

# The influence of elevated O<sub>3</sub> and CO<sub>2</sub> concentrations on secondary metabolites of Scots pine (*Pinus sylvestris* L.) seedlings

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## Abstract

Terpene, resin acid and total phenolic concentrations in five-year-old Scots pine (*Pinus sylvestris* L.) seedlings were analysed after exposure to ambient and realistically elevated (2 × ambient) O<sub>3</sub> and CO<sub>2</sub> concentrations and their combination in open-top chambers during two growing seasons. Under O<sub>3</sub> exposure, limonene concentration in needles and isopimaric concentration in stems decreased significantly. As a response to elevated CO<sub>2</sub>, α-pinene and total phenolic concentrations in needles increased significantly, while bornyl acetate concentration in needles and palustric + levopimaric and neoabietic acid concentrations in stems decreased significantly. Some terpenes and resin acids were found at lower concentrations in the combined O<sub>3</sub> and CO<sub>2</sub> treatment than in O<sub>3</sub> exposure or elevated CO<sub>2</sub>. A negative chamber effect was found: seedlings growing inside the chambers with ambient air had significantly lower concentrations of some terpenes and resin acids than seedlings growing outside the chambers. There was a lot of between-tree variation in terpene and resin acid concentrations, which is typical of open-pollinated populations. The results of this study suggest that, at least in short-term experiments, Scots pine secondary metabolites are relatively insensitive to climate change factors. Total phenolics in the needles were the most responsive group showing about 25% increase in elevated CO<sub>2</sub>, and O<sub>3</sub> exposure did not mitigate this CO<sub>2</sub> effect. Terpenes and resin acids were less responsive, although some individual compounds showed notable responses, e.g. α-pinene in needles, which increased about 50% in response to elevated CO<sub>2</sub>. As a consequence, although there were only slight effects on total pools of needle secondary metabolites, considerable O<sub>3</sub> and CO<sub>2</sub> effects on certain individual compounds might have ecological significance via trophic amplification, e.g. in decomposing processes of needle litter.

*Keywords:* climate change, resin acids, terpenes, total phenolics

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## Introduction

One of the main threats to the functioning, structure and diversity of natural and seminatural ecosystems is the increase in concentrations of air pollutants in this century (Bobbink 1998). Increasing anthropogenic emissions of reactive oxidized nitrogen (NO<sub>x</sub>) have led to an approximate doubling in tropospheric ozone (O<sub>3</sub>) concentrations since the last century (Fowler *et al.* 1998). Tropospheric O<sub>3</sub>

imposes a stress on forest trees. Decreased chlorophyll contents and photosynthetic rates, changes in carbon allocation and increased antioxidant activity have often been recorded, and O<sub>3</sub> appears to weaken the trees' resilience to abiotic and biotic stresses (Manning & Tiedemann 1995; Skärby *et al.* 1998). In Europe, O<sub>3</sub> is estimated to cause c. 10% forest yield losses as a whole. The generally used AOT40 (40 nL L<sup>-1</sup>) threshold concentration might not be low enough to predict O<sub>3</sub> effects on forest trees in Scandinavia, where chronic injury resulting

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from cumulative exposure may be observed in spite of relatively low O<sub>3</sub> concentrations (Broadmeadow 1998).

The increase in atmospheric carbon dioxide (CO<sub>2</sub>) and other greenhouse gases has produced serious concern regarding the heat balance of the global atmosphere. The annual increase rate of atmospheric carbon dioxide (CO<sub>2</sub>) is estimated to be currently about 2 ppm (roughly 0.6% per year) (Moore & Braswell 1994). For instance, stimulation of photosynthesis and growth (Saxe *et al.* 1998), increased starch and secondary compound accumulation, an increase in root:shoot ratio (Ceulemans & Mousseau 1994) and a reduction in tissue nitrogen concentration (McGuire *et al.* 1995) have been reported in response to elevated CO<sub>2</sub>. Moreover, CO<sub>2</sub>-mediated changes in host plants affect insect herbivores (Bezemer & Jones 1998) and are likely to promote plant diseases (Manning & Tiedemann 1995).

