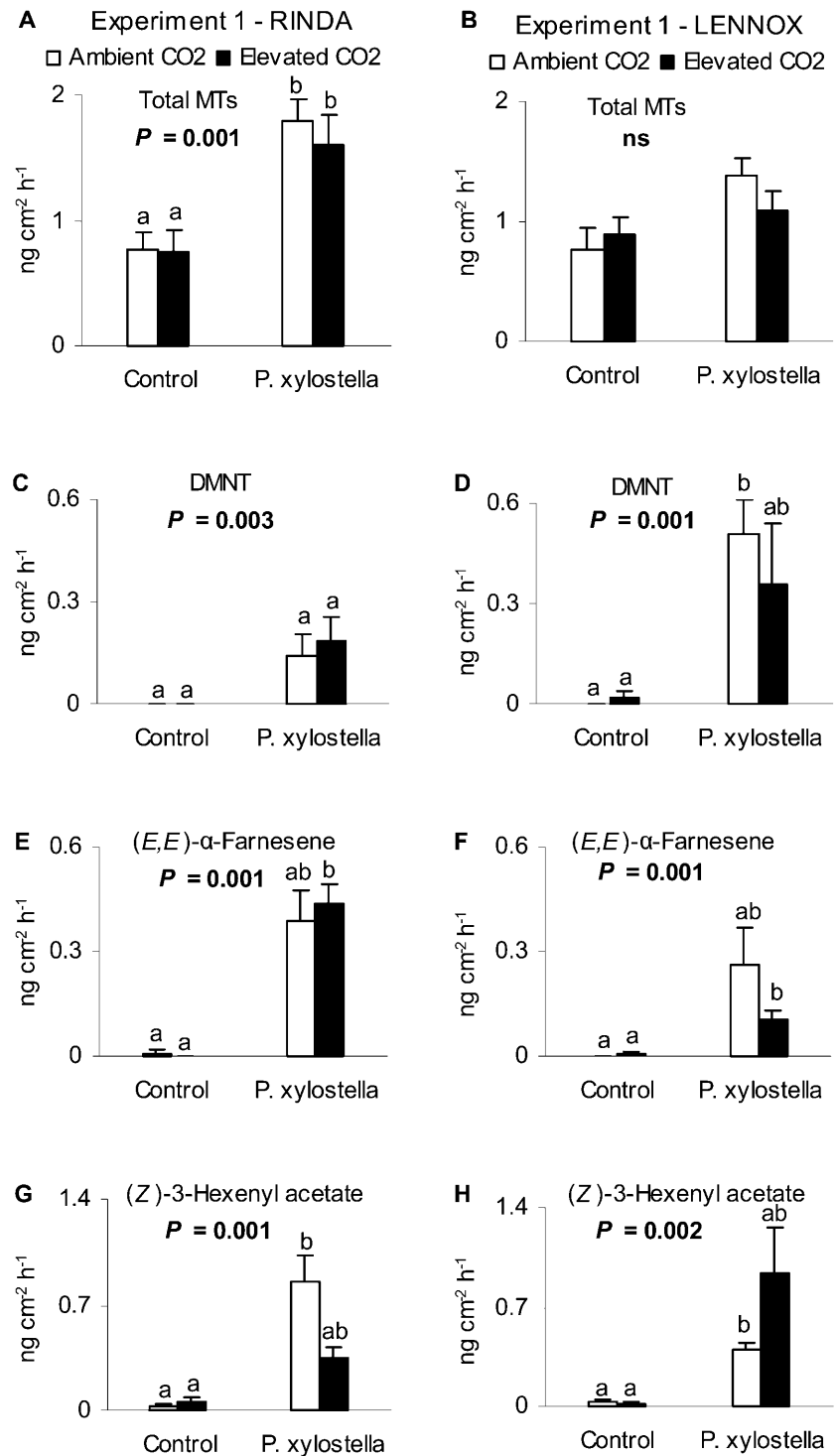
The Emission of Volatile Compounds

The composition of the cabbage emission profile in both experiments was identical. The emission of intact control cabbages consisted mainly of constitutive MTs (sabinene, limonene, β-pinene + myrcene [these two compounds were not separated on our HP-5 column], 1,8-cineol, α-thujene, and α-pinene), while *P. xylostella*-damaged cabbages emitted in addition to MTs also induced compounds, DMNT, sesquiterpene (*E,E*)-α-
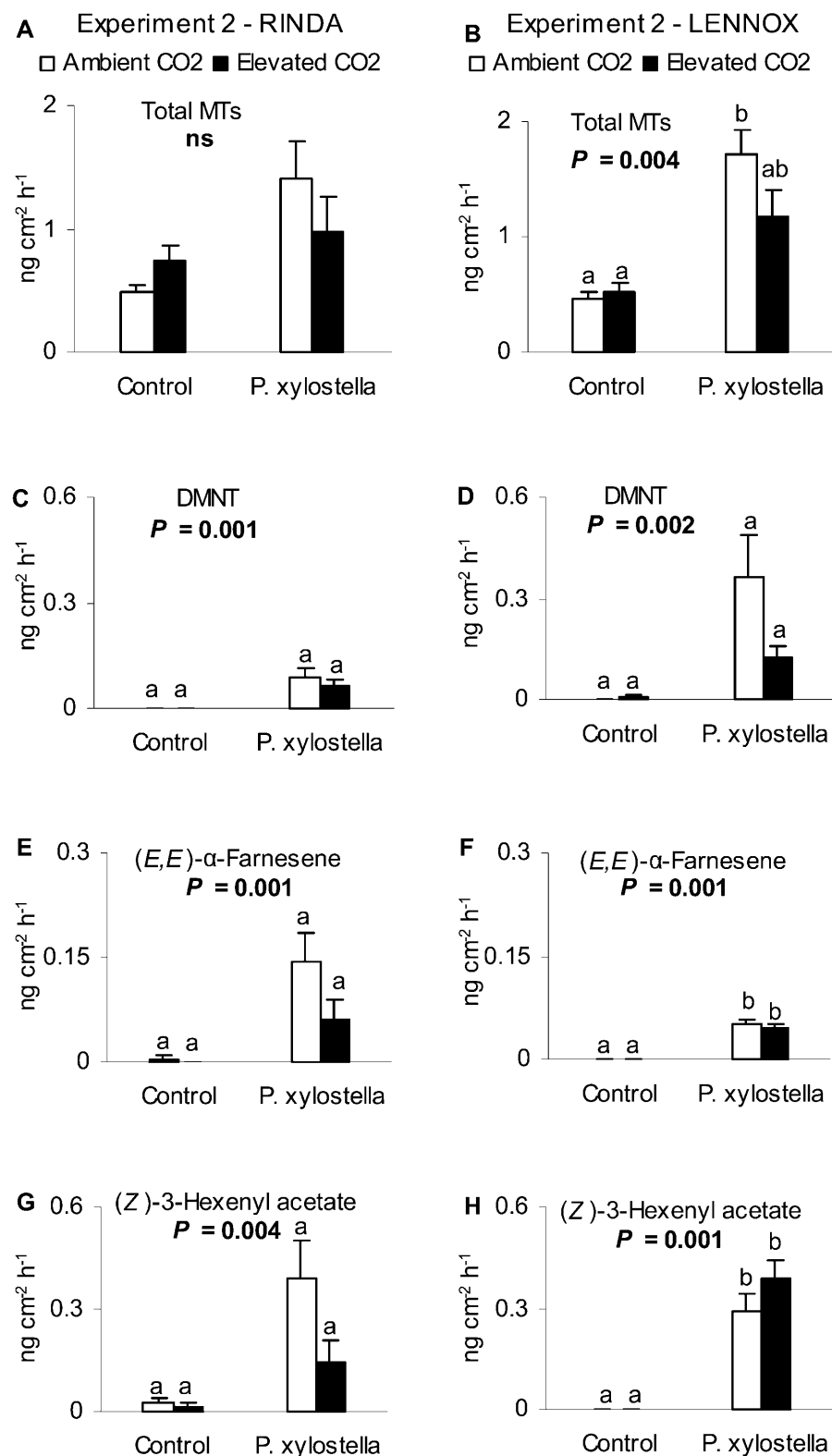

