

New critical levels for ozone effects on young trees based on AOT40 and simulated cumulative leaf uptake of ozone

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Received 9 July 2003; received in revised form 9 January 2004; accepted 14 January 2004

Abstract

Leaf or needle ozone uptake was estimated for young trees at seven experimental sites across Europe using a stomatal conductance simulation model. Dose–response relationships based on cumulative leaf uptake of ozone (CUO) were calculated using different hourly ozone flux thresholds and these were compared to dose–response relationships based on daylight AOT40, which is currently used within the UNECE Convention on Long-Range Transboundary Air Pollution (CLRTAP). Regression analysis showed that the CUO–biomass response relationships were highly significant for both coniferous and broadleaf trees, and independent of which ozone flux threshold was applied. On the basis of this regressions analysis, an hourly flux threshold of $1.6 \text{ nmol m}^{-2} \text{ s}^{-1}$ ($\text{CUO} > 1.6$) is proposed as the most appropriate for all species categories in deriving dose–response relationships. The analysis indicated that the current critical level for ozone impacts on European forests of AOT40 10 ppm h may not protect the most sensitive receptors and that critical levels for AOT40 and $\text{CUO} > 1.6$ of 5 ppm h and 4 mmol m^{-2} , respectively, are more appropriate. The research identified weaker dose–response relationships for the CUO exposure index compared with AOT40. Distinguishing between sensitive and less sensitive species substantially improved the CUO–biomass response relationships although, still, to a lesser extent than when exposure was expressed as AOT40.

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Keywords: Critical levels; Ozone uptake; Ozone flux; AOT40; Biomass reduction

1. Introduction

The ozone exposure indices for vegetation that have, to date, been used within the United Nations Economic Commission for Europe Convention on Long Range Transboundary Air Pollution (UNECE CLRTAP), are based on the ozone concentration in the air surrounding

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leaves and needles. However, it is widely recognised that only the ozone molecules that enter the leaves through the stomata are harmful to plants (Fuhrer, 2000). In addition, leaf detoxification systems are able to cope with some of the oxidative stress induced by ozone molecules taken up by the leaves, and the extent to which this represents a cost for the plants has been debated. The development of a physiologically based dose–response relationship for ozone requires that the ozone exposure should be described as leaf cumulative uptake of ozone (CUO), based on ozone flux estimates (Fuhrer, 2000; Karlsson et al., 2003c). Furthermore, the current critical level, expressed as AOT40, is based upon data from experiments on seedlings of a single species, *Fagus sylvatica* (Kärenlampi and Skärby, 1996), and thus may not be fully representative. Additional data sets covering a range of species and environmental conditions are required in order to develop a more robust basis for critical level exceedance mapping under CLRTAP.

The aim of this study was to derive CUO–biomass response relationships for young trees from existing experiments for a number of European broadleaf and conifer species, to compare these relationships with those based on daylight AOT40 and to use the relationships to propose new ozone critical levels for forest trees.

2. Methods

2.1. Description of data sets

Elevated levels of ozone frequently induce more or less reversible changes in the carbon allocation, growth pattern and physiology of trees (e.g. Miller et al., 1997). The response parameters used as indicators of ozone-related injury should be quantifiable, consistent and not dependent on the growth phase or recent exposure history, but rather, reflect the integrated, long-term impacts on the growth or condition of the trees. Thus in this analysis, only total and above ground biomass are used as response parameters. Data sets are only included where both daylight AOT40 and cumulative ozone uptake can be estimated. Brief descriptions of the individual data sets are given in Tables 1 and 2.

The Norway spruce (*Picea abies*) data sets from Östad, Sweden (60 m.a.s.l, mean annual precipitation 700 mm, mean annual temperature 7°C) have been described in detail by Wallin et al. (2002), Karlsson et al. (2002), Ottosson et al. (2003) and Karlsson et al. (2003b). One commercially available clone of Norway spruce was exposed to three different ozone levels in open-top chambers over the course of four growing seasons. The control treatment was charcoal-filtered air. The ozone treatments were applied within two physically and statistically separate experiments, with and without

combined drought and phosphorus deficiency stresses, respectively. With the exception of the phosphorus deficiency treatment, optimum nutrient levels were maintained. The birch (*Betula pendula*) data set from Östad has previously been described in detail (Karlsson et al., 2003a). Birch saplings of one half-sib family were planted in open-top chambers and exposed to three levels of ozone over two growing seasons. The control treatment was non-filtered air. Optimum nutrient levels were maintained.