The carbon-based secondary compounds in plants are of great ecological interest, because of their probable effects on the plant decomposition rates, plant digestibility and nutritional value for herbivores, and plant pathogen resistance (Peñuelas *et al.* 1997; Björkman *et al.* 1998). Although concentrations of plant defensive compounds are thought to be primarily genetically determined, their concentrations could also be influenced by environmental conditions (Kainulainen *et al.* 1996; Björkman *et al.* 1998). O<sub>3</sub> exposure has been shown to increase conifer phenolic concentrations (Tingey *et al.* 1976; Rosemann *et al.* 1991; Kainulainen *et al.* 1994), but low O<sub>3</sub> exposure had no effect on monoterpene and resin acid concentrations (Kainulainen *et al.* 1994; Kainulainen *et al.* 1998; Manninen *et al.* 2000). Studies with several plant species have provided evidence, that elevated CO<sub>2</sub> increases foliar concentrations of soluble phenolics and condensed tannins, but not of lignins or terpenoids (Peñuelas & Estiarte 1998). In conifers, as a response to elevated CO<sub>2</sub>, a decrease/increase in concentrations of some individual monoterpenes (Williams *et al.* 1994; Heyworth *et al.* 1998) and an increase in total phenolics (Gebauer *et al.* 1998) have been reported.

Much of our understanding of the impacts of climate change factors comes from studies of the effects of each individual factor. However, the interactions of environmental stresses on vegetation are rarely predictable (Runeckles & Krupa 1994). There are relatively few studies on responses of conifer secondary metabolites to increased O<sub>3</sub> and CO<sub>2</sub> in combination (e.g. Kainulainen *et al.* 1998). Because conifer species and even provenances differ in their responses to altered O<sub>3</sub> and CO<sub>2</sub> environments (Mortensen 1994), it is important to study each species separately. The objective of this study was to test whether realistically elevated tropospheric concentrations of O<sub>3</sub> and CO<sub>2</sub> and their combination alter the

concentrations of secondary metabolites in boreal Scots pine seedlings.

## Materials and methods

### *Plant material*

Three-year-old Scots pine (*Pinus sylvestris* L.) seedlings, originating from central-eastern Finland (M29-90-414), were planted in 7.5-L pots filled with a mixture of quartz sand and fertilized peat (3:1; Vapo peat PP6, basic fertilization 1 kg m<sup>-3</sup>, N-P-K 12:9:18 with micronutrients). The seedlings were transferred to the open-top chambers on 1 June 1995. During growing seasons seedlings were irrigated when necessary with tap water and fertilized (4 times and 7 times per growing season in 1995 and in 1996, respectively) with 0.1% full nutrient solution (Superex-5, N-P-K 11:4:25) receiving an additional nitrogen supply of 21.2 kg ha<sup>-1</sup> in 1995 and 55.7 kg ha<sup>-1</sup> in 1996.

### *O<sub>3</sub> and CO<sub>2</sub> fumigation*

The open-top chambers were situated on a natural pine heath in eastern Finland in the vicinity of Mekrijärvi Research Station (62°47'N, 30°58'E) of the University of Joensuu. Each chamber consisted of transparent plastic sheet used in greenhouses and a wooden frame built around a *c.* 20-y-old Scots pine tree with an average height of 3 m. There were four different treatments (four replicates) in 16 10-m<sup>3</sup>-volume chambers: ambient air, elevated O<sub>3</sub> (2 × ambient), elevated CO<sub>2</sub> (2 × ambient), and elevated O<sub>3</sub> and CO<sub>2</sub> in combination. In addition, five plots with the older tree in the centre served as chamberless controls. There were six potted seedlings in each treatment replicate. All of the seedlings were placed on a stand *c.* 60 cm above ground level on the eastern side of the tree.

Seedlings were exposed to studied gases in open-top chambers for two growing seasons between 1 June and 30 September in 1995, and 20 May and 18 September in 1996. The fumigation system operated continuously (16 h/d: 06.00–22.00 hours). Air was blown into the chambers through a circular perforated polyethylene tube located at the bottom of the chamber. The total air volume in each chamber changed once a minute. The exposure and gas controlling systems were computer-controlled. The O<sub>3</sub> concentration in the elevated O<sub>3</sub> treatment was continuously double the ambient (20–40 ppb). AOT40-values (=O<sub>3</sub> doses above 40 ppb) were 3.4 ppm h<sup>-1</sup> in ambient air and 33.3 ppm-h in elevated O<sub>3</sub> in 1995, and 3.0 and 39.9 ppm h<sup>-1</sup> in 1996, respectively. The system was not fully able to maintain twice the

ambient CO<sub>2</sub> concentration in the elevated CO<sub>2</sub> treatment, and the mean concentration of CO<sub>2</sub> remained *c.* 100 ppm below the target. For more detailed information about the fumigation system, see Palomäki *et al.* 1998.