Figure 2. Emission of intact control and *P. xylostella*-damaged cabbages (cvs Rinda and Lennox) grown at ambient or elevated CO$_2$ concentration. A and B, Emissions of total MTs (sabinene, limonene, β-pinene + myrcene, 1,8-cineol, α-thujene, and α-pinene; mean + SE). C and D, DMNT. E and F, (*E,E*)-α-farnesene. G and H, (*Z*)-3-hexenyl acetate (ng cm^{-2} h^{-1}) from intact control and *P. xylostella*-damaged cabbage plants ($n = 5$) grown at ambient (360 μmol mol^{-1}) or elevated (720 μmol mol^{-1}) CO$_2$ concentration in experiment 2. *P*-values indicate the main effect tested by Kruskal-Wallis-test. Different letters above the bars indicate significant differences between the treatments by Dunnett T3 post hoc test.

farnesene, and (Z)-3-hexenyl acetate. The emission data expressed on a leaf area basis and collated from both experiments revealed that growing the plants at elevated CO_2 had no effect on the emission of total (Figs. 1 and 2, A and B) or individual (data not shown) MTs of intact control and *P. xylostella*-damaged cabbage cvs Rinda and Lennox. Emissions of individual MTs of cv Rinda in experiment 1 were significantly higher from herbivore-damaged than intact control plants (main effect one-way ANOVA $P < 0.005$), while in experiment 2 only the emission of α-thujene was enhanced by *P. xylostella*-damage (main effect K-W $P = 0.037$). On the other hand, α-pinene and β-pinene emissions in experiment 1 and the emissions of all individual MTs of cv Lennox in experiment 2 were significantly higher from herbivore-damaged plants than from intact plants.

In both cultivars and both experiments, the emissions of induced compounds, DMNT, (E,E)-α-farnesene, and (Z)-3-hexenyl acetate, were not particularly responsive to the CO_2 concentration (Figs. 1 and 2) even though elevated CO_2 seemed to diminish the emission of DMNT from cv Lennox and (E,E)-α-farnesene from cv Rinda in experiment 2. The emissions of induced compounds were naturally higher from herbivore-damaged plants than from intact plants (Figs. 1 and 2, C–H). *P. xylostella*-damage tended to increase more clearly the emission of total MTs and induced compounds from plants grown at ambient CO_2 than at elevated CO_2.

The relative proportions of total MTs, total induced compounds, and total other compounds [hexanal, (Z)-3-hexenol, heptanal, nonanal and decanal] in relation to total volatiles were not affected by the elevated CO_2 concentration in either of the experiments. Again, *P. xylostella*-damage increased significantly ($P < 0.001$) the proportion of total induced compounds and thereby decreased significantly the proportion of total MTs ($P < 0.001$) in both cultivars and in both experiments (Fig. 3). In experiment 1, the percentage of induced compounds tended to be larger than in experiment 2 (Fig. 3).

Behavioral Response of *P. maculiventris* and *C. plutellae*

In experiment 1, the generalist predator *P. maculiventris* discriminated only between the odors of intact and *P. xylostella*-damaged cv Rinda plants, which were grown at ambient CO_2 concentration, preferring the odor of the damaged plants (Fig. 4A). In experiment 2, the specialist parasitoid *C. plutellae* preferred the odor of damaged plants (both cultivars) when the plants had been grown at ambient CO_2 but did not detect damaged cv Lennox plants when they were grown at elevated CO_2 (Fig. 4B).

