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Growth of Norway spruce (*Picea abies*) in relation to different ozone exposure indices: a synthesis

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Abstract

In the Göteborg Ozone-Spruce Project (GOSP), two independent open-top chamber experiments were conducted during four growing seasons, using one clone of Norway spruce (*Picea abies*). The experiments tested the impact of ozone, alone and in combination with low phosphorus supply and in combination with drought stress, respectively, on biomass accumulation. In this paper, the results from both experiments were combined for the first time in order to analyse the relationship between relative biomass accumulation and different exposure indices (accumulated exposure over a threshold (AOT) with different cut-off concentrations, and the sum of ozone concentrations above $60\,\mathrm{nl\,l^{-1}}$, referred to as SUM06). In addition, a pooled analysis was made on several European studies of Norway spruce as a first effort to synthesize independent data and test the relative growth in relation to the AOT40 index. Significant negative relationships between the relative biomass of the GOSP-clone and the different indices were obtained. AOT20 and AOT30 resulted in the highest correlations. Based on the regression model, ozone is predicted to reduce the biomass of the GOSP-clone by 1% at the critical level for forest trees in Europe, a seasonal AOT40 of $10\,\mathrm{\mu l\,l^{-1}\,h}$. A significant negative relationship between relative growth and AOT40 was obtained also with the European data set. At the present ozone critical level, the model predicted a 6% reduction in growth for the most sensitive Norway spruce trees in this data set.

Keywords: Biomass; Relative growth; AOT; SUM; Critical level

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1. Introduction

Clear evidence of negative effects of ozone on physiological parameters and the biomass accumulation of young trees has been provided by several experiments conducted in Europe and the eastern United States (Sandermann Jr. et al., 1997; Skärby et al., 1998; Chappelka and Samuelson, 1998). The available information about the impact of ozone on forest trees makes it possible to identify critical levels of ozone exposure and to test different exposure indices. Different kinds of numerical definitions of exposure can be tested by using retrospective data analysis or by setting up experiments to test different indices. Lee and Hogsett (1999) re-evaluated the role of concentration and time of day in developing ozone exposure indices for crops and trees. They came up with the same conclusions as Lee et al. (1988) 11 years earlier, i.e. that indices giving preferential weight to higher concentrations are better predictors of response than mean or peak indices. One objective in this work is to find out the most realistic and useful indices to develop international control policies to reduce ozone exposure. A so-called critical level concept

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is currently used in order to protect European forests from growth reductions and to try to assess forest areas at risk (Fuhrer et al., 1997). A critical level, in terms of a cumulative exposure index, for ozone impact on forest trees in Europe was adopted at the United Nations Economic Commission for Europe (UN/ECE) workshop in Kuopio, based on the best available knowledge (Kärenlampi and Skärby, 1996). It was agreed that exposure of forest trees should be characterized by the same index as crops: AOT40 (Accumulated exposure Over a Threshold of 40 $\mathrm{nl\,l^{-1}}$ during daylight hours defined as clear-sky calculated global radiation $>50\,\mathrm{W\,m^{-2}}$). When AOT40 is calculated for a certain time period all hourly concentrations above 40 $\mathrm{nl\,l^{-1}}$ are first identified. Then 40 $\mathrm{nl\,l^{-1}}$ is subtracted from each of all the identified hourly concentrations above 40 $\mathrm{nl\,l^{-1}}$ and finally the sum of all such exceedances is calculated.

The AOT40 index describes the portion of exposure, which is thought to be biologically effective. It is easily measured, calculated, mapped and modeled and this simplification has been accepted to be used for a general risk assessment (Fuhrer et al., 1997). In this approach, a single level is defined, which is set to protect the most sensitive receptor under the most sensitive environmental conditions. Furthermore, the objective of this approach is to define where adverse effects of ozone may occur. A critical level for forest trees has been defined as an AOT40 of 10 $\mathrm{\mu l\,l^{-1}\,h}$, accumulated during daylight hours over a 6-month growing season (Fuhrer et al., 1997). A cumulative-type index using a discontinuous weighting function, such as AOT, is based mainly on statistical criteria using the ozone concentration in the air surrounding the plant, and a scientifically clear mechanistic basis is still lacking. It is still, however, biologically relevant and also simple, which is a very important requirement when used in mapping and modeling of ozone exposure over Europe to define areas where adverse impacts of ozone may occur. In the US a corresponding standard has been set for vegetation, a so-called Secondary Standard, which is also a cumulative standard, using a threshold of 60 $\mathrm{nl\,l^{-1}}$expressed as as SUM06 (Lefohn and Foley, 1992). A SUM06 of 25–38 $\mathrm{\mu l\,l^{-1}\,h}$ over a running 90-day maximum, using values from a 12-h daily window, has been agreed upon for the protection of vegetation (Heck and Cowling, 1997; US EPA, 1996). The SUM06 is calculated by identifying all hours with ozone concentrations above 60 $\mathrm{nl\,l^{-1}}$ and take the sum of these, using no threshold in the accumulation in this case.

