FOLIAR SYMPTOMS, ETHYLENE BIOSYNTHESIS AND WATER USE OF YOUNG NORWAY SPRUCE (*PICEA ABIES* (L.) KARST.) EXPOSED TO DROUGHT AND OZONE

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Abstract. Four-year old Norway spruce (*Picea abies* (L.) Karst.) trees were pretreated at low and high water supply, and then placed into a growth chamber containing four compartments so that two levels of ozone exposure, 0.02 and 0.4 $\mu l\ l^{-1}$, could be replicated. They were exposed to ozone and drought stress for 59 days, and water use was determined by periodic weighing. Small effects of ozone treatment were detected on new shoot dry weight, and water use by trees receiving the high ozone treatment appeared higher. Both visual symptoms and ethylene biosynthesis showed that drought stress reduced damage to trees exposed to high ozone. Ethylene emission and 1-aminocyclopropane-1-carboxylic acid (ACC) levels increased after 18 days of exposure to 0.4 $\mu l\ l^{-1}$ ozone, while visual symptoms were seen at 30 days. After 59 days of exposure to the combined stresses, ethylene and ACC levels were lower, but showed an ozone x water interaction. Most ethylene and ACC were produced by wet trees at high ozone concentration, but dry trees also had high ethylene and ACC levels at low ozone. Levels of needle malonyl-ACC (MACC) were not significantly affected by treatment, and did not change with time, but root MACC levels, which were twice needle levels, were high in wet trees at high ozone concentration, but also high in dry trees at low ozone concentration. These results suggest that drought stress occurring during ozone exposure could be expected to reduce damage to young Norway spruce, and that this damage may be related to ethylene biosynthesis.

Introduction

Ozone is recognized as a phytotoxic air pollutant and is known to reduce photosynthesis and growth under controlled conditions (Reich, 1987; Pye, 1988; Edwards *et al.*, 1992). Susceptibility to ozone damage is partly dependent on stomatal conductance because plants with higher conductance allow greater entry of ozone into leaves and develop more damage (Reich and Amundson, 1985). For an equivalent dose, agricultural crops are most sensitive to ozone, broadleaved trees intermediate, and conifers most tolerant (Reich, 1987). Drought stress causes reduction in stomatal conductance and can therefore reduce the extent of damage caused by ozone (Tingey and Hogsett, 1985). This effect has been demonstrated for soybean (*Glycine max* L. Merrill) where drought stressed plants showed less ozone damage than well watered plants (Amundson *et al.*, 1986).

Recent decline of Norway spruce is thought to be related to interactions between pollutants and natural stresses (Rehfuess, 1987). Ozone pollution is suspected to

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play a rôle, especially at higher elevation in Germany (Rehfuess, 1987), although some results indicate it may not be important (Schulze, 1989). Ozone exposure over three years has been shown to reduce net photosynthesis in Norway spruce (Wallin *et al.*, 1990), and increase transpiration rate (Barnes *et al.*, 1990a). In North America ozone is implicated in damage to a number of conifer and broadleaf trees (Woodman, 1987).

Ethylene emission is known to respond to various environmental stresses, including drought, chilling, pathogen infection and exposure to air pollutants, and it is suggested that endogenous ethylene mediates changes in gas exchange known to occur under adverse conditions (Gunderson and Taylor, 1991). Recent work has questioned whether ethylene biosynthesis is promoted by drought stress in beans (*Phaseolus vulgaris* L.) and cotton (*Gossypium hirsutum* L.) (Morgan *et al.*, 1990). Ozone exposure promoted ethylene formation in various plant species (Tingey *et al.*, 1976), and the level of 'stress' ethylene induction is considered a major determinant of ozone tolerance (Mehlhorn and Wellburn, 1987; Langebartels *et al.*, 1991). Moreover, ethylene emission was postulated as a site independent factor suitable for prediction of forest decline (Mehlhorn *et al.*, 1989).

The purpose of this work was to confirm that drought stress could reduce ozone damage in young Norway spruce (*Picea abies* (L.) Karst.), and to test the hypothesis that ozone induced changes of ethylene biosynthesis might precede appearence of foliar visual symptoms.

Methods

Plant culture

Four-year-old, bare root, Norway spruce (provenance 840 21, from 900–1300 m Alpine source) were obtained from Laufen nursery, southeast Bavaria, and potted into 30 cm deep × 10 cm diameter cylindrical containers (volume 2.36 L), on 16–18 October 1991. The potting soil was a 1 peat:1 vermiculite mixture containing 1 kg $CaCO_3$ and 2 kg slow release NPK fertilizer (Kaliphos 18:10:10) per m^3. The plants remained outside over winter and roots were protected by mulching to the top of the pots. On 16 January 1992, they were brought into a greenhouse providing 20 °C day and 15 °C night temperatures, and a 16 h photoperiod. On 10 February temperatures were increased to 25 °C day and 20 °C night. An Adelgid *Sacchiphantes viridis* Ratz.) infestation was controlled with a Metasystox (BASF) spray on 7 February. Bud flushing was evident on 20 February.