DISCUSSION

Elevated CO_2 and Plant Properties

As indicated by the decreased SLA and the increased leaf thickness in experiment 1, cv Lennox was more responsive to elevated CO_2 than cv Rinda. The SLA of Lennox was reduced at elevated CO_2 in both experiments although in experiment 2 only in the plants fed by *P. xylostella*. This intraspecific variation in the SLA response to elevated CO_2 is well known in the family Brassicaceae (Van Der Kooij et al., 2000). The analysis of more than 100 plant species has revealed that there is an average 11% reduction of stomatal density attributable to doubled atmospheric CO_2 (Hetherington and Woodward, 2003). We did not find significantly diminished stomatal density in cabbages grown at elevated CO_2, suggesting that the small
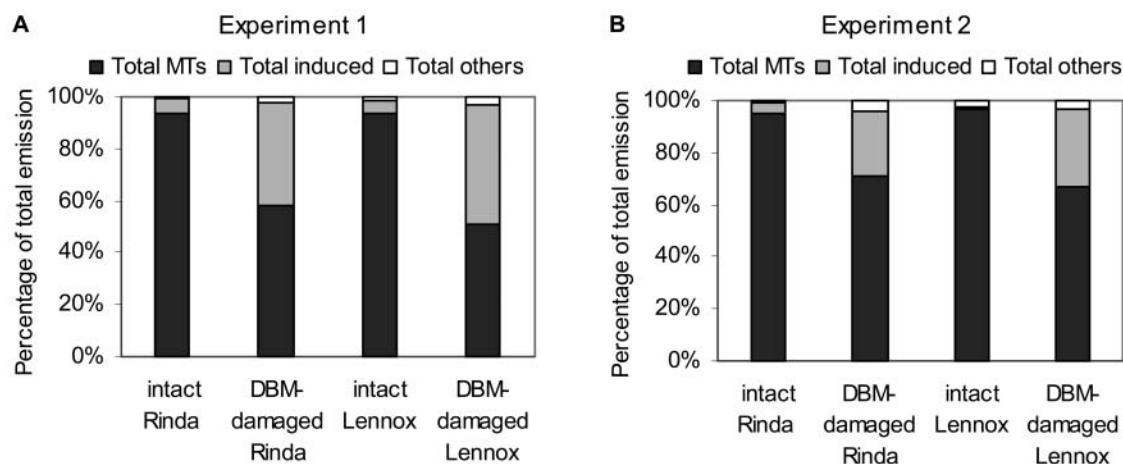

Figure 3. Percentage of MTs and induced and other compounds in the total emission. A, Emission of total MTs (sabinene, limonene, β-pinene + myrcene, 1,8-cineol, α-thujene, and α-pinene), total induced compounds [DMNT, (E,E)-α-farnesene, (Z)-3-hexenyl acetate, and (Z)-3-hexenol], and total other compounds [hexanal, (Z)-3-hexenol, nonanal, and decanal] in experiment 1 and B, emissions of total MTs, total induced compounds, and total other compounds [hexanal, (Z)-3-hexenol, heptanal and nonanal] in experiment 2 from intact control and *P. xylostella*-damaged cabbage cvs Rinda and Lennox ($n = 10$).
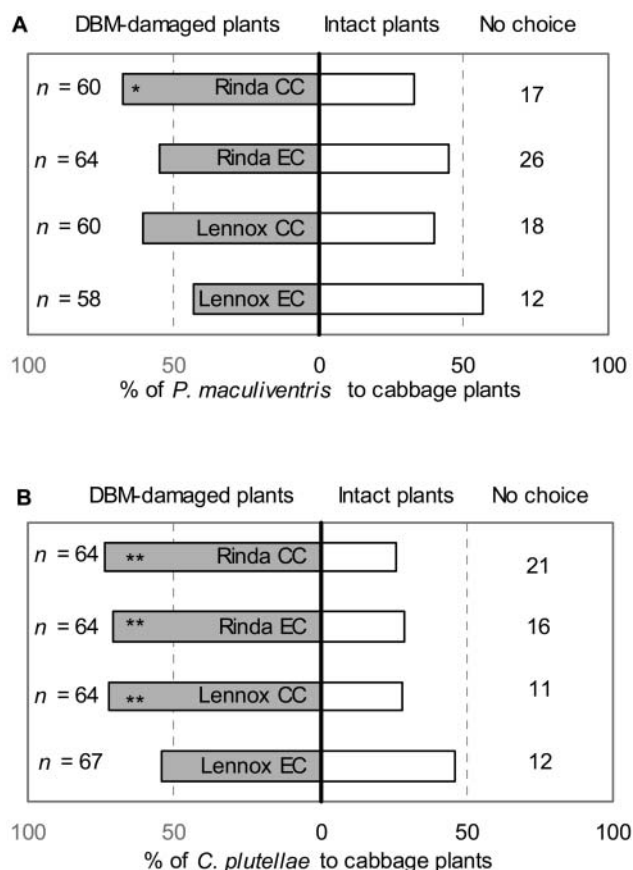
Figure 4. The orientation behavior of *P. maculiventris* and *C. plutellae*. The percentage of A, *P. maculiventris* in experiment 1 and B, *C. plutellae* in experiment 2 choosing for *P. xylostella*-damaged or intact control cabbage (cvs Rinda and Lennox) plants grown at ambient (CC) or elevated CO$_2$ (EC) in the Y-tube olfactometer. Asterisks indicate significant (*$P < 0.05$, **$P < 0.01$, in binomial test) preference toward either odor source. *n* indicates the total number of predators or parasitoids used in the assay including the individuals that did not show any preference for either odor source. DBM, diamondback moth, *P. xylostella*.