Norway spruce is the economically most important forest tree species in southern Sweden, where the ozone levels often exceed the present critical level for forest trees (Kindbom and Pleijel, 1999). Many experiments with ozone and Norway spruce have been conducted and the main part is concerned with physiological and biochemical effects (Langebartels et al., 1997; Selldén et al., 1997). Considerably less is known about the effects of ozone on biomass growth. Landolt et al. (2000) and Braun and Flückiger (1995) found decreased biomass, however non-significant, in Norway spruce seedlings after one or two seasons of ozone exposure in open top chambers (OTCs). Significant decreases in biomass growth in one clone of Norway spruce of 8% and 5%, respectively, were obtained in the Göteborg Ozone-Spruce Project (GOSP), after four seasons of ozone exposure in OTCs (Karlsson et al., 2002; Ottosson et al., 2003). These results are further explored, for the first time in this investigation, by making an analysis of relationships to different exposure indices. The aims of this study were; (a) to test the exposure–response relationship for biomass accumulation of the GOSP-clone versus AOT with different cut-off concentrations and SUM06, using linear regression, and (b) to synthesize and test published European data concerning ozone effects on growth of Norway spruce, in relation to the present ozone critical level (AOT40) set for forest trees.

2. Materials and methods

According to the aim, this investigation of Norway spruce and exposure indices covers two parts, one testing a single clone and the other testing published data on effects of ozone on growth.

2.1. Test of indices on the clone used in GOSP

GOSP was carried out at the Östad field station, located 50 km north–east of Göteborg, Sweden, and included two statistically independent experiments in OTCs: the *main experiment* and the *drought experiment*. Both experiments used the same clone, the GOSP-clone, and a detailed description of the experimental setup was given in Wallin et al. (2002).

2.1.1. Plant material and maintenance

Three-year-old Norway spruce saplings (*Picea abies* (L.) Karst., of one clone, C77-0068 Minsk) were grown individually in sand in 120 l pots. The saplings were propagated from cuttings in the autumn 1989 and planted at Östad in July 1991. Water and nutrients were supplied daily by a computerized irrigation system.

2.1.2. Treatments

During the vegetation season ozone was generated from pure oxygen (ozone generator: Model GL-1, PCI Ozone Corp., NJ, USA) and the addition corresponded to 1.4 times the ozone concentration in the ambient air. In the *main experiment* the ozone treatments were charcoal-filtered air (CF), non-filtered air (NF) and non-filtered air with extra ozone (NF+). A low phosphorus level (LP) was applied to half of the chambers with charcoal-filtered air (CF/LP) and additional ozone (NF + /LP), respectively, in order to test the possible interaction between nutrient stress and ozone. Except for LP, all other treatments were supplied with high phosphorus (HP) (Ottosson et al., 2003). The abbreviation HP is only used for treatments that were paired with LP treatments, i.e. CF/HP and NF + /HP. Each of the five treatments in the *main experiment* had 6 replicate chambers, using 30 OTCs and each chamber had 18 trees. In the *drought experiment* the ozone treatments were CF and NF + with 6 replicate chambers using 12 OTCs and 24 trees per chamber. Each chamber had 12 trees randomly selected for the drought stress (D) treatment while another 12 were kept well-watered (W) (Karlsson et al., 2002).

2.1.3. *Harvest procedure*

In order to make possible the aggregation of data from different harvests, each plot was divided into 3 statistical blocks with 6 trees in each block in the *main experiment* and 4 trees per block and water regime in the *drought experiment*. Whole trees, including the roots, were harvested regularly. The *main experiment* included 6 harvest occasions within each block. Block 1 was harvested in 1992 and 1993, block 2 in 1994 and block 3 in 1995 and 1996. Since the first harvest in block 1 was made in May 1992, before exposure started, it was not included in block 1 in this analysis. Thus, a total of 510 trees were harvested in the main experiment. In the *drought experiment* a total of 144 trees were harvested, including two harvest occasions per block, in April and September in 1993, 1994 and 1995, respectively. The plant material was dried at 70°C until constant weight.

2.1.4. *Calculations*

The geometric block mean mass for the different treatments were calculated from the harvest means (Tables 1 and 2). The daylight AOTX ($X = 0$, 10, 20, 30, 40, 50 or 60) and SUM06 for each harvest occasion was accumulated and again used to calculate the block means (Tables 3 and 4). The cumulation of all AOTX and SUM was made between April and October. The theoretical absolute biomass at an exposure of $0\,\mu l\,l^{-1}\,h$ was determined at the y-intercept, using a linear regression of biomass versus exposure index, for each

Table 1
Mean of total dry weight biomass (g) and coefficient of variation (CV) for each harvest and statistical block in the *main experiment*