Preconditioning with drought stress

To precondition plants to drought stress before ozone exposure four blocks of 22 plants were established along the greenhouse bench, and two allocated at random to low water treatment and two for controls. Within each block trees were randomly

allocated for two future ozone treatments, and 5 were marked separately for periodic removal of branches for water potential measurement with a pressure bomb. To impose two levels of water supply, soil weight at field capacity was determined for each potted plant by subtracting empty container weight and an average seedling fresh weight. Soil moisture content at field capacity was determined from subsamples to be 352% of soil dry weight, and pots were rewatered to return controls to field capacity and low supply pots to a water content of 250% of dry soil. Weighing and rewatering were done at weekly intervals from 26 January until 26 March. In the greenhouse average water potentials, at noon on 27 Februsry, were –0.89 and –1.19 MPa for controls and stressed trees respectively, and pre-dawn values on 6 March, were –0.37 and –0.61 MPa (Table I).

OZONE EXPOSURE AND DROUGHT STRESS

Plants were exposed to two levels of ozone, replicated twice, with two levels of moisture stress randomized within each ozone level, and replicated 7 times. The four ozone treatment plots were therefore split by the two watering treatments which were randomised among 7 replicates. In addition, the 20 plants designated for water potential measurements were allocated five per chamber so that at least two plants from each watering treatment were placed in every chamber. Plants were treated with ozone and drought stress in closed chambers from 27 March to 25 May 1992. Four plexiglass chambers, of 0.9 m^3 with 0.83 m^2 floor , contained in a growth room, provided 25 °C day and 20 °C night temperatures, a constant relative humidity of 75 ± 5%, and a 16 hr photoperiod. The photosynthetic photon flux density (PPFD), over the waveband 400–700 nm, varied from 90 to 100 μmol m^{-2} s^{-1} within each chamber, except for one 16 hr photoperiod per week when a PPFD of 400 to 450 μmol m^{-2} s^{-1} was provided. Light intensities increased and decreased during the first and last hour of the photoperiod. These light conditions were representative of the floor of a dense spruce stand (Johansson, 1987), as might be experienced by natural regeneration. Day temperature at the high PPFD rose to 30 °C. Ozone was generated from pure O_2 and was analysed as described previously (Langebartels *et al.*, 1991). A background ozone level of 0.02 ± 0.005 μl l^{-1} was maintained in all chambers, and a treatment of 0.4 ± 0.04 μl l^{-1} ozone, between 13:00 and 23:00 daily, was allocated to two chambers. The concentration of 0.4 μl l^{-1} was used because Norway spruce is relatively resistant to ozone over exposure periods of less than a season (Wallin *et al.*, 1990). The treatment was not maintained on the 12th day of the fumigation when ozone levels in the two treatment chambers rose from 0.4 μl l^{-1} at 16:00 to 1.2 μl l^{-1} at 9:00 the following morning due to a computer malfunction. Control levels were unaffected. Wind speed within chambers was in the range 0.3 to 1.0 m s^{-1}, and flow rate through the chambers was 60 m^3 hr^{-1}.

TABLE I

Mean water potentials (Mpa), measured with a pressure bomb on excised shoots, for watering and ozone treatments by dates, together with level of significance between means, and error degrees of freedom (d.f.) and mean square (E.M.S.)

Date	Watering treatment					Ozone treatment				
	Wet	Dry	p	d.f.	E.M.S.	0.02 μl l^{-1}	0.4 μl l^{-1}	p	d.f.	E.M.S.
Preconditioning period										
27 Feb	–0.89	–1.19[a]	0.30	1	0.114					
06 Mar	–0.37	–0.61	0.03	1	0.001					
Ozone exposure period										
02 Apr	–0.87	–0.96	0.41	14	0.049	–0.87	–0.97	0.20	1	0.005
16 Apr	–0.50	–0.54	0.47	14	0.013	–0.54	–0.50	0.29	1	0.002
24 Apr	–0.73	–0.75	0.70	14	0.040	-0.69	-0.79	0.48	1	0.043
07 May	–0.50	–0.73	0.02	14	0.033	–0.64	–0.58	0.14	1	0.001
10 May	–1.07	–1.31	<0.01	42	0.064	–1.15	–1.24	0.64	1	0.221
25 May	–0.55	–0.99	<0.01	42	0.051	–0.80	–0.73	0.55	1	0.054

[a] The numbers of observations per date were 20, except for 10 May and 25 May when the numbers were 48 and 56 respectively. All measurements were made pre-dawn except on 27 February and 10 May.