reduction in the emission of DMNT and (E,E)-α-farnesene from *P. xylostella*-damaged cabbages is not a consequence of a lower emission rate through stomata but probably is a reflection of restricted synthesis of volatile compounds. However, at the moment the importance of the density and behavior of stomata for controlling total emission is still unclear (Niinemets and Reichstein, 2003).

Elevated CO$_2$ and Plant Emissions

The elevated CO$_2$ concentration per se had no significant effect on constitutive MT emission from intact control plants, while it seemed to have overall reducing, although nonsignificant, impact on MT emission from herbivore-damaged plants. In an earlier study, we detected decreased MT emissions per shoot dry weight from intact and herbivore-damaged cv Lennox cabbages grown at elevated CO$_2$ (Vuorinen

et al., 2004). At least in the case of intact cabbages, the observed decrease in the MT emission might have been a consequence of expressing emission results per shoot dry weight since the intact control plants grown at elevated CO$_2$ concentration had higher shoot dry weights than plants grown at ambient CO$_2$ (Vuorinen et al., 2004). In general, emissions from plants have been expressed on a dry weight basis (e.g. Peñuelas and Llusià, 1997; Constable et al., 1999b; Staudt et al., 2001) or on a leaf area basis (e.g. Loreto et al., 2001, 2004). If the MT emission in this study was expressed on a shoot dry weight basis (data not shown), then it was significantly decreased from herbivore-damaged cabbages grown at elevated CO$_2$.

In earlier studies, the reduction of MT emission from species which do not store terpenes, such as *Quercus ilex* (Loreto et al., 2001) and cabbage (Vuorinen et al., 2004), has been detected at elevated CO$_2$ concentration. In contrast, Staudt et al. (2001) found that *Q. ilex* leaves grown at elevated CO$_2$ exhibited higher MT emission per projected leaf area and per leaf dry mass than leaves grown at ambient CO$_2$ regardless of the CO$_2$ concentration during sampling. We collected volatile compounds at ambient CO$_2$. In a trial test, *P. xylostella*-damaged plants grown and sampled at elevated CO$_2$ had significantly lower MT emission than those plants grown at elevated CO$_2$ and sampled at ambient CO$_2$, while CO$_2$ concentration during sampling of volatile compounds did not affect MT emissions from intact cabbages (data not shown). Assumingly the decrease in emission of MTs at elevated CO$_2$ would have been stronger if the sampling would have been conducted at elevated CO$_2$. When one considers terpene storing plants such as conifers (Constable et al., 1999b) or *Rosmarinus officinalis* (Peñuelas and Llusià, 1997), elevated CO$_2$ concentration appeared to have no impact on MT emission. Litvak et al. (2002) detected a lower total MT pool size in wounded Douglas fir needles than in intact needles at ambient and elevated CO$_2$ concentrations, suggesting that the rate of MT accumulation decreases in relation to other compounds such as starch at elevated CO$_2$. They also concluded that Douglas fir depends more on constitutive MTs for defense than on induced compounds, which is not the case with cabbage. The *Brassica* species do not have MT pools in their leaves but are able to produce herbivore-induced volatile compounds.