The data sets from Vosges, France (730 m.a.s.l, mean annual precipitation 1000 mm, mean annual temperature 7°C) represent 10 years of research carried out in open-top chambers at Col du Donon in the Vosges mountains of north-east France, close to Nancy. In all experiments, the charcoal-filtered treatment was used as the control. A more detailed description of the facility is given in Dixon et al. (1998). The first experiment assessed the effects of ozone on two clones of Norway spruce (*P. abies*). The plants were exposed to ozone in combination with two water supply treatments. As a result of the limited replication in this experiment, data from both clones and for the two water supply treatments were aggregated. In the second experiment, plants of Aleppo pine (*Pinus halepensis*) were exposed to ozone for two years in combination with two water supply treatments. Finally, a 2-year experiment was performed on both pedunculate and sessile oak (*Quercus robur* and *Q. petraea*), again in combination with two water supply treatments. In the case of the Aleppo pine and oak experiments, data from the two water supply treatments were not aggregated.

The experiment at Ebro, Spain (0 m.a.s.l, mean annual precipitation 580 mm, mean annual temperature 17°C) was carried out in open-top chambers located in the Ebro Delta, Spain. Two-year-old Aleppo pine (*P. halepensis*) seedlings were exposed continuously throughout the 3-year experimental period to three different ozone levels with charcoal filtered air as the control treatment.

The three data sets from Kuopio, Finland (84 m.a.s.l, mean annual precipitation 600 mm, mean annual temperature 3°C) for birch (*B. pendula*) are from different open-field experiments conducted at a free-air ozone exposure facility at Kuopio in central Finland, as described by Oksanen (2003). The birch saplings were exposed to ambient air and approximately 1.5 times ambient ozone levels. In the first experiment, two potted clones were exposed over one growing season (Oksanen and Saleem, 1999). In the second experiment, ten birch clones were planted in the ground and exposed to ozone over five growing seasons (Oksanen, 2001). In the final experiment, the two clones used in the first experiment were exposed to the experimental treatments over the course of two growing seasons. In all experiments, ambient air was used as the control treatment. The data

Table 1
Description of the conifer tree species data sets

	Östad, SE	Schönen-buch/ Zugerberg, CH	Ebro, ES	Headley, UK	Vosges, FR	Vosges, FR
Experimental period	4 years, Apr–Sep	1–3 years. Jun–Oct	3 years. Continuous exposure	2 years Apr–Nov	2 years. Apr–Oct	5 years, 3 years exposure
Species	<i>Picea abies</i> , 1 clone	<i>P. abies</i>	<i>Pinus halepensis</i>	<i>P. sylvestris</i>	<i>P. halepensis</i>	<i>P. abies</i> , 2 clones
Plant initial age	3 years	Seedling	2 years	1 year	2 years	5 years
Growth system	1201 pots. Sand	Pots. Forest or compost soil	181 pots. Peat, sand, vermiculite	In the ground	231 pots. Peat and sand	In the ground
Exposure system, replicates	OTC, CF and NF+, 6 repl.	OTC, CF and NF. 2–4 repl.	OTC CF, NF, NF+, 3 repl.	OTC, NF, NF+, 2–4 repl.	OTC, CF, NF, NF+, 2 repl.	OTC, CF, NF, NF+ 25 and 50, 2 repl.
Response parameter	Total biomass	Total biomass	Total biomass	Above ground biomass	Total biomass	Above ground biomass
Number of plants per data point	12–36	14–30	18	24	32	12
Number of data points contributed	27	10	2	4	4	4
Water supply	Well-watered, drought	Well watered	Well watered	Well-watered, drought	Well-watered, drought	Well-watered, drought

OTC, open-top chambers; CF, charcoal filtered air; NF, non-filtered air; NF+, non-filtered air with additional ozone.