MEASUREMENTS

Shoot height, length of new apical shoot, and stem diameter above soil, were measured for all plants at the start, and end of the ozone exposure period. The tops and bottoms of the containers were sealed with polyethylene sheet shortly before the experiment started, so that water loss from the pots occurred primarily as transpiration from the shoot. The effectiveness of the sealing was verified by measuring weight loss from four containers without trees which lost only 0.42% of average weight lost by containers with trees over the 59 day ozone exposure. On 7 occasions, 26 March, 7, 16 and 28 April, and 8, 18 and 25 May, pots were weighed and watered to return control pots to 352%, and stressed pots to 250% during the ozone exposure period. Differences between weights at the start and the end of each interval were assumed to represent transpirational water loss, and total water use for each plant was obtained for the experimental period.

At the start of the ozone exposure period shoot volume of all 88 plants was determined by displacement (cf. Burdett, 1979). Six plants from each of the four blocks in the greenhouse were randomly chosen for destructive measurement of new and old shoot and root dry weight after determination of shoot volumes. Regression of shoot dry weight over shoot displacement for the 24 plants in the starting sample had a coefficient of determination (r^2) of 0.95, and the regression equation (intercept 4.39, slope 0.290) was used to estimate the shoot dry weights of remaining plants at the start of the ozone exposure period.

Ethylene evolution, and ACC and MACC concentrations, were determined after 18 days of ozone exposure by sampling the 5 plants designated for water potential measurement, plus one other, in each chamber. The same measurements were repeated again at the end of ozone exposure using 5 other moisture stressed and 5 control trees from each chamber. Ethylene evolution was determined by placing a current shoot (0.3–0.4 g fresh weight) into a silicone stoppered, 25 ml, conical flask for 2 hr in the growth room, under the same temperature and light conditions to which the whole plants were exposed. Two 1 ml aliquots of head gas were withdrawn for ethylene determination by gas chromatography (Langebartels *et al.*, 1991). New fine root samples, extending 5–8 cm from the tip, were also obtained at the end of ozone exposure from 3 moisture stressed and 3 control trees in each chamber for ACC and MACC determination. Current shoots and new root samples were prepared for ACC and MACC determination by freezing in liquid N_2, grinding with pestle and mortar in the presence of liquid N_2, and extracting with 2% (w/v) metaphosphoric acid (Chen and Wellburn 1989) and 1% (w/v) PVPP. ACC and MACC were quantified according to Langenartels *et al.* (1991).

An assessment of needle mottling and colour change caused by ozone was made after 39 days, and again after 53 days, and was subjectively scored on a scale of 0 (healthy) to 4 (severe), using half unit intervals. Nineteen trees were available for scoring in each treatment (both blocks combined), except for wet trees at 0.4 $\mu l\ l^{-1}$ ozone, where 20 trees were available. Net photosynthesis was measured

on 6 moisture stressed and 6 control trees in each chamber after 48 days ozone exposure, using an ADC system (LCA 2, cuvette C, ADC, Hoddesdon, U.K.). The QFD to which measured branches were exposed averaged 190 ± 12 μmol m^{-2} s^{-1}. Cuvette temperature was 30 °C, and relative humidity was 28%. Needle projected area was measured with a Delta-T area meter (Delta-T Devices Ltd., Cambridge, U.K.).

At the end of the ozone exposure period, after 59 days, shoots were separated into current and old growth, and dry weights of current and old needles, stems and roots were obtained. Lengths of 28 root systems (16 from replicate 1 and 12 from replicate 2), representing moisture, ozone and replicate combinations, were determined using the method of Tennant (1975). Dry weights of new shoots, removed for measurement of photosynthesis, ethylene, ACC and MACC, were determined from fresh weights and fresh weight:dry weight ratios, and added to final shoot weights.

Data were treated by analysis of variance, using either anova or general linear models procedures (SAS Institute Inc., 1989). Tests showed injury scores of ozone damage were not normally distributed so they were treated by contingency table analysis (Sokal and Rohlf, 1981). Two large values occurring in needle ACC measurements after 59 days of ozone exposure (23 and 39 nmol g^{-1} fresh weight) were excluded from analysis because both Student and Press statistics showed them to be outliers.

Results

The dry treatment consistently reduced xylem water potentials compared with the wet treatment, and dry trees had significantly lower water potentials on four occasions (Table I). Height growth was largely completed during the preconditioning period, and averaged 6.9 cm for dry trees and 8.9 cm for wet trees. Root dry weight approximately doubled during the ozone exposure period, during which time neither shoot height nor root dry weight were affected by treatments (Table II).