Treatment											
		CF/HP		NF		NF + /HP		CF/LP		NF + /LP	
Block	Harvest date	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV
1	1992-11-04	75	16	68	16	74	31	64	41	71	16
	1993-03-31	86	13	67	23	58	41	76	29	87	15
	1993-05-10	86	16	116	21	85	27	96	31	105	34
	1993-07-26	206	19	236	23	224	21	218	26	216	25
	1993-11-08	321	19	291	28	336	21	328	20	272	18
	Aritm.mean	155	16	155	22	156	28	156	29	150	22
	Geom.mean	130	16	129	22	122	28	127	29	130	22
2	1994-04-13	284	15	367	27	308	14	323	19	328	31
	1994-05-18	471	21	414	28	383	20	405	17	413	24
	1994-06-22	556	15	565	16	512	16	523	23	546	18
	1994-07-27	702	27	797	14	699	8	756	23	694	9
	1994-09-07	878	9	901	15	958	16	840	23	663	21
	1994-11-09	1012	18	870	18	972	19	790	13	814	13
	Aritm.mean	651	18	652	19	639	15	606	20	577	19
	Geom.mean	600	18	614	19	583	15	569	20	550	19
3	1995-04-05	1064	38	1129	13	966	22	968	12	969	18
	1995-05-17	1388	12	1331	17	1294	12	1283	8	1056	19
	1995-06-21	1574	10	1271	10	1466	17	1258	12	1151	13
	1995-07-26	2081	6	1912	14	1876	18	1401	18	1523	13
	1995-11-09	2699	10	2651	16	2619	7	1933	18	1809	23
	1996-05-22	3287	12	3251	5	3195	12	2409	6	2441	20
	Aritm.mean	2016	15	1924	13	1902	15	1542	12	1492	17
	Geom.mean	1871	15	1776	13	1750	15	1472	12	1412	17

CF, charcoal filtered air; NF, non-filtered air; NF +, non-filtered air with extra ozone; P, phosphorus; H, high; L, low.

Table 2
Mean of total dry weight biomass (g) and coefficient of variation (CV) for each harvest and statistical block in the *drought experiment*

Treatment									
		CF/W		NF+/W		CF/D		NF+/D	
Block	Harvest date	Mean	CV	Mean	CV	Mean	CV	Mean	CV
1	1993-04-01	77	20	78	25	80	21	77	26
	1993-09-13	254	30	236	26	243	18	230	25
	Aritm.mean	166	25	157	26	162	20	154	25
	Geom.mean	140	25	136	26	140	20	133	25
2	1994-04-07	327	23	321	31	240	18	261	18
	1994-09-13	989	10	812	24	767	11	726	17
	Aritm.mean	658	17	567	27	504	14	494	18
	Geom.mean	569	17	511	27	429	14	435	18
3	1995-04-10	1229	16	948	16	840	15	794	12
	1995-09-25	2390	11	2620	11	1737	14	1585	7
	Aritm.mean	1810	14	1784	13	1289	14	1190	10
	Geom.mean	1714	14	1576	13	1208	14	1121	10

W, well-watered; D, drought.

Table 3
AOT0 and AOT40 ($\mu l\, l^{-1}\, h$) for each harvest and each statistical block in the *main experiment*

Block	Harvest date	Treatment AOT0			Treatment AOT40		
		CF	NF	NF+	CF	NF	NF+
1	1992-11-04	8.1	42.7	55.5	0.0	2.1	8.3
	1993-03-31	8.1	42.7	55.5	0.0	2.1	8.3
	1993-05-10	16.6	63.2	81.8	0.4	4.1	14.8
	1993-07-26	23.6	103.4	137.6	0.4	6.0	25.2
	1993-11-08	27.3	124.0	169.0	0.4	6.0	27.9
	Mean	16.8	75.2	99.9	0.2	4.0	16.9
2	1994-04-13	33.6	130.4	175.5	1.0	6.7	28.6
	1994-05-18	39.8	150.4	202.2	1.2	8.8	36.3
	1994-06-22	44.1	169.9	230.6	1.2	9.4	43.3
	1994-07-27	48.4	191.8	262.9	1.2	12.1	54.1
	1994-09-07	51.6	210.8	293.8	1.2	13.6	63.2
	1994-11-09	53.7	217.6	301.2	1.2	13.6	63.6
	Mean	45.2	178.5	244.4	1.2	10.7	48.2
3	1995-04-05	55.8	219.7	303.3	1.3	13.7	63.7
	1995-05-17	63.3	241.1	330.2	1.3	14.9	69.0
	1995-06-21	67.0	260.7	359.6	1.3	15.9	76.6
	1995-07-26	70.9	277.1	383.3	1.3	16.3	80.0
	1995-11-09	76.9	302.9	422.0	1.3	17.6	87.7
	1996-05-22	86.1	326.6	445.7	1.3	18.7	88.8
	Mean	70.0	271.4	374.0	1.3	16.2	77.6

index and each block. This procedure was made separately for the different water and nutrient regimes. In the next step the theoretical absolute biomass at $0\,\mu l\, l^{-1}\, h$ was used as the reference ($=1$) to calculate the relative biomass for each treatment as described by Fuhrer (1994). The relative biomass from all blocks and treatments, totally 27 data-points, were combined and fitted again by using linear regression to test the indices and define confidence limits. The linear regression model is based on the assumption that y (relative biomass) is