Table 2
Description of the deciduous tree species data sets

	Schönen-buch/ Zugerberg, CH	Headley, UK	Vosges, FR	Östad, SE	Kuopio, FI
Experimental period	1–3 years. May–Oct	2–3 years Apr–Nov	2 years, Apr–Oct.	2 years May–Sep	1–5 years. May–Sep
Species	<i>Fagus sylvatica</i>	<i>Quercus. robur, Q. petraea</i>	<i>Quercus robur, Q. petraea</i>	<i>Betula pendula</i>	<i>Betula pendula</i>
Plant initial age	Seedlings	1 year	2 years	5 months	1 year
Growth system	Pots. Forest, comp. soil	In the ground	231 pots	1201 pots	7 and 201 pots/ in the ground
Exposuresystem, replicates	OTC. CF, NF, 2 repl.	OTC, NF, NF+, 2–4 repl.	OTC. CF, NF+, 2 repl.	OTC, NF, NF+, NF++, 4 repl.	Open field exposure, 2 repl. plots
Response parameter	Total biomass	Above ground biomass	Total biomass	Perennial biomass	Perennial biomass
Number of plants per data point	15	40	16	24	10–100
Number of data points contributed	14	8	12	5	6
Water supply	Well watered	Well-watered, drought	Well-watered, drought	Well watered	Well watered

OTC, open-top chambers; CF, charcoal filtered air; NF, non-filtered air; NF+, non-filtered air with additional ozone; NF++, non-filtered air with more additional ozone, compared to NF+, in experiments several levels of ozone additions.

for the different clones were aggregated, and thus all clones included in each experiment are represented by a single data-point.

The experiments underlying the data sets from Schönenbuch (400 m.a.s.l, mean annual precipitation 800 mm, mean annual temperature 10°C) and Zugerberg

(990 m.a.s.l, mean annual precipitation 1300 mm, mean annual temperature 7°C), Switzerland, have been described by Braun and Flückiger (1995). Data are included from a total of 10 experiments using beech (*F. sylvatica*) and Norway spruce (*P. abies*) seedlings, with the duration of the experiments ranging from a few months to three years. All experiments involved the exposure of tree seedlings to either ambient or charcoal-filtered air as treatments.

The data from Headley, UK (80 m.a.s.l, mean annual precipitation 780 mm, mean annual temperature 10°C) are sub-sets from two factorial CO₂–O₃ experiments carried out in open-top chambers (Medlyn et al., 1999; Broadmeadow and Jackson, 2000). In the first experiment, sessile oak and Scots pine (*Q. petraea* and *Pinus sylvestris*) were exposed to experimental conditions for 3 years, with water supply an additional treatment in the final 2 years. In the second experiment, sessile and pedunculate oak (*Q. petraea* and *Q. robur*) were exposed to ozone in combination with two water supply treatments. In both experiments the ambient (unfiltered) air control was compared with the elevated ozone treatment.

2.2. Estimates of leaf ozone flux and daylight AOT40

Ozone flux was estimated on the basis of the multiplicative stomatal conductance simulation model (Jarvis, 1976; Emberson et al., 2000a, b; UN ECE, 2003). The application of the model to estimating ozone flux is still under development, and there is also some uncertainty in model parameterisation for individual tree species. As a result of this uncertainty in model parameterisation and the potential for both phenotypic and genotypic variation in stomatal responses to environmental variables, site specific information was used where available. Complete local parameterisations of the stomatal conductance response functions were used for estimating ozone uptake for Norway spruce (Karlsson et al., 2000, 2003b) and birch (Uddling et al., submitted, Table 3) at Östad, for Aleppo pine at Ebro (Table 3), and for birch (Uddling et al., submitted, Table 3) at Kuopio.

A single value of g_{\max} was assumed for each of the species (Table 3), with the exception of Aleppo pine. This distinction was made because in the Mediterranean coastal region, a large proportion of annual gas exchange occurs during the winter months in Aleppo pine (Elvira and Gimeno, 1996). To account for this, a relatively high value of g_{\max} together with a g_{age} function was applied to the Ebro simulations (Table 3), based on extensive field measurements. In contrast, a single value of 300 mmol m⁻² s⁻¹ for g_{\max} was applied to simulations of stomatal uptake for Aleppo pine at Vosges because of the temperate nature of the climate at this site. For oak, a value of 310 mmol m⁻² s⁻¹ was adopted for g_{\max} , based

on extensive measurements at Headley (Medlyn et al., 2001). For all other simulations, the current EMEP default value for g_{\max} was assumed.