Current season stem and needle dry weights were only measured at the end of the ozone exposure period and therefore showed preconditioning amd ozone exposure effects. When adjusted on the basis of starting shoot dry weights, current season stem dry weight was 21% less, and current needle dry weight was 20% less for dry trees; ozone treatment reduced current season stem weight by 14% (Table II). Root length showed an ozone x water interaction in which dry trees had 24% more root length than wet trees at 0.02 μl l^{-1}, but there was little difference between dry and wet trees at 0.4 μl l^{-1} (Table V).

Water use of dry trees was less than that of wet trees throughout the ozone exposure period (Figure 1). Wet trees receiving 0.4 μl l^{-1} ozone also used more water than wet trees receiving 0.02 μl l^{-1} ozone. This difference was already present at the first measurement period, and did not develop progressively, suggesting either

TABLE II

Mean shoot height growth, shoot and root starting dry weights and dry weight growth during ozone exposure period, current year stem and needle dry weight growth during conditioning and exposure periods combined, and associated probabilities

Measurement	Treatments Ozone μl L^{-1}				Probabilities and error degrees of freedom for effects							
	0.02	0.02	0.4	0.4	Ozone (O)		Water (W)		O × W		Covariate[a]	
	Water											
	Dry	Wet	Dry	Wet	p	d.f.	p	d.f.	p	d.f.	p	d.f.
Ozone exposure period												
Height growth cm	0.51	0.23	0.64	0.00	0.91	1	0.12	50	0.53	50		
Starting shoot weight g	20.8	20.2	20.2	22.6	0.66	1	0.60	50	0.40	50		
Shoot weight growth g	2.41	1.32	1.78	2.42	0.86	1	0.11	50	0.21	50		
Starting root weight g	4.22	3.96	4.99	4.81	0.53	1	0.75	18	0.95	18		
Root weight growth g	4.83	3.94	2.86	3.91	0.18	1	0.93	18	0.29	18		
Preconditioning and ozone exposure period												
Current[b] stem weight growth g	1.32	1.48	0.99	1.42	0.07	1	0.01	25	0.21	25	<0.01	25
Current[b] needle weight growth g	3.70	4.21	3.39	4.70	0.74	1	<0.01	25	0.08	25	<0.01	25

[a]Probability for covariate starting shoot weight.

[a b]Means adjusted for starting shoot weight.

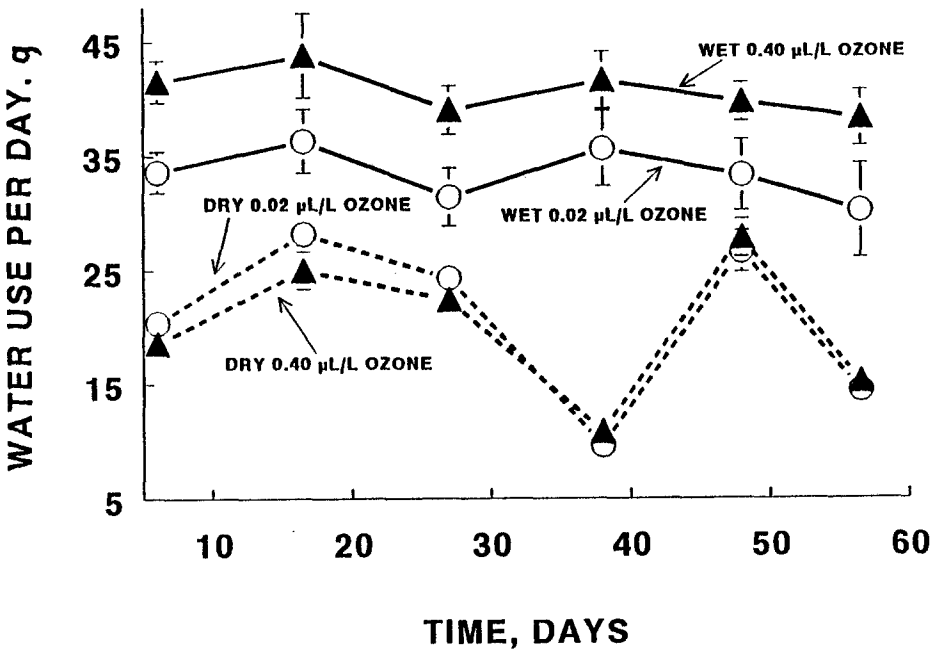

Fig. 1. Mean water use per day for six intervals during exposure to ozone at either 0.02 μl l^{-1} or 0.40 μl l^{-1}, and adequate water or drought. Values are plotted at mid points between weighings, and analysis of variance showed the interval × water treatment significant (p = <0.01) and the ozone × water treatment significant (p = <0.01). Standard errors are shown except where symbols exceed the size of error bars.

development during the first 10 days of ozone exposure, or that the 14 wet trees in the high ozone treatment had higher transpiration at the start of the ozone exposure period. The latter could have resulted from these 14 trees having larger shoots and therefore more transpiring surface. To see if this might be so, water use was adjusted for starting shoot weight and final shoot weight by covariance. Both covariates were significant, but the ozone by water interaction remained significant, indicating that the effect was not entirely due to shoot size (Table III).