#### *Temperature and light intensity*

Two degrees higher mean air temperature and a slightly lower light intensity compared to open air treatment were measured in the open-top chambers. Temperature differences between separate chambers were not statistically significant. The light intensity in the measurements varied between 147 and 554  $\mu\text{mol m}^{-2}\text{s}^{-1}$  in open air, and between 136 and 318  $\mu\text{mol m}^{-2}\text{s}^{-1}$  in ambient chamber being significantly higher in open air treatment when compared to O<sub>3</sub> and O<sub>3</sub> + CO<sub>2</sub> chambers in cloudy afternoon measurements, but not in cloudy or sunny morning measurements (Palomäki *et al.* 1998). The light intensity in the chambers was reduced mainly by the transparent plastic sheet used to build the chambers, but because the open-top field fumigation system was built in a pine stand on a natural heath, the light intensity between each chamber and also in the open air varied also depending on the time of the day and the amount and position of trees around the chambers. The older sapling in the chamber affected the light regime mostly in the late afternoon since the potted experimental seedlings were situated on the eastern side of the chambers or open air plots. For more detailed information about the chambers, see Palomäki *et al.* 1998.

#### *Sampling and chemical analysis*

Samples for analysis of secondary metabolites were collected on 24 September 1996. The samples for terpene and resin acid analyses were frozen immediately in liquid nitrogen and stored at  $-80^{\circ}\text{C}$  before analysis. Mono- and sesquiterpenes were extracted from frozen needles with *n*-hexane (Kainulainen *et al.* 1992) using 1-chloro-octane as an internal standard. Resin acids were extracted from freeze-dried and milled stems with petroleum ether-diethyl ether following the procedures of Gref & Ericsson (1985), because concentrations of most resin acids in stems appeared to be more responsive to environmental disturbances than resin acids in needles (Sallas *et al.* 1999). The internal standard was heptadecanoic acid. Both extracts were analysed by gas chromatography-mass spectrometry (Hewlett Packard GC-type 5890, MSD type 5970) using a 30-m-long HP-5MS capillary column (0.25 mm ID, 0.25  $\mu\text{m}$  film thickness, Hewlett Packard) and helium as the carrier gas. The temperature programme for terpenes was from  $40^{\circ}\text{C}$  to  $250^{\circ}\text{C}$  and for resin acids from  $150^{\circ}\text{C}$  to  $250^{\circ}\text{C}$  at the rate

of  $5^{\circ}\text{C min}^{-1}$ . In both analyses, the technique of Selected-Ion-Monitoring (SIM) was used. Individual substances were quantified by their peak areas and identified by comparison of their mass spectra and retention times with pure reference compounds. Analysed individual terpenes and resin acids were summed to total terpenes and total resin acids.

For total phenolic analysis dried samples were stored in a desiccator at  $4^{\circ}\text{C}$ . Fine powdered needles and stems were extracted separately with 80% acetone, and the residue washed three times with acetone. Total phenolics were analysed using Folin-Ciocalteu reagent as reported by Julkunen-Tiitto (1985). The absorbances of the samples were measured with a spectrophotometer (Shimadzu UV-2100) at 735 nm.