The current parameterisation of the ozone deposition module of the EMEP model was used (Emberson, personal communication, Table 3), with the exception of the temperature and soil moisture response functions as described below. The EMEP formulation of the function describing temperature limitation of stomatal conductance (g_{temp}) in beech, with an optimum of 24°C declining to a minimum at 13°C, was replaced by a linear function based on the observed temperature dependency of photosynthesis in beech (Larcher, 1969). The new function has an optimum at 20°C and a minimum conductance at -5°C. Maximum conductance was assumed between 20°C and 24°C, with the original EMEP g_{temp} function retained above 24°C. In the case of the function describing soil water potential limitation of stomatal conductance (g_{SWP}), the EMEP formulation assumes no limitation to conductance from 0 to -1.0 MPa, and a linear decrease from -1.0 MPa to a minimum conductance at -1.9 MPa. This function was replaced by a new function based on conventional soil physics theory in which it is assumed that water is freely available from 0 to -0.05 MPa and that there is a linear reduction in availability from -0.05 to a minimum at -1.5 MPa (Hall et al., 1977). The g_{SWP} function assumed a value of 1.0 for all well-watered treatments. This new g_{SWP} function was adopted for all species in experiments for which local parameterisations were not available, and is supported by experimental data describing the stomatal response to soil water potential for several North American conifer species (Kramer and Kozlowski, 1979).

Daylight AOT40 was calculated according to Kärenlampi and Skärby (1996) for the same periods as used for estimates of cumulative ozone uptake (i.e. between SGS and EGS, Table 3).

2.3. Calculations and statistics

Each data point included in the regression analysis represents a single value for each species and treatment in each experiment. This approach is justified by the observation that the main source of variation in the response of young trees to ozone is at the individual plant level (e.g. Ottosson et al., 2003). In most cases, individual treatment combinations were included as separate data-points. However, in some experiments where replication was limited at the individual plant level and no significant interaction was observed between ozone and the additional treatment(s), each ozone treatment was aggregated across the additional treatments. Further details of replication and experimental design for each study are given in Tables 1 and 2.

Table 3
Methodology applied for ozone flux simulation. SGS, start of growing season

Data set	EMEP parameter	g_{\max} (mmol H ₂ O m ⁻² s ⁻¹) ^a	g_{\min} (fraction)	g_{light} (α)	g_{VPD}		g_{SWP}		g_{temp}			g_{age}				
					VPD _{max} (kPa)	VPD _{min} (kPa)	SWP _{max} (MPa)	SWP _{min} (MPa)	T_{\min} (°C)	T_{opt} (°C)	T_{\max} (°C)	SGS (yearday)	EGS (yearday)	$g_{\text{age-a}}$ (fraction)	$g_{\text{age-b}}$ (days)	$g_{\text{age-c}}$ (days)
<i>Picea. abies</i> , Vosges, FR	Spruce	155	0.02	-0.01	3.5	0.6	-0.05	-1.5	3	20	37	01 Apr	30 Sep	0.5	1	80
<i>P. abies</i> , Schön./Zug., CH	Spruce	155	0.02	-0.01	3.5	0.6	-0.05	-1.5	3	20	37	01 Apr	30 Sep	0.6	30	36
<i>Pinus sylvestris</i> , Headley, UK	Spruce	155	0.02	-0.01	3.5	0.6	-0.05	-1.5	3	20	37	01 Apr	30 Sep	0.5	1	80
<i>P. halepensis</i> , Vosges, FR	Med. pine	300	0.13	-0.013	1.6	0.4	-0.05	-1.5	4	20	36	01 Apr	30 Sep	0.2	130	130
<i>P. halepensis</i> , Ebro, SP	Med. pine	350	0.13	-0.013	3.2	0.8	-0.05	-1.5	10	27	38	01 Jan	31 dec	0.09 ^b	0 ^b	305 ^b
<i>Fagus. sylvatica</i> Schön./Zug.,CH	Beech	220	0.13	-0.006	3.1	1.1	-0.05	-1.5	-5	20–24	35	01 Apr	30 Sep	0.3	50	50
<i>Quercus sp</i> Headley, UK	Beech	310	0.13	-0.006	3.1	1.1	-0.05	-1.5	-5	20–24	35	01 Apr	30 Sep	0.3	50	50
<i>Q. sp</i> Vosges, FR	Beech	310	0.13	-0.006	3.1	1.1	-0.05	-1.5	-5	20–24	35	01 Apr	30 Sep	0.3	50	50
<i>Betula pendula</i> Östad, SE ^c	—	220	—	—	—	—	—	—	—	—	—	3 jun/28 Apr ^d	—	0.2	16	—
<i>B. pendula</i> Kuopio, FI ^c	—	220	—	—	—	—	—	—	—	—	—	18–20 May	—	0.2	16	—

EGS, end of growing seasons; $g_{\text{age-a}}$, minimum g_{age} , $g_{\text{age-b}}$, number of days from SGS for g_{age} to reach its maximum; $g_{\text{age-c}}$, number of days during the decline for g_{age} to again reach its minimum.