Water use efficiency, calculated as whole tree increase in dry weight per unit water use over the 59 day ozone exposure period, showed dry trees to be more than twice as efficient (0.0080 g dry weight g^{-1} water) as wet trees (0.0034 g dry weight g^{-1} water), with p<0.01. Water use efficiency values obtained for 0.02 μl l^{-1} and 0.4 μl l^{-1} ozone treatments were similar (0.0055 g and 0.0059 g dry weight g^{-1} water, respectively).

Visible symptoms consisted of transverse yellow banding on current year needles, which was initially subtle, but later gave a yellow hue to the shoot. Symptoms became visible after 30 days of ozone exposure, and contingency table analysis showed a significant effect of ozone and ozone × water interaction on symptom

TABLE III

Covariance analysis of water use, in which shoot dry weights at the start and at the end of the ozone exposure period were tested as covariates, and mean water use per day during ozone exposure, adjusted for starting weight

Analysis of covariance						Treatments		Water use
Source	d.f.	Mean square	p	Mean square	p	Ozone $\mu l\,l^{-1}$	Water	g day^{-1}
Replicate	1	3.6	0.74	2.8	0.76	0.02	Dry	20.9
Ozone (O)	1	115.0	0.52	107.2	0.48			
						0.02	Wet	33.7
Error 1	1	129.2		91.7				
						0.4	Dry	20.5
Water (W)	1	3620.0	<0.01	3677.0	<0.01			
O × W	1	154.2	0.03	120.6	0.05	0.4	Wet	40.0
Covariate	1	409.6[a]	<0.01	473.9[b]	<0.01			
Error 2	49	31.7		30.4				

[a] Covariate = starting dry weight.

[b] Covariate = final dry weight.

TABLE IV

Percentage frequency of visual symptom scores shown by ozone and watering treatments after 39 and 53 days of ozone exposure, and chi-square tests of effects

Score	39 days exposure				53 days exposure			
	Ozone $\mu l\, l^{-1}$				Ozone $\mu l\, l^{-1}$			
	0.02	0.02	0.4	0.4	0.02	0.02	0.4	0.4
	Water				Water			
	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet
Low[a] (0-0.5)	100.0	100.0	57.9	25.0	94.7	100.0	10.5	0.0
Medium (1.0-2-0)	0.0	0.0	42.1	45.0	5.3	0.0	84.2	45.0
High (2.5-4-0)	0.0	0.0	0.0	30.0	0.0	0.0	5.3	55.0

Chi-square test results

Difference tested	d.f.	Chi.square	p
39 day ozone exposure			
Ozone 0.02 $\mu l\, l^{-1}$ v. 0.4 $\mu l\, l^{-1}$	2	25.0	<0.01
Dry v. wet in 0.02 $\mu l\, l^{-1}$ ozone	1	0.0	1.00
Dry v. wet in 0.4 $\mu l\, l^{-1}$ ozone	1	8.3	0.02
53 day ozone exposure			
Ozone 0.02 $\mu l\, l^{-1}$ v. 0.4 $\mu l\, l^{-1}$	2	65.6	<0.01
Dry v. wet in 0.02 $\mu l\, l^{-1}$ ozone	1	1.0	0.31
Dry v. wet in 0.4 $\mu l\, l^{-1}$ ozone	1	12.3	<0.01

[a] Testing showed that the original 9 scores could be collapsed into low, medium and high categories, as indicated by bracketed scores, without significant loss of information.

scores after 39 and 53 days exposure to ozone (Table IV). At the 0.4 $\mu l\, l^{-1}$ ozone level the dry trees showed less visible symptoms than the wet trees. Net photosynthesis also showed an ozone × water interaction in which wet trees had the highest rate at 0.02 $\mu l\, l^{-1}$ ozone, but the lowest rate at 0.4 $\mu l\, l^{-1}$ ozone (Table V).

Ethylene emission by current year shoots of trees receiving the high ozone concentration was significantly greater than for controls 18 days after the start of ozone exposure (Figure 2). Both the mean ethylene and ACC concentration were highest in wet trees receiving high ozone, but it was not possible to demonstrate significant differences between wet and dry treatments. MACC concentrations in needles appeared not to vary with treatment, and it was noticeable that the mean

TABLE V

Net photosynthesis and root length, together with probabilities for the ozone × water stress interaction

Treatment levels		Net photosynthesis[a] μmol CO_2 m^{-2} s^{-1}	Root length[b] m
Ozone μL/L	Water	Measured at 44 days	Measured at 59 days
0.02	Dry	1.41	38.0
0.02	Wet	1.92	29.0
0.4	Dry	0.94	28.3
0.4	Wet	0.70	30.4
Probability for ozone × water			

[a] Observations per mean = 12.
[b] Observations per mean = 7.