In our earlier study we found no impact of CO$_2$ levels on the emission of induced compounds (Vuorinen et al., 2004), while in this study CO$_2$ concentration seemed to decrease the emissions of *P. xylostella*-induced compounds. The variation in the emission of induced compounds between individual plants was notable, and therefore no clear statistically significant CO$_2$ effect was observed. Also, feeding habits and the type of damage caused by *P. xylostella* larvae is not necessarily equal among the plants that might have great impact on the emission rates of induced compounds. Herbivore-induced compounds are derived

mainly from the jasmonic acid signaling pathway, which modulates the lipoxygenase pathway producing green leaf volatiles, and two terpenoid pathways (Dicke et al., 2003), mevalonic acid pathway in the cytosol and the deoxyxylulose phosphate/methylerythritol pathway in plastids (Sponsel, 2002). In parallel to our earlier results (Vuorinen et al., 2004), at least volatile compounds from the jasmonic acid signaling pathway were detectable from cabbage after *P. xylostella*-damage.

Trophic Interactions

P. maculiventris nymphs preferred the odor of damaged plants grown at ambient CO_2, but showed little preference between damaged and intact plants when the cabbages were grown at elevated CO_2. According to the opinions of Vet and Dicke (1992) herbivore-derived volatiles are the most reliable cues for generalist predators for prey locating. Therefore predators should not innately react to herbivore-induced plant-derived semiochemicals, which are more abundant but less reliable and which can be used for prey locating after associative learning process. However, Reddy et al. (2002) observed that the generalist predator *Chrysoperla carnea* is attracted to the odor of (Z)-3-hexenyl acetate, which is released in large amounts from herbivore-damaged cabbages. Furthermore, Dickens (1999) detected that *P. maculiventris* has olfactory receptors for (Z)-3-hexenyl butyrate which is induced by the Colorado potato beetle, and the predator was also attracted to a blend comprising (E)-2-hexenol, (Z)-3-hexenol, (±)-linalool, nonanal, and MeSA. Sant'Ana et al. (1999) found that third- and fifth-instar nymphs reacted to common plant green leaf volatiles (E)-2-hexenol and (E)-2-hexenal, which is also the dominant odor of the male-produced aggregation pheromone. The fifth-instars responded also to nonanal and (±)-linalool (Sant'Ana et al., 1999). Neither (E)-2-hexenol nor (E)-2-hexenal was emitted from the damaged cabbages in this study. The greatly reduced emission of (Z)-3-hexenyl acetate from *P. xylostella*-damaged cv Rinda plants grown at elevated CO_2 may be an explanation for the reduced host-searching efficiency of *P. maculiventris* in this study. On the other hand, there seemed to be elevated emission of (Z)-3-hexenyl acetate at elevated CO_2 from *P. xylostella*-damaged cv Lennox plants, but nonetheless the predator still did not show a preference for the odor of damaged plants.

Orientation of the parasitoid *C. plutellae* toward damaged plant was stronger than that of the generalist predator. The response of parasitoids to plant-herbivore complexes differs among the plant species and herbivores involved. For instance, *C. plutellae* shows a specific response toward the host-plant complex, unlike *C. glomerata*, and the presence of the nonhost affects the specificity of the response of the wasps (Shiojiri et al., 2000). Liu and Jiang (2003) showed that volatile compounds from Chinese cabbage were more attractive to female *C. plutellae* than those from white cabbage when both plant species were either intact or infested with *P. xylostella*. DMNT and other terpenes appeared to be important cues for orientation of *C. plutellae* to *P. xylostella*-damaged plants (Shiojiri et al., 2001). (E,E)-α-Farnesene is not one of the *P. xylostella*-induced compounds in all cabbage varieties and in this respect differs from DMNT and (Z)-3-hexenyl acetate (Shiojiri et al., 2001). The decreasing impact of elevated CO_2 on DMNT and MT emissions from cv Lennox probably explain the reduction in host-searching efficiency of *C. plutellae*. As our results point to a reduced orientation efficiency of the specialist parasitoid toward herbivore-damaged plants grown at the forecasted elevated atmospheric CO_2 concentration, this finding deserves further and more extensive research on other plant species. *C. plutellae* and other Braconids at the top of food chains maintain important position in terms of global biodiversity (Dolphin and Quicke, 2001). In the future, these parasitoid species may become threatened also by elevated atmospheric CO_2 concentration if their host-searching efficiency is impaired.