^a Projected leaf and needle area basis.

^b Function used for g_{age} : If $yd < (EGS - g_{\text{age-c}})$; $g_{\text{age}} = 1$. If $yd > (EGS - g_{\text{age-c}})$; $g_{\text{age}} = ((0.00003 * yd^2) - (0.0128 * yd) + 1.66) + g_{\text{age-a}}$. The function in Emberson et al. (2000b) involving the parameter $g_{\text{age-b}}$ is not used. yd , year day.

^c For the birch data-sets from Östad and Kuopio the following functions were used to calculate stomatal conductance (g_s); $g_s = \max \{g_{\text{dark}}, g_{\text{max}} f(\text{PPFD}) f(D) f(T)\}$, $f(\text{PPFD}) = g_{\text{dark}}/g_{\text{max}} + (1 - g_{\text{dark}}/g_{\text{max}})(1 - \exp(-k_1 \text{PPFD}))$, $f(T) = \max \{g_{\text{dark}}/g_{\text{max}}, \min \{1, k_2 + k_3 T\}\}$, $f(D) = \min \{1, (k_4/1 + Dk_5)\}$, where g_{dark} and g_{max} are the g_s in darkness and under non-limiting conditions, respectively, D is the water vapour pressure deficit in the air, PPFD is the photosynthetically active photon flux density, T is air temperature, $k_1 = 0.00453$, $k_2 = 0.490$, $k_3 = 0.0253$, $k_4 = 1.498$, $k_5 = 0.641$.

^d Values for the 1997 and 1998 growing seasons, respectively.

The approach suggested by Fuhrer (1994) was applied to all data sets, with exposure and flux calculated for all treatments including charcoal filtered controls. For each experiment, the hypothetical biomass at zero exposure was calculated as the y -axis intercept, using extrapolation of the linear regression of biomass versus AOT40 or CUO. In the next steps the reduction in biomass for each treatment was calculated relative to the hypothetical biomass at zero AOT40 or CUO.

The experimental data included multi-year experiments from one to five growing seasons. In order to obtain a common time basis for the dose–response relationships for all experiments, both the ozone exposure index and the effect parameter were recalculated as a function of the number of growing seasons over which the individual experiments were conducted. The ozone exposure indices were divided by the number of growing seasons. The effect parameter (% biomass reduction) was recalculated according to the formula:

$$RB_{\text{year}} = RB_{\text{tot}}^{(1/\text{yr})}$$

where RB_{year} is the relative biomass per growing season (a value between 0 and 1, relative to the control at zero ozone exposure); RB_{tot} is the relative biomass at the time of harvest; yr is the number of growing seasons from the start of the experiment until the time of harvest. Thus, both exposure and effects are expressed on the basis of a single growing season. The relationships with a number of different exposure indices were fitted by simple regression. The general assumptions on which the regression models are based (Underwood, 1997) were

satisfied in all cases. There were no repeated measurements on the same plant individuals, 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 analysis in this study was restricted to daylight AOT40 and CUO with thresholds of 0, 1.6, 3.2 and $4.8 \text{ nmol m}^{-2} \text{ s}^{-1}$. The threshold of $3.2 \text{ nmol m}^{-2} \text{ s}^{-1}$ corresponds to the ozone flux at an ozone concentration of 40 nl l^{-1} and maximum stomatal conductance for Norway spruce ($155 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$). Regression analysis was performed using STATGRAPHICS PLUS for Windows software.

3. Results and discussion

3.1. Comparison of AOT40 and CUO indices

The relationships between CUO and daylight AOT40 for different ozone flux thresholds are shown in Fig. 1. In general, correlations were strong between the two exposure indices. However, CUO was high at low values of AOT40 when the CUO threshold was zero, while AOT40 for conifers was high at low CUO when the threshold was set to $3.2 \text{ nmol m}^{-2} \text{ s}^{-1}$.