#### *Statistical analysis*

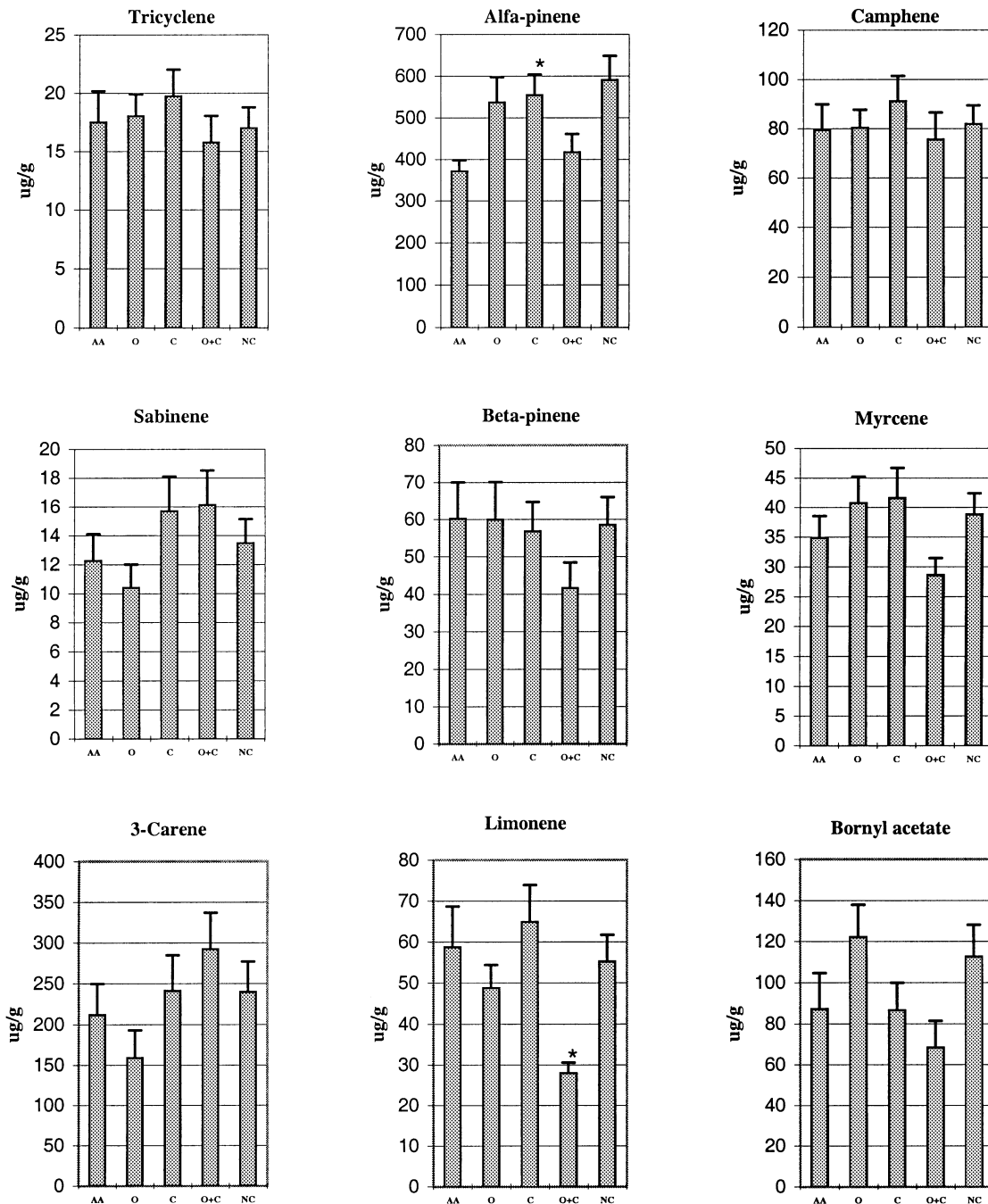
When necessary, data were normalized by  $\log(x+1)$  transformations for statistical analysis. GLM multivariate (SPSS for Windows, v. 8.0) was used to test for interaction effects of O<sub>3</sub> and CO<sub>2</sub> using whole plant biomass as a covariate. Differences between single treatments were tested with Tukey's *post hoc* test. An independent samples *t*-test was used to test for a chamber effect.

## **Results**

#### *Terpene concentration in needles*

According to GLM analysis, exposure to O<sub>3</sub> affected significantly concentration of one individual monoterpene, limonene ( $P=0.013$ ) (Fig. 1), while elevated CO<sub>2</sub> decreased concentration of bornyl acetate ( $P=0.033$ ). There was a significant two-way interaction between O<sub>3</sub> and CO<sub>2</sub> treatments in concentrations of  $\alpha$ -pinene ( $P=0.009$ ), limonene ( $P=0.048$ ) and bornyl acetate ( $P=0.041$ ), and a marginally significant two-way interaction between O<sub>3</sub> and CO<sub>2</sub> treatments in total terpene ( $P=0.057$ ) concentration (Fig. 2).

Tukey's *post hoc* test revealed a marginally significant ( $P=0.077$ ) increase in  $\alpha$ -pinene concentration under O<sub>3</sub> exposure and a significant ( $P=0.035$ ) increase in  $\alpha$ -pinene concentration in elevated CO<sub>2</sub>, when compared to ambient air. Seedlings in combined O<sub>3</sub> and CO<sub>2</sub> treatment had significantly ( $P=0.035$ ) less bornyl acetate in their needles than seedlings exposed to O<sub>3</sub>. Seedlings in combined O<sub>3</sub> and CO<sub>2</sub> treatment had significantly ( $P=0.025$ ) less limonene than seedlings in ambient air and significantly ( $P=0.004$ ) less limonene than seedlings in elevated CO<sub>2</sub>.



**Fig. 1** Mean concentrations ( $\mu\text{g g}^{-1}$  fwt) of monoterpenes (+SEM) in pine needles in different treatments. Values differing significantly from ambient air are marked with an asterisk (\*). AA, Ambient air; O, Ozone; C, Carbon dioxide; OC, Ozone + carbon dioxide; NC, No chamber.

#### Resin acid concentration in stems

Only concentrations of some individual resin acids were affected by  $\text{O}_3$  or  $\text{CO}_2$  treatments. Exposure to  $\text{O}_3$  decreased concentration of isopimaric acid ( $P=0.047$ ) (Fig. 3), and exposure to elevated  $\text{CO}_2$  decreased con-

centrations of palustric + levopimaric ( $P=0.010$ ) and neoabietic ( $P=0.005$ ) acids. There was a significant two-way interaction between  $\text{O}_3$  and  $\text{CO}_2$  treatments in pimaric acid ( $P=0.024$ ) concentration.