MATERIALS AND METHODS

The Experiments, Plant Material, and CO_2 Exposure

We performed two separate experiments under similar growth conditions for the plants, produced similar *Plutella xylostella*-damage, sampled volatiles, and used the Y-tube olfactometer in a similar manner in both experiments. In the first experiment, we studied the orientation behavior of the generalist *Podisus maculiventris*, and in addition to plant growth measurements we also measured leaf thickness and stomatal density from intact control plants. In the second experiment, we tested the orientation behavior of the specialist *Cotesia plutellae*. Emission data from both experiments are presented.

White cabbage (*Brassica oleracea* ssp. *capitata* cvs Lennox and Rinda) seedlings were sown in 1-l plastic pots filled with Sphagnum peat and sand (3:1, v/v) and grown for 24 to 26 d at ambient (360 μmol mol^{-1}) or elevated (720 μmol mol^{-1}) CO_2 concentration in growth chambers (Bioklim 2600T, Kryo-Service Oy, Helsinki) at 23°C:18°C, 70%:80% relative humidity, and 22:2-h photoperiod (250–300 μmol m^{-2} s^{-1} photosynthetically active radiation during the light period). CO_2 enhancement was maintained for 24 h d^{-1}. The CO_2 treatments and seedlings were rotated among the two chambers weekly to randomize any systematic chamber effect across the seedlings. The seedlings were watered daily and fertilized weekly with 0.1% of 9-Superex (19:5:20 N:P:K; Kekkilä, Finland) at a rate of 0.05 to 0.1 L $plant^{-1}$, starting 2 weeks after sowing.

Leaf Properties

The SLA was determined by scanning the leaf with a Logitech-scanner and analyzing the leaf area using a Logitech Photo Touch Color-program (Logitech, Morges, Switzerland) and dividing the leaf area by the leaf dry weight. Leaf thickness was measured from the 3rd and 5th youngest leaves between the veins in the left top one-quarter of the leaf with a micrometer (Mitutoyo Mod. 1D-C112CB, Mitutoyo Kanagawa, Japan). Stomatal density was determined after collection of volatiles by pressing a small proportion of the adaxial and abaxial right top quarter of the leaf surface against a glass microscope slide painted with instant glue (Loctite Super Glue +, Henkel Loctite, Cleveland). After drying for 1 to 2 min, the leaf was peeled off and replicas were examined at 200× magnification with a light microscope (Oksanen, 2003).

Insects and *P. xylostella*-Damage

Larvae of the crucifer specialist *P. xylostella* (Lepidoptera: Plutellidae) were reared at 25°C, 50% relative humidity, and 16:8-h photoperiod on broccoli (*B.*

oleracea ssp. *italica*) seedlings. Feeding damage was caused by transferring eight third-instar larvae onto five randomly selected plants grown at ambient or elevated CO_2 for 48 h. Five intact plants from both CO_2 concentrations were used as controls. Plants with the feeding larvae were kept in a separate growth chamber in similar environmental conditions as described earlier.

Spined soldier bug *P. maculiventris* (Hemiptera: Pentatomidae) nymphs were obtained from Koppert Biological Systems, Berkel en Rodenrijs, The Netherlands. The nymphs were fed mainly with larvae of yellow mealworm, *Tenebrio molitor* (Coleoptera: Tenebrionidae) at $23°C \pm 1°C$, $75\% \pm 5\%$ relative humidity, and a 16:8-h photoperiod. Fifth instars were used in the behavioral assay.

C. plutellae Kurdjumov (Hymenoptera: Braconidae) pupae were obtained from the Centre de Coopération Internationale en Recherche Agronomique pour le Développement (Montpellier, France). Second-instar larvae of *P. xylostella* feeding on broccoli were offered to *C. plutellae* females for egg laying at $25°C \pm 1°C$, 50% relative humidity, and 16:8-h photoperiod. Pupae were placed in a clean cage until emergence. Adults were provided with 20% honey-water solution for feeding. From 3- to 5-d-old *C. plutellae* females were used in the behavioral assay. Predators and parasitoids had not experienced the odor of *P. xylostella*-damaged white cabbages before the behavioral assay.