ACC level in wet trees receiving high ozone for 18 days was 230% of the MACC level, while the ACC level in wet trees receiving low ozone was only 6% of the MACC level.

At the end of the ozone exposure period, wet trees subjected to high ozone showed the highest production of ethylene, but dry trees in the low ozone concentration also had high values. Measurement of ACC revealed an ozone × water interaction similar to that shown by ethylene (Figure 2). Regression showed that daily water use per unit shoot weight explained 56% (p=<0.01) of the variation in needle ACC values of trees exposed to 0.4 μl l^{-1} ozone. This correlation was obtained with the relationship: ACC concentration = exp $(0.5x^2)$-1, where x is water use. None of the variation in ACC shown by trees receiving low ozone could be explained by water use. Similarly, xylem water potential measured at the end of the experiment explained 29% (p=0.02) of the variation in needle ACC values of trees exposed to 0.4 μl l^{-1} ozone, but none of the variation shown by trees receiving 0.02 μl l^{-1} ozone. Root ACC levels measured after ozone exposure averaged 14% of needle ACC values, and showed no significant treatment effects (Figure 3). Root MACC levels averaged almost exactly twice the needle MACC levels and showed a significant (p=0.04) ozone x water interaction (Figure 3). Shoot MACC concentrations were significantly correlated with root MACC concentrations (r^2 = 0.49, p = <0.01), although there was no significant correlation between shoot and root ACC.

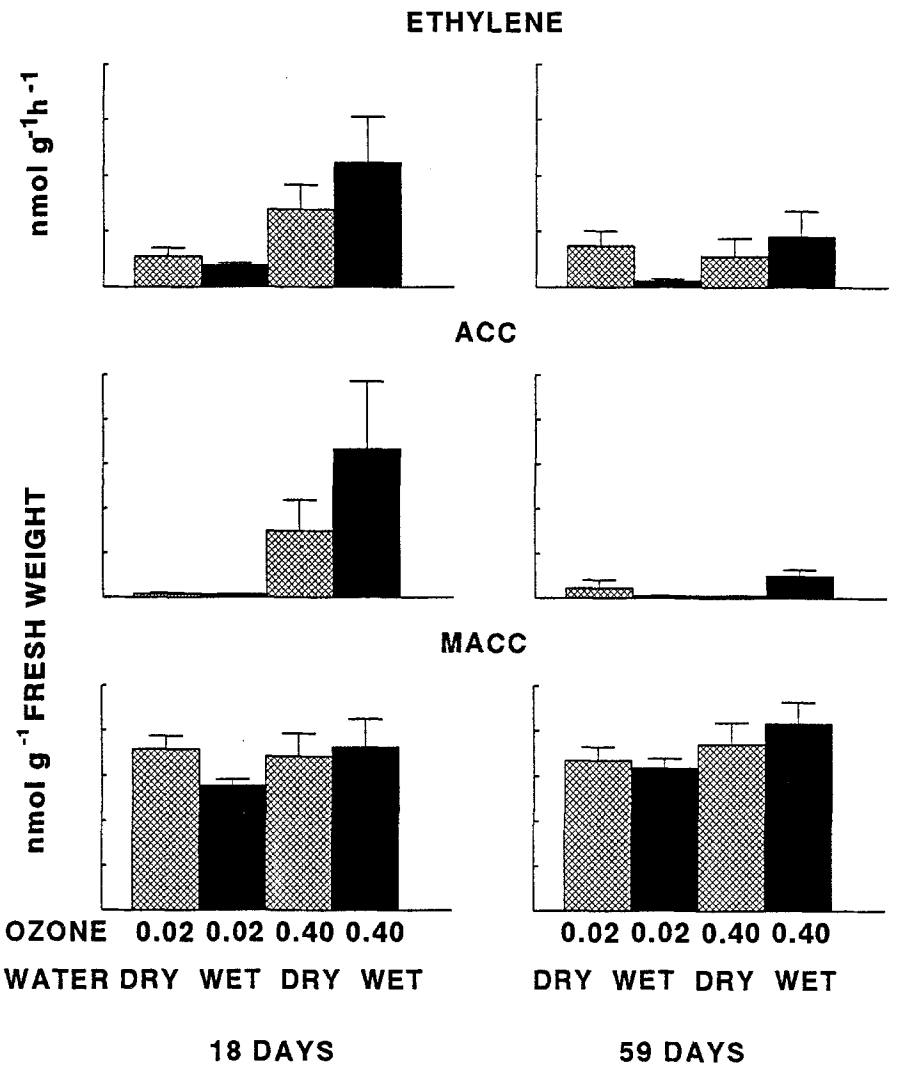

Fig. 2. Ethylene evolution per hour of current year spruce shoots, and ACC and MACC concentrations per g current year spruce needle fresh weight after 18 and 59 days exposure to ozone. Trees were exposed to either 0.02 or 0.4 $\mu l\ l^{-1}$ ozone, and adequate water or drought. Analysis of variance showed the ozone effect significant (p = 0.04) for ethylene at 18 days, and the ozone × water interaction effect significant for ethylene (p = 0.08) and ACC (p = <0.01) at 59 days. Standard errors are shown above histogram bars.