3.2. Dose–response relationships based on daylight AOT40 and cumulative ozone uptake

In a preliminary regression analysis performed on pooled data for all broadleaf and conifer species

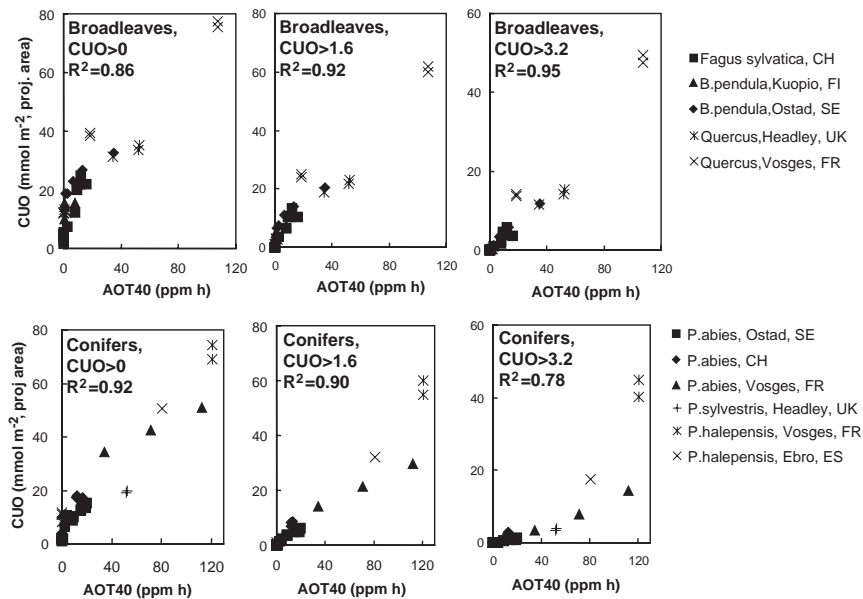


Fig. 1. The relationships between daylight AOT40 and CUO, calculated for individual tree species and experiments, using different ozone flux thresholds. $\text{CUO} > t$, represents the cumulative leaf uptake of ozone calculated for an hourly ozone flux threshold of $t \text{ nmol m}^{-2} \text{ s}^{-1}$. R^2 values for linear regression analysis are indicated in each panel.

separately, a large variation in ozone sensitivity was evident between different species. Furthermore, these preliminary linear regressions generally resulted in significant, negative y -axis intercepts, thus precluding any assessment of critical levels (data not shown). Consequently, both the conifer and broadleaf species pooled data sets were sub-divided into sensitive (Norway spruce, Scots pine, birch and beech) and less sensitive (Aleppo pine and oak) categories. This procedure substantially increased the coefficient of determination (R^2) for the individual linear regression analyses, with the exception of Aleppo pine, for which the regressions were non-significant. It also eliminated the significant y -axis intercepts (Table 4).

A note of caution over the definition of sensitive and less sensitive species is warranted. The technical manual of the European Intensive Forest Health Monitoring Programme (EC, 1998) includes a list of ozone-sensitive species, which includes Norway spruce, but not Scots pine. The list includes species that show visible injury in the field, although no effect on growth is implied, and it is explicitly stated that one does not necessarily indicate the other. Furthermore, both pedunculate oak (*Q. robur*) and Aleppo pine are included on the list of sensitive species, although they are classed as less sensitive in this study, based on the response of growth to ozone.

In general, the highest R^2 for all species categories was observed when a threshold of $1.6 \text{ nmol m}^{-2} \text{ s}^{-1}$ was applied to hourly CUO values (Table 4). Moreover, at higher thresholds, an undesirable clustering of the residuals was evident at low values of CUO (data not shown). Thus, the threshold value of $1.6 \text{ nmol m}^{-2} \text{ s}^{-1}$ appears to be the most appropriate to apply across all species categories. For all CUO thresholds and all target receptors tested in this analysis, the daylight AOT40 index always had a higher R^2 , compared to CUO. This observation was unexpected and the reasons behind it may be complex. One possible explanation is that where climatic conditions do not lead to significant limitation of stomatal conductance, the external exposure will correlate closely with internal dose. It is only when the two indices diverge that plant responses can be expected to correlate better with an uptake-based index (CUO) than an external exposure index (AOT40). This point is illustrated in Fig. 1, where the correlation between AOT40 and CUO was strong. Soil water potential also had little impact on modelled stomatal conductance even in the drought treatments (data not shown), and only the Aleppo pine data set from Ebro was representative of a dry Mediterranean climate. Even in this data set, the difference between CUO and AOT40 was minimal as Aleppo pine growing in the coastal Mediterranean region does not have a dormant season (Elvira and Gimeno, 1996) and CUO was therefore estimated for the entire year, with low uptake in the dry