Collection of Volatile Compounds

Intact control plants and *P. xylostella*-damaged plants from both CO_2 concentrations from both cultivars ($n = 5$) were used for the collection of the volatile compounds. Cabbages with rinsed and a slightly pruned root system in a 15-mL vial filled with tap water were individually enclosed inside 1.5-L glass vessels, which were closed with a glass lid sealed with Teflon tape and parafilm. After 5 min adjustment, a sample was collected for 30 min on approximately 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, Freiburg, Germany, Model N022AN.18). An inlet for purified air and an outlet for sampling were on the top of the vessel. The airflow was calibrated with the mini-Buck calibrator (Model M-5, A.P. Buck, Orlando, FL), and flow rate was set to 0.215 L min^{-1} for filtered and pressurized air and 0.200 L min^{-1} for sampling. The collection was performed at 22°C and at 300 μmol m^{-2} s^{-1} photosynthetically active radiation at ambient CO_2 concentration.

The samples were analyzed by gas chromatography-mass spectrometry (Hewlett-Packard GC type 6890, MSD 5973). Trapped compounds were desorbed (Perkin Elmer ATD400 Automatic Thermal Desorption system; Norwalk, CT) at 250°C for 10 min, cryofocused at −30°C, and injected onto a HP-5 capillary column (50 m × 0.2 mm i.d. × 0.5-μm film thickness; Hewlett-Packard, Palo Alto, CA). 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. The reference substances for α-thujene, DMNT, and (E,E)-α-farnesene were not available; therefore, the concentration of these compounds were calculated by assuming that the responses would be the same as the responses of α-pinene, (Z)-ocimene, and (E)-β-farnesene, respectively. After sampling, the leaf area of the plants was determined and emissions were calculated as ng cm^{-2} h^{-1}.

Behavioral Assay

The Y-tube olfactometer (main arm 10.5 cm, other arms 10 cm, i.d. 1.6 cm, and angle between two arms approximately 90°) was used to test the behavior of *P. maculiventris* and *C. plutellae*. The insects were given a choice between *P. xylostella*-damaged and intact cabbages grown at ambient or elevated CO_2 concentration. The plants were carefully placed in the 1-l vessels which were closed with Teflon sealed lids with two inlets. The pressurized air was purified with activated carbon and divided via Teflon tubing into two separate flows (0.500 L min^{-1}) which both passed through a 1-l glass vessel containing one cabbage as an odor source and leading to either arm of the Y-tube. The airflow was adjusted with pressure and needle valves and calibrated daily with M-5 mini-Buck calibrator. The insects were introduced to the downwind end of the Y-tube and observed for 5 min or until they made their final choice. The choice was recorded when the insect passed two-thirds or the far end of the Y-tube arm for *P. maculiventris* and *C. plutellae*, respectively. Odor sources, vessels, Y-tube, and lids were replaced after testing six *P. maculiventris* or eight *C. plutellae* to avoid any errors caused by the olfactometer system itself. All the

glassware was heat-treated at 120°C before use, and the Y-tube was turned horizontally around after each tested insect and rinsed with 96% ethanol at least after every third tested insect. In the experiments, approximately 60 *P. maculiventris* nymphs and approx. 64 *C. plutellae* females were tested on 10 and 8 different odor source pairs, respectively, from both CO_2 concentrations and cultivars.

Statistical Analyses

Statistical analyses were performed using SPSS 11.5 for Windows statistical package. The main effect of treatments was tested by nonparametric Kruskal-Wallis-test and one-way ANOVA and multiple comparisons by Dunnett T3 and Tukey post hoc tests. ARCSIN-transformed relative proportions of compound groups were tested by independent samples t test. The data from the behavioral assay were analyzed with the nonparametric binomial test to test whether there was a significant difference in attraction between the two odor sources from plants receiving different treatments (test proportion was set to 0.50).

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