Discussion

Ozone treatment produced symptoms on current year foliage of Norway spruce which first became visible after 30 days. This type of symptom is indicative of acute ozone toxicity which is consistent with the elevated ozone concentration applied (0.4 $\mu l\ l^{-1}$). Chronically injured Norway spruce show chlorosis after pro-

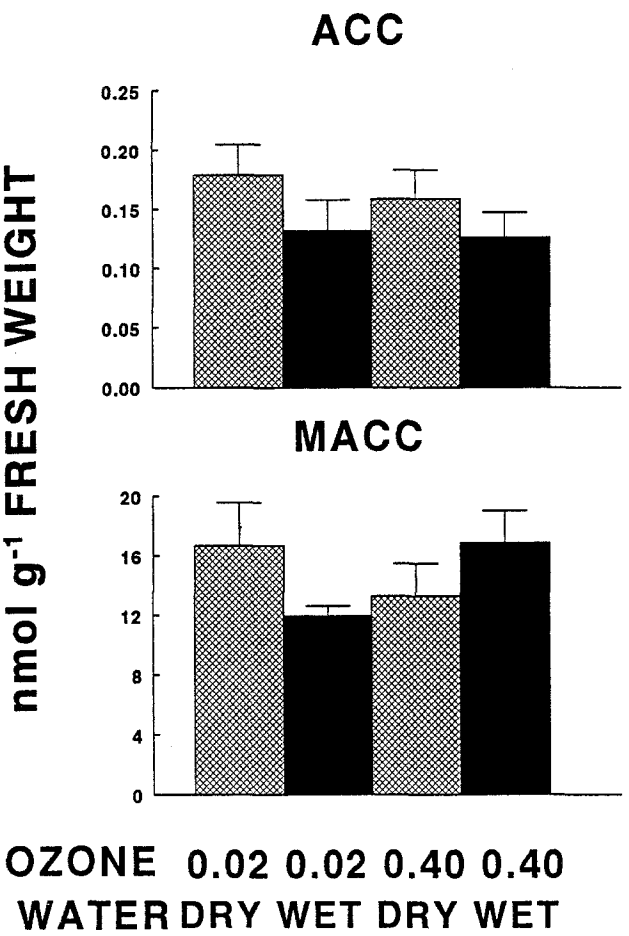

Fig. 3. Concentrations of ACC and MACC in new white roots, 5–8 cm long, at end of ozone exposure period. Trees were exposed to either 0.02 or 0.4 $\mu l\, l^{-1}$ ozone, and adequate water or drought. Analysis of variance showed the ozone × water interactions was significant ($p = 0.04$) for MACC. Standard errors are shown above histogram bars.

longed periods of time, even in the year after ozone treatment, although changes in cell wall enzymes, polyamines and other secondary compounds can be detected during the exposure period (Langebartels *et al.*, 1990). In the present experiment, low water supply resulted in substantially less symptom development. Water use measurements showed that dry trees transpired, on average, 56% as much water as wet trees, and therefore had lower shoot conductances. As a result, they presumably absorbed less ozone into the needles and received less damage. The significant regression between water use and ACC accumulation in wet trees, but not dry trees exposed to high ozone concentration, is in agreement with ozone damage depending on high shoot conductance. Even so, after 44 days, net photosynthesis of wet trees was lower than dry trees exposed to 0.4 $\mu l\, l^{-1}$ ozone. This implies damage to

a non-stomatal photosynthetic process. In contrast, young loblolly pine (*Pinus taeda* L.), previously exposed to 0.1 μl l^{-1} ozone , showed increased photosynthesis under well watered conditions, but more rapid decline as drought stress increased (Woong *et al.*, 1990), suggesting that only a stomatal process was affected at the lower ozone concentration.