Table 4
AOT0 and AOT40 ($\mu l\,l^{-1}\,h$) for each harvest and each statistical block in the *drought experiment*

Block	Harvest date	Treatment AOT0		Treatment AOT40	
	Treatment	CF	NF+	CF	NF+
1	1993-04-01	8.3	55.7	0.0	8.3
	1993-09-13	26.3	162.6	0.4	27.5
	Mean	17.3	109.2	0.2	17.9
2	1994-04-07	30.1	171.9	0.4	28.0
	1994-09-13	52.2	296.7	1.2	63.6
	Mean	41.1	234.3	0.8	45.8
3	1995-04-10	58.1	305.6	1.3	63.8
	1995-09-25	76.5	420.1	1.3	87.6
	Mean	67.3	362.9	1.3	75.7

normally distributed and that the variance of y is not dependent on x (AOT or SUM). In order to investigate if these assumptions were satisfied the residuals were studied with respect to normal distribution, if the residuals had a sum of zero and if their variance was independent of x (Underwood, 1997).

2.2. Test of the AOT40 index on the European data

Data from peer-reviewed, published papers were collected. In order to be included in the test of the AOT40 index for Norway spruce the following selection criteria were used: *Exposure system*: closed environmental chambers, OTCs, open field fumigation systems; *Growth parameter*: total biomass, shoot or stem biomass, stem volume; *Exposure duration*: at least 50 days, i.e. approximately one growing season; *Growing conditions*: pot size that does not limit growth; *Modifying factors*: acid mist, drought and nutrient deficiency; *Data availability*: possibility to obtain and/or calculate daylight AOT40, accumulated over the whole duration of each experiment.

2.2.1. Calculations

In a few cases the European data set presented CF treatments with an AOT40 above $0\,\mu l\,l^{-1}\,h$. In these cases the theoretical absolute biomass at an exposure of $0\,\mu l\,l^{-1}$ was calculated, from which a new relative biomass was obtained, as described in Section 2.1.4. Two linear regression analyses were performed: (1) using all data points or (2) using only those data points where the ozone effects were statistically significant in the individual experiments. In order to try to reveal possible weaknesses, such as systematic bias hidden in the model, further tests were performed. For each original data set the relative growth at 10 and $50\,\mu l\,l^{-1}\,h$ was taken out from the dose-response curve. This particular relative growth for each experiment was plotted versus seedling age and versus number of seasons of exposure, respectively, in order to test for systematic bias in the data set due to these factors.

3. Results

3.1. Test of exposure indices on the clone used in GOSP

The accumulated biomass at each harvest and the arithmetic and geometric means of each block are presented in Table 1 (the *main experiment*) and Table 2 (the *drought experiment*). The corresponding AOT0 and AOT40 are presented in Tables 3 and 4. Significant negative correlations between the relative total biomass, aggregated for each block, and all tested cases (AOT0-60 and SUM06) were obtained. The coefficients of determination (r^2) ranged from 0.42 to 0.47 and the highest correlations were obtained for AOT20 and AOT30 (Fig. 1). The intercepts varied between 0.997 and 0.998 and the p-values between 0.00008 and 0.00025. The assumptions, on which the regression model was based, were satisfied for all cases. The residuals were normally distributed and the sum of the residuals was close to zero in all cases and did not vary systematically with the index used. The relationship between relative biomass of the GOSP-clone and ozone exposure expressed as daylight AOT40 is shown in Fig. 2. After four growing seasons, ozone had reduced the biomass by 3.5% at an AOT40 of $40\,\mu l\,l^{-1}\,h$. At the critical level ($10\,\mu l\,l^{-1}\,h$) the biomass reduction was approximately 1%. At approximately $15\,\mu l\,l^{-1}\,h$ a reduction of 1.5%, predicted by the regression model, becomes statistically significant ($p = 0.05$; Fig. 2). At the US Secondary Standard, SUM06 of $25–38\,\mu l\,l^{-1}\,h$, the predicted relative biomass decrease was 2–3%.

3.2. Test of the AOT40 index on the European data set

Based on type of exposure system and selection criteria a great number of published experiments were examined in detail. Data from 16 independent European experiments, published between 1990 and 2002, were finally used to test the relationship between relative growth and AOT40 (Table 5). Regardless of the direction of the effect (stimulations or reductions in growth) all data were used in the analyses. In Table 5, however, only the maximal reduction in growth is presented from each publication. In the following, these experiments are highlighted in cases where additional information is considered necessary.