According to Tukey's *post hoc* test, seedlings in combined  $\text{O}_3$  and  $\text{CO}_2$  treatment had significantly less

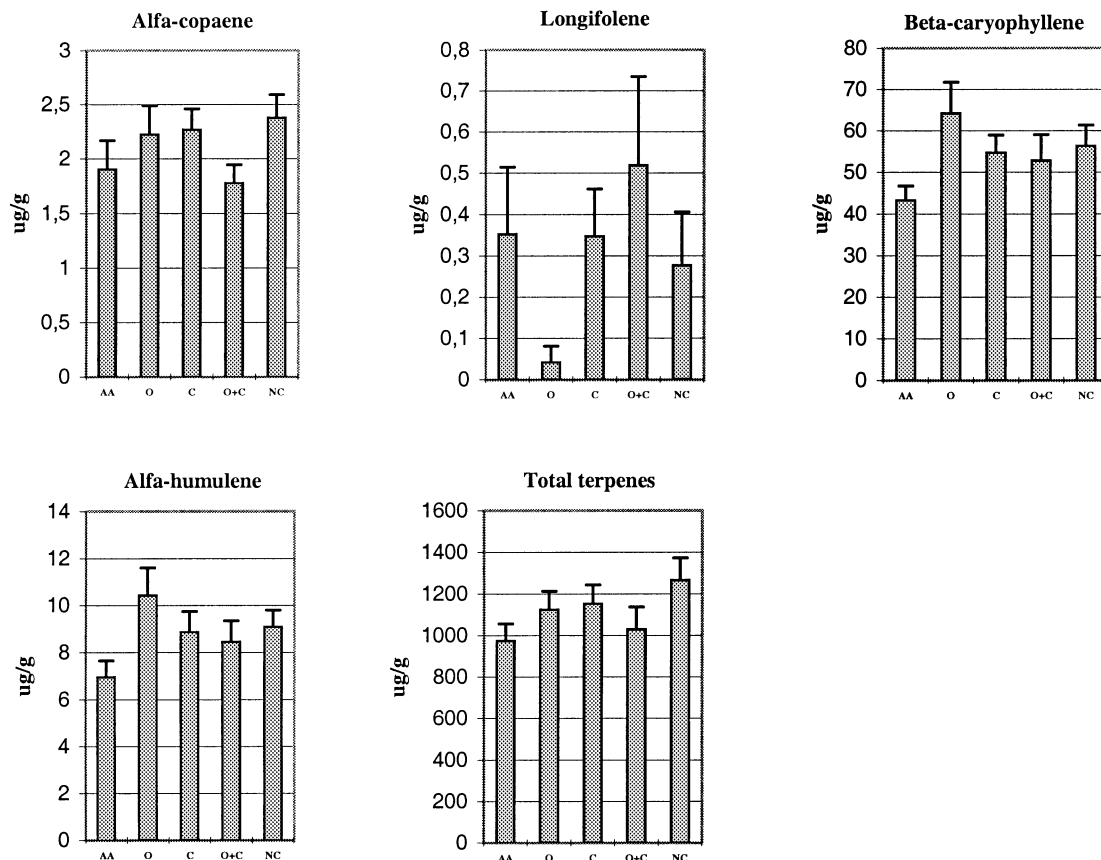


Fig. 2 Mean concentrations ( $\mu\text{g g}^{-1}$  fwt) of sesquiterpenes and total terpenes (+SEM) in pine needles in different treatments. Symbols as explained in caption to Fig. 1.

palustric + levopimaric ( $P=0.006$ ) and neoabietic ( $P=0.010$ ) acids than seedlings under  $\text{O}_3$  exposure.

#### Total phenolic concentration in needles and stems

Total phenolic concentration in needles increased significantly ( $P=0.005$ ) in seedlings grown in elevated  $\text{CO}_2$  (Fig. 4). According to Tukey's *post hoc* test, seedlings in elevated  $\text{CO}_2$  ( $P=0.017$ ) and in the combined  $\text{O}_3$  and  $\text{CO}_2$  treatment ( $P=0.016$ ) had more total phenolics in needles, when compared to ambient air.

In stems, differences in total phenolic concentrations between treatments were not statistically significant.

#### Chamber effect

Seedlings growing outside the chambers had significantly higher concentrations of several terpenes and resin acids. Concentrations of  $\alpha$ -pinene ( $P=0.018$ ),  $\beta$ -caryophyllene ( $P=0.042$ ),  $\alpha$ -humulene ( $P=0.045$ ) and total terpenes ( $P=0.047$ ) in needles, and concentrations of sandaracopimaric acid ( $P=0.011$ ), dehydroabietic acid

( $P=0.028$ ) and total resin acids ( $P=0.001$ ) in stems were significantly higher compared to seedlings grown in chambers with ambient air. In addition, seedlings in chambers had significantly lower stem base diameters than seedlings in open air, but no statistical difference was found in current-year main shoot length, plant height, root and plant total dry masses and root-to-shoot dry weight ratio in the final harvest (24 September 1996) (Utriainen *et al.* 2000).