The experiment suggested that the high concentration of ozone increased water use. Differences in shoot size did not explain the ozone × water interaction evident for well watered trees, so that increased water use of trees exposed to 0.4 μl l^{-1} remained a possibility. The gradual increase in water use of dry trees exposed to 0.4 μl l^{-1} ozone, compared with dry trees exposed to 0.02 μl l^{-1} (Figure 1) also supported this view. Increased transpiration and needle water loss of four-year old Norway spruce exposed to 0.08 μl l^{-1} ozone have been observed previously (Barnes *et al.*, 1990a). Ozone can inhibit closure of stomata so that water loss occurs at night (Barnes *et al.*, 1990b), and increased water use shown by trees exposed to 0.4 μl l^{-1} ozone could have been partly due to night time water loss. Norway spruce receiving long term ozone exposure showed no increase in gas phase limitation to photosynthesis during mild drought stress, whereas control plants showed a normal increase (Wallin and Skärby, 1992). Therefore, in this experiment, trees receiving high ozone treatment were expected to show lower WUE than trees receiving low ozone treatment, but this was not demonstrated. However, WUE also included root dry weights which showed no effects of treatments, although roots almost doubled in dry weight during the ozone exposure period. These Norway spruce had similar WUE values to two-year old Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), which varied from 0.0062 to 0.0087 (Smit and van den Driessche, 1992).

High ozone treatment resulted in increased ethylene biosynthesis after 18 days. This increase was detectable some 12 days visible symptoms were first observed, and the treatment means of ethylene and ACC at 18 days reflected the assessment of symptoms two weeks later (Figure 2, Table IV). A similar relationship between ethylene production and needle damage in adult silver fir (*Abies alba* L.) trees suffering decline has also been observed (Fuhrer, 1985). Under the conditions used in this study, ozone was more effective than drought in inducing ethylene. It has been suggested that intact plants do not respond to drought stress by producing ethylene (Morgan *et al.*, 1990), and greater evolution of ethylene in stems of 24-yr old Norway spruce was associated with adequate water supply and high water potential, compared with trees of low water status (Eklund *et al.*, 1992). Needle ACC measurements made on ozone treated trees only reached high levels at high rates of water use (Figure 2). Low rates of water use probably reflected reduced stomatal conductance and gas exchange which prevented ozone induced ethylene stimulation. The effect of moisture stress in stimulating this pathway was not clear. Trees exposed to low ozone showed higher mean levels of ethylene and ACC under dry conditions, than under wet at both measurements dates. On the other hand, it was not possible to demonstrate any relationship between either tree water

use, or water potential, and ACC concentration in trees grown at low ozone level, suggesting that the dependence of ACC production on moisture stress was weak.

Needle concentrations of MACC were not significantly affected by treatment, but judging from standard errors, variation of MACC levels about the mean increased in both shoot and roots as a result of stress treatments (cf. Fuhrer, 1985). Root MACC levels were two-fold higher than shoot MACC levels, and the two measurements were correlated. These results suggested that shoot MACC was translocated into the roots of Norway spruce trees in this experiment. MACC mobility has been studies before in herbaceous plants (Fuhrer and Fuhrer-Fries, 1985; Machackova *et al.*, 1992), and if production of MACC is a mechanism for ethylene biosynthesis regulation (Chen and Welburn, 1989), roots may be a storage site when stress results in high levels of MACC production.

The production of ethylene by current year needles and concentration of ACC declined from the 18 day to the 59 day measurement, indicating a transient induction due to daily ozone exposure. Herbaceous plants are known to respond to ozone with a rapid surge of ethylene within a few hours (Tingey *et al.*, 1976; Mehlhorn and Wellburn, 1987; Langebartels *et al.*, 1991). Conifers, on the other hand, show long term increases in ethylene biosynthesis depending on the nature and magnitude of the stress applied. Elevated ACC levels and stress ethylene emissions were sustained for several weeks in acid mist treated red and white spruce (Chen and Wellburn, 1989). Sulphur dioxide fumigation of larch and Scots pine resulted on one to two peaks of ethylene emission (Bucher, 1981). The first lasted for 5 to 10 days and occurred earlier at higher levels of treatment. Ozone-exposed Norway spruce seedlings also showed dose dependent peaks of ethylene formation and ACC levels occurring during the first two weeks of treatment (S. Simons and C. Langebartels unpublished). Results obtained in the present work are consistent with an extended induction of ethylene biosynthesis which declined to the end of the ozone exposure period. This induction of the senescence-promoting hormone, ethylene, may contribute to the appearance of visible symptoms. More frequent observations would be necessary to confirm the exact time course of these changes for comparison with long term effects of chronic ozone exposure.

In this work low water supply resulted in reduced symptoms in young Norway spruce exposed to a high concentration of ozone. The trees were grown at low irradiance, such as natural regeneration might experience. Although short term experiments may not simulate long term exposure, it suggests that Norway spruce regeneration would show reduced damage from ozone exposure occurring during periods of drought. Ethylene promotes maturation and senescence in many plants and may have similar effects in Norway spruce. Promotion of ethylene biosynthesis by ozone treatment may offer a mechanism for how ozone accelerates the ageing process (Reich and Amundson, 1985; Wallin *et al.*, 1990). The possible ability of spruce to regulate ethylene production by translocating MACC to the root system could, therefore, be a factor in resistance of this species to ozone.

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