3.2.1. Closed chambers

Five clones of 3-year-old Norway spruce saplings were grown in two different soil types and exposed to an

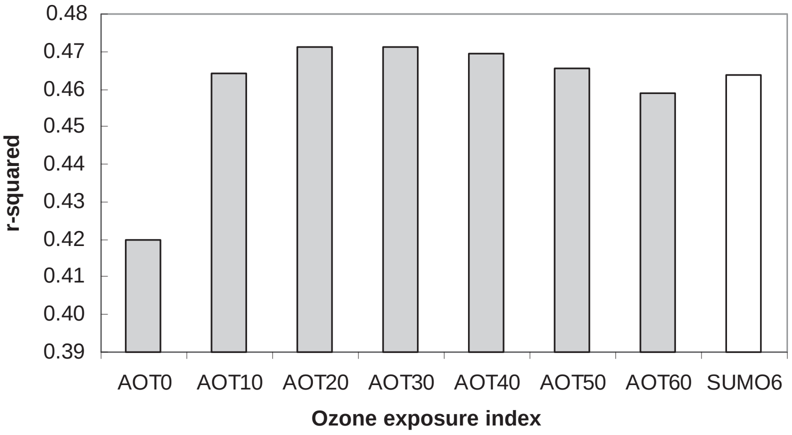

Fig. 1. Coefficient of determination (r^2) values for the regression between relative biomass of the GOSP-clone of Norway spruce and AOTX $\mu l\, l^{-1}\, h$ ($X = 0, 10, 20, 30, 40, 50, 60$) and SUM06 $\mu l\, l^{-1}\, h$. Ozone data for $X = 0$ and 40 are shown in Tables 2 and 4. Ozone data for $X = 10, 20, 30, 50, 60$ and SUM06 are not shown.

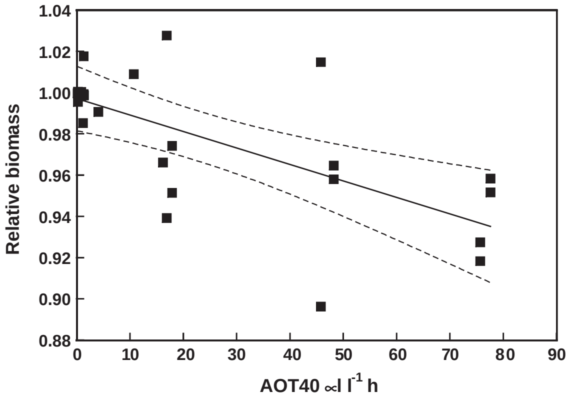

Fig. 2. The relationship between relative biomass of the GOSP-clone of Norway spruce and ozone exposure expressed as AOT40 ($\mu l\, l^{-1}\, h$). $y = -0.0008x + 0.9971$; $r^2 = 0.47$; $p < 0.001$. The regression line is shown with the 95% confidence intervals.

AOT40 of 64 $\mu l\, l^{-1}$ h ozone and acid mist in closed environmental chambers (Payer et al., 1990). After two growing seasons the stem volume was measured. Stem volume of three out of the five clones was reduced by ozone and acid mist in the neutral soil and a fourth clone was affected in the acidic soil only. It was not possible to test for an interaction between acid mist and ozone, but we consider ozone to be the most important pollutant in this case. In another study, where the same chamber system was used, the shoot biomass of Norway spruce was not changed significantly after one season of an AOT40 of 110 $\mu l\, l^{-1}$ h (Polle et al., 1993). Information about light regimes and ozone exposure in these two studies was received from H.D. Payer (personal communication) and from Blank et al. (1990).

3.2.2. OTCs

Dixon et al. (1998) reported a significant decrease (−77%) in growth in one out of two clones of 9-year-old Norway spruce tested, after three growing seasons and an AOT40 of 337 $\mu l\, l^{-1}$ h and two seasons of drought as compared to filtered air and drought. A significant biomass decrease of 8% and 5%, respectively, was detected after four growing seasons and an AOT40 of 76 and 78 $\mu l\, l^{-1}$ h in the GOSP-clone reported separately by Karlsson et al. (2002) and Ottosson et al. (2003). These two experiments also tested drought and LP, respectively, and no interactions were found.

3.2.3. Open-release systems

Three long-term studies have been performed in Finland, using open-release fumigation systems. None of these studies resulted in any significant effects. Rantanen et al. (1994) found that total seedling dry weight tended to increase compared to the control seedlings. Wulff et al. (1996) found that seedlings from a northern provenance behaved different from a southern provenance of Norway spruce, where the northern appeared to be more ozone sensitive, after transplantation to a more southern location. Similarly, Utriainen and Holopainen (2001) found negative effects of ozone in combination with nutrient stress (nitrogen and phosphorus), however non-significant, on total biomass of Norway spruce after two seasons.

3.2.4. Plant and pot size

The plant size varies considerably between experiments and the majority of the experiments in Table 5 used pots. The size of the pot in relation to the final dry mass of the individual tree was considered the most critical factor in order to make sure that uncontrolled growth limitation was avoided. In all experiments the

Table 5
Publications used to test the AOT40 index for Norway spruce (*Picea abies*)