#### Discussion

Overall,  $\text{O}_3$  and/or  $\text{CO}_2$  had only slight effects on concentrations of monoterpenes in needles as shown in earlier studies with loblolly pine (Williams *et al.* 1994) and Scots pine (Heyworth *et al.* 1998; Kainulainen *et al.* 1998). Increased concentrations of the most abundant monoterpene,  $\alpha$ -pinene, in elevated  $\text{CO}_2$ , are consistent with the study of Heyworth *et al.* (1998). In contrast to this observation, Williams *et al.* (1994) found decreased concentrations of  $\beta$ -pinene in needles under elevated  $\text{CO}_2$ . In addition, wounded and half-eaten needles of

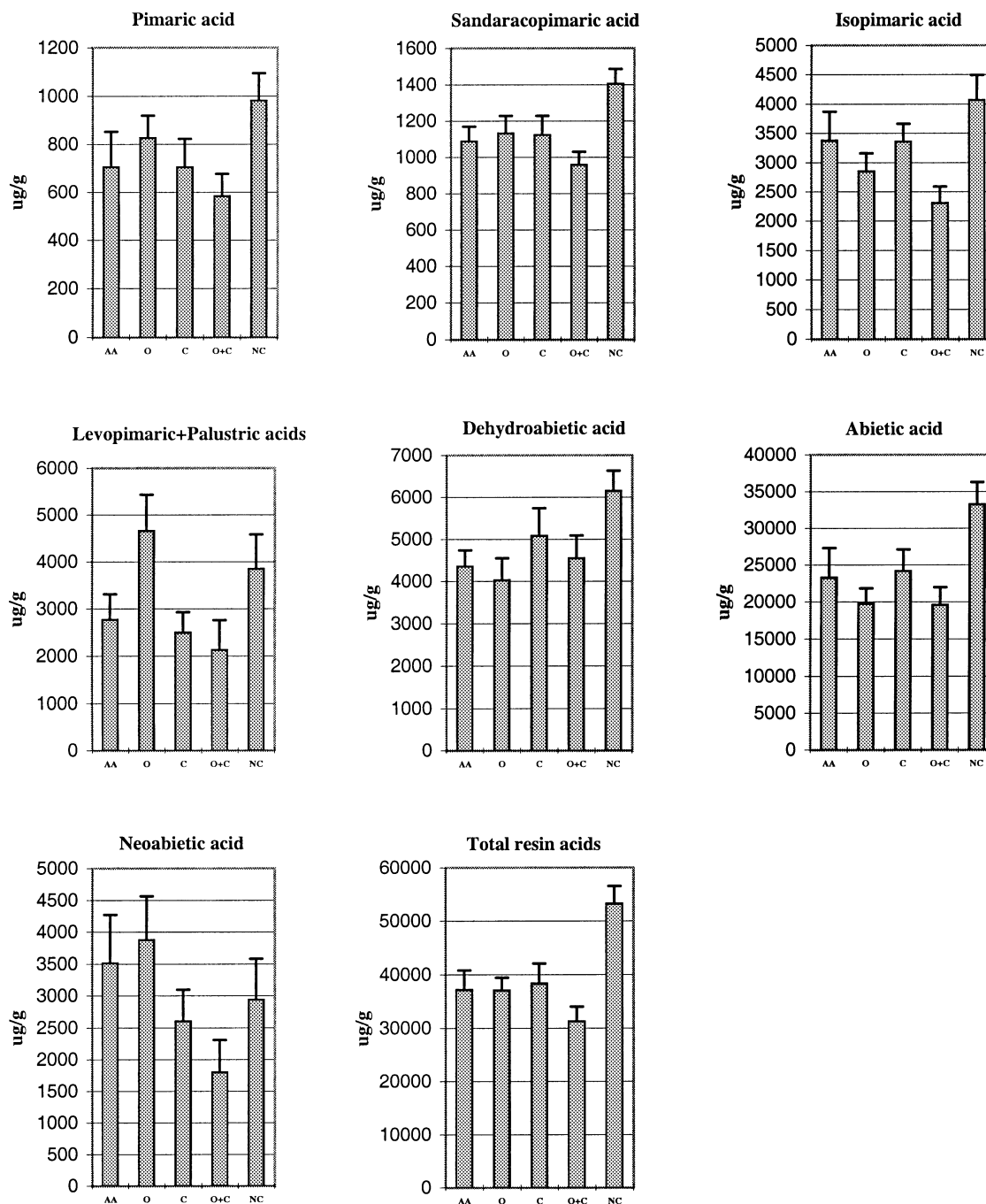
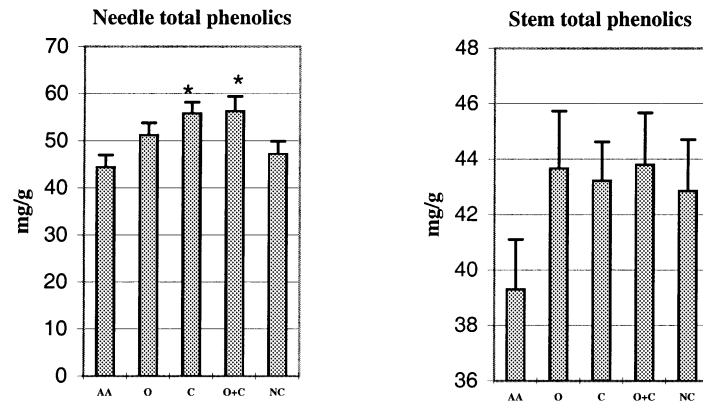