Exposure technique	Growing seasons	Age at harvest	Dry mass of control at harvest (g)	Pot size (l)	AOT40 ($\mu l\,l^{-1}\,h$) DL	Max. reduction of growth parameter vs. control	Reference
CIC	2 (+acid mist)	4	140 (fresh mass)	2	64	−**22%** (clone 16, stem volume)	Payer et al. (1990) and Payer (pers. comm.)
						−**28%** (clone 133, stem volume)	
						−**28%** (clone 14, stem volume)	
						−**24%** (clone 11, stem volume)	
CIC	1 (176 days)	5	250 (fresh mass)	50 kg (3 plants per pot)	40	−4% (shoot biomass)	Polle et al. (1993)
CIC	1 (205 days)	3	440	40 (4 plants per pot)	22	−3% (total biomass)	Lippert et al. (1996)
CIC	1 (60 days)	3	3.5	0.3	80	−**18%** (clone F1) (total biomass)	Skre and Mortensen, 1990
						−**30%** (clone F3)	
CIC	1 (105 days)	0	1.5	0.05	42	−**8%** (total biomass)	Mortensen (1990)
	1 (55 days)	1	3	0.5	18	−**22%** (total biomass)	
COC	3	3	nm	20	30	9% (stem volume)	Lucas and Diggle (1997)
OTC	2	0-3	0.05–13	1.3 (3 plants per pot)	35	−15% (total biomass)	Braun and Flückiger (1995) and Braun (pers. comm.)
OTC	5	9	nm	soil grown	75	−12% (stem biomass)	Skärby et al. (1995)
OTC	1 (141 days)	1	8	1.5	11	−25% (clone M)	Karlsson et al. (1997)
			5			25% (clone L)	
OTC	3 (+drought)	9	1200	soil grown	337	−**77%** (clone I, shoot biomass)	Dixon et al. (1998) and Le Thiec (pers. comm.)
						−53% (clone G, shoot biomass)	
OTC	1 (183 days)	0	0.04	120 seeds in 25	20	−8% (total biomass except needles)	Landolt et al. (2000)
OTC	4 (+drought)	7	2600	120	76	−**8%** (total biomass)	Karlsson et al. (2002)
OTC	4 (+P-deficiency)	7	3300	120	78	−**5%** (total biomass)	Ottosson et al. (2003)
ORS	2	5	80	7.5	15	−18% (total biomass)	Rantanen et al. (1994)
ORS	3	7	60-70		34	−13% (south. prov.) (total biomass)	Wulff et al. (1996)
		5	20	7.5	34	−11% (north. prov.)	
ORS	2 (+N and P-deficiency	6	88	7.5	10	−32% (total biomass)	Utriainen and Holopainen (2001)

CIC, Closed indoor chambers; COC, Closed outdoor chambers; OTC, Open-top chambers; ORS, Open release system; nm, not measured; DL, daylight. The AOT40 values correspond to the % maximal reduction in growth presented in each study. Statistically significant effects are printed in bold.

description of how the plants were grown gave a general insight whether there was a risk for pot limitation. In no case the dry mass was larger than approximately 50 g l^{-1} at the final harvest, which was considered to be within the limit for free growth of a tree seedling, according to nursery cultural practices (Grossnickle, 2000).

3.2.5. The relation between relative growth and the AOT40 index

When the literature data, using different growth parameters, was plotted in relation to AOT40 (Figs. 3 and 4) the results pointed in a similar direction as with the single GOSP-clone. Both the regressions shown in Figs. 3 and 4 were significant and had residuals with a normal distribution. The regression using all data-points had a low coefficient of determination ($r^2 = 0.2$) and an AOT40 of 40 μl l^{-1} h corresponded to approximately 3% decrease in biomass. At the current critical level, an AOT40 of 10 μl l^{-1} h, no effect on the biomass was predicted. In the other regression model (Fig. 4) the data-points represent only significant ozone effects, reported from individual experiments. These were taken from six out of the 16 publications in Table 5. The relative growth was predicted to decrease by 6% and 13%, respectively, at an AOT40 of 10 and 40 μl l^{-1} h, with a coefficient of determination of 0.80. Several tests were made to investigate possible factors that may influence the variation of the ozone effect on growth: indoor experiments excluded, Dixon et al. (1998) excluded, age of seedlings, number of seasons of exposure, normalization to seasonal values, pot size. The exclusion of indoor experiments resulted in a very similar regression equation as in Fig. 3. To test whether the data points originating from Dixon et al. (1998), with much higher exposure dose than any other experiment (Table 5), dominated the slope of the relationship, shown in Figs. 3 and 4, in an inadequate way, this data set was excluded from the regressions. The regressions were, however, similar to those presented in Figs. 3 and 4, and still significant ($y = -0.0017x + 1.0278$ and $y = -0.0023x + 0.9696$, respectively), while the r^2 values were much lower (0.079 and 0.38, respectively). Young seedlings (0–4 years) tended to be more sensitive to ozone than older (5–9 years), while longer duration of exposure did not change the regression equation. Normalization of all data to annual averages did not change the regression equation substantially. Pot size influenced the model in a way that large pots gave a significant equation, similar to the equation in Fig. 3 ($y = -0.0011 + 0.9968$) and increased r^2. Finally, the three experiments from Sweden with exposure periods of 4 (Karlsson et al., 2002; Ottosson et al., 2003) or 5 seasons (Skärby et al., 1995) resulted in a regression equation similar to the one in Fig. 3 ($y = -0.0012 + 0.9876$; $p = 0.01$), but with much higher r^2 (0.61). This increase in r^2 demonstrates the importance of variation in experimental procedures and of climate.

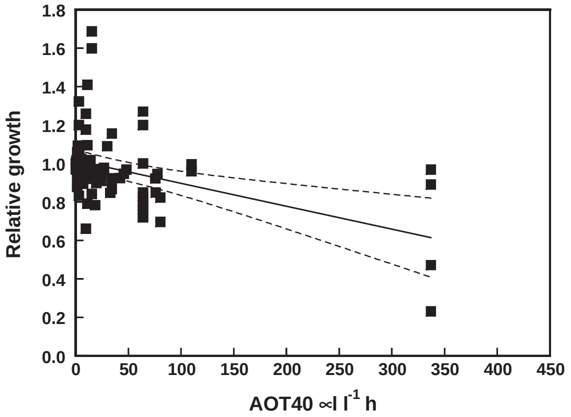

Fig. 3. The relationship between relative growth of Norway spruce and ozone exposure (AOT40 μl l^{-1} h). Data originate from the references in Table 5. $y = -0.0012x + 1.02$; $r^2 = 0.19$; $p < 0.001$. The regression line is shown with the 95% confidence intervals.

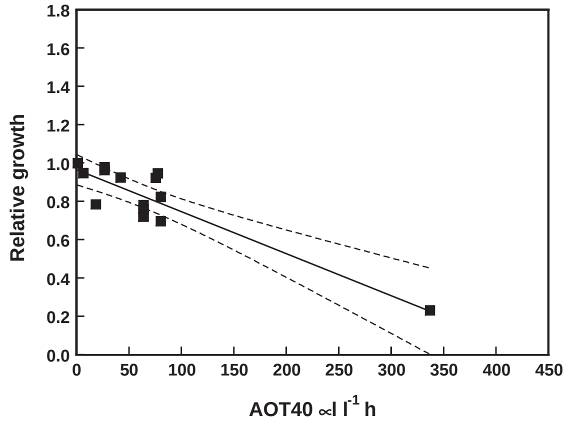

Fig. 4. The relationship between relative growth and ozone exposure (AOT40 μl l^{-1} h). Data originate from the references showing significant reduction in growth vs. control (in bold) in Table 5. $y = -0.0022 + 0.97$; $r^2 = 0.80$; $p < 0.001$. The regression line is shown with the 95% confidence intervals.

Seedling age and exposure duration was the two most important factors that varied between different experiments. The effect of these factors was tested for separately on the data used in Fig. 3 (all data), in order to find systematic bias in the model. No significant correlations were obtained, however, indicating that these factors had minor influence on the model and that the model assumptions were valid (Table 6). Fig. 4 contained too few data pairs to be able to make a reliable test of the risk for systematic bias.

Table 6
Test of the regression model in Fig. 3 concerning systematic bias due to the possible influence of age of seedlings and exposure duration

AOT40	Influence of age				Influence of exposure duration			
	x-variable	Intercept	r^2	p	x-variable	Intercept	r^2	p
10	−0.0032	1.052	0.002	0.85	−0.02	1.09	0.02	0.51
50	−0.0047	1.209	0.0002	0.95	−0.107	1.49	0.02	0.48

A regression is performed for the relative biomass at an AOT40 $\mu l\,l^{-1}$ of 10 versus seedling age and for the relative biomass at an AOT40 of 50 versus exposure duration. $y = kx + i$, where i is intercept, r^2 is the coefficient of determination for the equation and p is the level of significance.

4. Discussion

4.1. Test of exposure indices on the clone used in GOSP

All regressions had very similar r^2. Such a narrow range of r^2 (0.42–0.47) for AOT with different cut off values is expected, due to auto-correlation. In an experiment the fumigation procedure itself, e.g. when ozone is added on a proportional basis, predetermines the sequence of the treatments and their respective effects, which results in strong auto-correlation for time and ozone accumulation (Pleijel, 1996). Also, when only one set of data from one experimental site is used this is a drawback for the possibilities to generalize the results. One site only represent one climate zone and one frequency distribution of ozone, while if experiments from many sites are used, different frequency distribution of ozone and other site conditions will provide to a more representative data base, with a wider range of r^2. When Pleijel et al. (2002) used a data set originating from four independent experiments and compared the r^2 values for relative yield of potato versus CUO_3^t (Cumulative Uptake of Ozone), using different thresholds (t) for the ozone uptake rate, the r^2 values varied between 0.3 and 0.42. As indicated in the introduction it is only when experiments are set up to specifically test different indices that the sequence/order possibly can be changed (Lee and Hogsett, 1999).