Fig. 3 Mean concentrations ( $\mu\text{g g}^{-1}$  dwt) of resin acids (+SEM) in pine stems in different treatments. Symbols as explained in caption to Fig.1.

ponderosa pine had significantly higher concentrations of  $\alpha$ -pinene (Litvak & Monson 1998), which might be one of the most responsive monoterpenes to environmental stresses.

Isopimaric acid concentrations in stems decreased in  $\text{O}_3$  exposure. The slight increase in palustric + levopimaric acids in stems in  $\text{O}_3$  exposure is in agreement with

earlier studies, where some individual resin acids increased in pine shoots (Kainulainen *et al.* 1995a,b, 2000) in response to higher levels of  $\text{O}_3$  than used in this study. In the seedlings of this study current-year needle resin duct areas increased slightly in  $\text{O}_3$  exposure (Utriainen *et al.* 2000), which may indicate a defence reaction against  $\text{O}_3$ . Elevated  $\text{CO}_2$  caused a reduction in



**Fig. 4** Mean concentrations ( $\text{mg g}^{-1}$  dwt) of total phenolics (+SEM) in pine needles and stems in different treatments. Values differing significantly from ambient air (Tukey's test) are marked with an asterisk (\*). Symbols as explained in caption to Fig. 1.

palustric + levopimaric and neoabietic acid concentrations in stems. In elevated  $\text{CO}_2$ , current-year needle resin duct areas increased during the first exposure year significantly (Utriainen *et al.* 2000), which could be related with increased production of secondary compounds. The reduction in resin duct area during the second exposure year might indicate acclimation of carbon assimilation of the seedlings in elevated  $\text{CO}_2$  or slight nitrogen deficiency of all the seedlings used in this study.

Some terpenes and resin acids were found at lower concentrations in the combined  $\text{O}_3$  and  $\text{CO}_2$  treatment when compared with  $\text{O}_3$  exposure or elevated  $\text{CO}_2$  treatment. Higher levels of  $\text{CO}_2$  could have diminished detrimental effects of  $\text{O}_3$  by decreasing  $\text{O}_3$  flux into needles by reduced stomatal conductance or by providing more substrates to counteract  $\text{O}_3$  effects (Allen 1990; Kellomäki & Wang 1997). However, when compared to  $\text{O}_3$  or  $\text{CO}_2$  as single variables, Norway spruce seedlings concurrently exposed to elevated  $\text{O}_3$  and  $\text{CO}_2$  exhibited the lowest superoxide dismutase and catalase levels, enzymes that characterize the oxidative stress tolerance (Polle *et al.* 1993). The joint application of elevated  $\text{O}_3$  and  $\text{CO}_2$  also resulted in the lowest values of carboxylation efficiency and maximum photosynthetic capacity in Norway spruce seedlings (Lippert *et al.* 1997). In this study,  $\text{O}_3$  and  $\text{CO}_2$  in combination reduced significantly current-year main shoot length and caused a 15% reduction in total plant dry mass (Utriainen *et al.* 2000), which might indicate reduced photosynthetic capacity and thus depletion of substrates needed for secondary metabolites production.

A significant negative chamber effect was found in the case of terpenes and resin acids: seedlings in chambers accumulated mostly lower levels of secondary compounds than seedlings grown in open air. This might be a consequence of reduced light intensity in the chambers (Palomäki *et al.* 1998). Gref & Tenow (1987) detected lower concentrations of resin acids in shade needles, which is probably caused by reduced photosynthesis and

thus depleted substrates needed for resin acid biosynthesis. Monoterpene synthesis is also light dependent (Gleizes *et al.* 1980). Another possible explanation for the lower levels of terpenes detected in the chambers might be that the higher mean temperatures in the chambers increased the emission of monoterpenes, which is generally regarded to be temperature dependent (Kesselmeier & Staudt 1999).

There was a lot of variation in concentrations of individual and total terpenes and resin acids between individual seedlings, which is typical of open-pollinated tree populations. Genetic differences most likely lead to variation in responses to stress factors as well. Yet it is considered more realistic herein to test climatic change effects with naturally regenerated trees than with cloned trees, which might represent only a small fraction of traits existing in real forests. The problem of inheritable tree variation in concentrations of secondary metabolites could have been avoided by making a preliminary study of the levels of the secondary metabolites in the studied seedlings and using these as covariates in the statistical analysis. However, this was not done to avoid the possible induced production of oleoresins as a result of sampling.

The relatively high tolerance of conifers to  $\text{O}_3$  (Fuhrer *et al.* 1997) and relatively low levels of  $\text{CO}_2$  used in this study might be other reasons that might explain the rather small responses observed in terpenes and resin acids. This is in agreement with an earlier study of Kainulainen *et al.* (1998) using naturally growing trees in the same chambers. At elevated  $\text{O}_3$ , seedling growth was retarded leading to a nonsignificant 11% reduction in total plant dry mass, and at elevated  $\text{CO}_2$  growth was accelerated leading to a nonsignificant 19% increase in total plant dry mass (Utriainen *et al.* 2000) causing seedlings to be of different size and developmental stage at a given age. Gebauer *et al.* (1998) suggest that plant size should be used as a covariate when evaluating direct effects of  $\text{CO}_2$  on carbon investment into secondary

compounds, and therefore seedling biomass was used as a covariate in this study.

Total phenolics in needles increased significantly in elevated CO<sub>2</sub> as was observed in the study of Gebauer *et al.* (1998) with *Pinus taeda*. Peñuelas & Estiarte (1998) suggested that increases in phenolics under elevated CO<sub>2</sub> could be an indirect effect of decreases in organic N compounds, which causes a reduction of protein synthesis (growth) consequently increasing substrate availability for the synthesis of phenolics. In this study, Ca and Mn concentrations increased and Cu concentration decreased significantly in response to elevated CO<sub>2</sub>, and elevated O<sub>3</sub> decreased concentration of Mn in current-year needles. No statistically significant differences between treatments were found in current-year needle concentrations of Fe, K, Mg and P (Utriainen *et al.* 2000). Needle N concentrations in the current experiment (current-year needle concentration was *c.* 9–10 mg g<sup>-1</sup> dwt and in response to elevated CO<sub>2</sub> nitrogen content in the current-year needles decreased about 24%) were comparable to the concentrations in natural boreal pine forests, where relatively low N availabilities prevail throughout the growing seasons (Helmisaari 1990; Raitio 1990; Attiwill & Adams 1993). The present experiment might simulate natural conditions quite well, because boreal pine forests are usually poor in nitrogen. However, growing the experimental plants in containers may present several problems: root constriction possibly resulting to source-sink imbalance and greater down-regulation in photosynthesis (Arp 1991), possible greater plasticity of individually grown plants compared to plants grown in competition, and potentially different responses of seedlings vs. mature trees (Pritchard *et al.* 1999). The higher responsiveness of the phenolics to CO<sub>2</sub>, compared with the terpenoids, may be the result of different biochemical pathways involved in their synthesis. Only phenylpropanoids compete directly with protein synthesis and plant growth for the same substrate (phenylalanine), while the synthesis of terpenoids proceeds via mevalonic acid pathway (Haukioja *et al.* 1998).

In summary, the results of this study suggest that, at least in short-term experiments like this, Scots pine secondary metabolites as total pools are relatively insensitive to climate change factors, and especially to elevated atmospheric concentrations of O<sub>3</sub>. Total phenolics in needles were the most responsive group of the secondary metabolites, showing about 25% increase in elevated CO<sub>2</sub>. Although total terpenes and total resin acids increased in elevated CO<sub>2</sub> only 18% and 3%, respectively, some individual compounds showed notable responses, e.g.  $\alpha$ -pinene in needles, which increased almost 50% in response to elevated CO<sub>2</sub>. These global change effects on specific compounds and their ratio

might have larger ecological significance, e.g. for host plant selection of herbivores than effects on less responsive total pools of groups of secondary metabolites. Certain monoterpenes have, e.g. antifungal properties (Gershenzon & Croteau 1991) which might be amplified in decomposing processes of needle litter and thus affect on further trophic levels.

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