Having made these reservations we still see the value of testing different cut-off values and indices. The results of the present investigation showed that a cut-off concentration is relevant, that there were small differences between the different cut-off concentrations, but that 20 or 30 $nl\,l^{-1}$ provided the best correlations. AOT40 and SUM06 seem to explain the effect approximately equally well. The distinct increase in the r^2 between the cut-off concentrations 0 and 10 $nl\,l^{-1}$ (Fig. 1), might indicate a biological threshold, above which ozone is starting to become toxic. A similar pattern has been documented with potato at approximately 20 $nl\,l^{-1}$ (Pleijel et al. 2002). These results can be compared with the boundary-line and linear regression analysis presented by Taylor Jr. (1994) for loblolly pine (*Pinus taeda*). He used data from the literature in order to examine growth at ozone levels currently found in the south eastern forests in the US. Generally, effects occurred at or above a daytime 12-h mean of 45 $nl\,l^{-1}$ during more than 110 days during one growing season. This is in agreement with the threshold value of 40 $nl\,l^{-1}$ used in the AOT40 index. The most sensitive loblolly pine trees exhibited growth loss at a threshold mean of 25 $nl\,l^{-1}$, which is in agreement with a cut-off value lower than 40 $nl\,l^{-1}$ found for the GOSP-clone. An independent study of loblolly pine showed similar results with mature trees in the field (McLaughlin and Downing, 1996). They defined a rough ozone response threshold at 40 $nl\,l^{-1}$ and concluded that negative ozone effects on stem growth of mature trees can start to occur at ambient levels of ozone.

4.2. Test of the AOT40 index on the European data

Fig. 3 shows a weak correlation, which is still significant, when all data were included, i.e. the model cannot fully explain the variability in growth. In addition to a variation in climate and experimental procedures, the plot most likely demonstrates a range of individual responses of spruce trees of different genetic origin and a potentially large pool of genetic variation. Several studies of loblolly pine report family and genotype as a source of variation in governing the response of this species to ozone (Taylor Jr., 1994; McLaughlin et al., 1994). Taylor Jr. (1994) calculated this factor to explain between 50 and 75% of the variation in ozone sensitivity. A general conclusion for all studies of loblolly pine was that ozone can affect growth, but the response will be strongly dependent on genetic variation associated with family origin. This statement is probably valid also for Norway spruce. It is hard to explain the very strong stimulation of growth by ozone in some of the experiments. The rationale for also using growth data presented as shoot biomass only (Skärby et al., 1995; Polle et al., 1993) or calculated stem volume (Payer et al., 1990; Lucas and Diggle, 1997), based on diameter and height, is that these parameters for Norway spruce typically correlate well with total biomass (unpublished observations). However, this could be another source of variation.

When only the data showing statistically significant effects were included, the regression had a much stronger correlation ($r^2 = 0.80$). It could be debated which regression model to use for the European data, the one including all data or the one with statistically significant data only. Studies with small sample sizes, as is often the case in the studies presented in Table 5, are less likely to have significant results compared to studies with large sample sizes, even if both have effects of the same magnitude. This is a strong reason for using Fig. 3 representing all data. Another argument is that by including also "no effect" studies, a dose–response relationship is generated that is more representative for populations of Norway spruce trees, i.e. Norway spruce forests, still ignoring all other problems with scaling the effects from young to mature trees. On the other hand using Fig. 4, with data where the effects were statistically significant is sensible, according to the Level I approach, since it obviously includes the most sensitive Norway spruce clones and the most clear-cut experimental results. The results, using only significant data, suggest that the critical level for forest trees does not fully protect the most sensitive spruce trees at an AOT40 of $10\,\mu l\,l^{-1}\,h$ since the relative growth was reduced by 6% at this exposure. When dealing with trees, multi-year exposures and cumulative effects have to be taken into account. The linear regression models presented in Figs. 3 and 4 take the time dimension into account more or less indirectly only, via the cumulated dose. This can be questioned, since both short term (50 days) and long term exposures (4 growing seasons) were included in the same plot. Equally important to consider is the age of the seedlings used in the different experiments. If these aspects have an influence on the result, the model can be questioned. However, neither age nor exposure duration seemed to have a significant influence at an AOT40 of 10 or $50\,\mu l\,l^{-1}\,h$ when this was tested for the regression model in Fig. 3. Finally, the inclusion of indoor studies in the model can be debated. The exclusion of these experiments, however, resulted in almost the same regression equation, which means it is not a problem to use the data in risk assessments even if emphasis should be made on outdoor chamber studies. Having made this systematic review of ozone experiments on Norway spruce, it seems more correct to include all data sets rather than to exclude some of them and thereby lose important information. Finally, climate is probably a very important factor to explain the variation and sufficient pot size seems to be a critical prerequisite for a sound scientific experiment with the aim to test the influence of ozone on tree growth.

5. Conclusions

The regression model showed a significant correlation for the relationship between relative biomass of the Norway spruce clone used in GOSP and the AOT as well as SUM06 indices, among which AOT20 and AOT30 were the best. The model predicted that an AOT40 of $10\,\mu l\,l^{-1}\,h$ would decrease the biomass of this clone by 1%. The European data concerning Norway spruce, available to test the AOT40 index, suggest that the current critical level, an AOT40 of $10\,\mu l\,l^{-1}\,h$ during one growing season may result in a 6% growth reduction in the most sensitive trees. The results suggest that the critical level set for forest trees does not fully protect from growth reductions of Norway spruce in Europe.

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