Table 4

Statistical data for regression analysis of the relationships between ozone exposure indices and % reduction of biomass for different tree species categories

Ozone index/ plant category	Linear regression			
	R^2	p , slope	p , intercept	Slope
<i>AOT40</i>				
Birch, beech	0.62	***	Ns	-0.732
Oak	0.65	***	Ns	-0.214
Norway spruce, Scots pine	0.79	***	Ns	-0.154
Aleppo pine	0.35	Ns	Ns	—
<i>CUO>0</i>				
Birch, beech	0.43	**	Ns	-1.01
Oak	0.49	***	Ns	-0.348
Norway spruce, Scots pine	0.70	***	Ns	-0.366
Aleppo pine	0.34	Ns	Ns	—
<i>CUO>1.6</i>				
Birch, beech	0.50	***	Ns	-1.31
Oak	0.49	***	Ns	-0.348
Norway spruce, Scots pine	0.77	***	Ns	-0.571
Aleppo pine	0.41	Ns	Ns	—
<i>CUO>3.2</i>				
Birch, beech	0.41	***	Ns	-1.96
Oak	0.54	***	Ns	-0.384
Norway spruce, Scots pine	0.78	***	Ns	-1.28
Aleppo pine	0.42	Ns	Ns	—
<i>CUO>4.8</i>				
Birch, beech	0.18	**	*	-3.39
Oak	0.54	***	Ns	-0.567
Norway spruce, Scots pine	0.65	***	**	-2.87
Aleppo pine	0.43	Ns	Ns	—

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; Ns, $p > 0.05$; CUO $> t$, cumulative leaf uptake of ozone over a threshold $t \text{ nmol m}^{-2} \text{ s}^{-1}$; AOT40, accumulated exposure of ozone over a threshold of 40 ppb.

summer months and high uptake in winter diminishing the difference between the two exposure indices.

3.3. New ozone critical levels

The current approach adopted for determining new ozone critical levels for trees is a matter of debate. For agricultural crops, the critical level is set on the basis of a 5% annual yield loss (Kärenlampi and Skärby, 1996;

Karlsson et al., 2003c). The process is not as simple for trees, as their (commercial) rotation may exceed one hundred years. Impact studies have primarily been carried out on young trees, and little is known about the relative sensitivities of juvenile and mature plant material (Kelly et al., 1995; Kolb and Matyssek, 2001). Putting this last issue aside, if the ozone sensitivity was to remain constant over the entire life span of a tree, then there is a strong argument that the critical level should be set at the lowest exposure/dose for which a statistically significant effect is demonstrable.

The critical level was defined in this study as the exposure index where the 99% confidence limit of the dose–response relationship indicated a significant reduction in biomass (i.e. the x-axis intercept of the 99% confidence interval in the dose–response graph). On this basis, new critical levels of daylight AOT40 and CUO assuming a threshold of $1.6 \text{ nmol m}^{-2} \text{ s}^{-1}$ ($\text{CUO} > 1.6$) are presented in Table 5 (also see Figs. 2 and 3), expressed on a single growing season basis. The new critical levels were similar for the sensitive broadleaf and sensitive conifer categories, with proposed values for daylight AOT40 of 2–5 ppm h, corresponding to

Table 5

Proposed new ozone critical levels based on daylight AOT40 and $\text{CUO} > 1.6$, for different tree categories, expressed as the value accumulated over one growing season

Species category	Critical levels based on deviation of the 99% confidence intervals of the linear regression from a zero response		% Biomass reduction per growing season at the proposed critical level	
	Daylight AOT40 (ppm h)	CUO > 1.6 (mmol m ⁻² , proj. area)	Based on AOT40	Based on CUO > 1.6
Broadleaf, sensitive (Birch, beech)	2.4	3.4	3	5.6
Broadleaf, less sensitive (Oak)	18	12	4.5	5.7
Conifer, sensitive (Norway spruce, Scots pine)	4.7	1.8	0.8	0.9

The values represent the exposure index at which the 99% confidence intervals of the linear regression deviate from a zero response. $\text{CUO} > 1.6$, cumulative leaf uptake of ozone over a threshold $1.6 \text{ nmol m}^{-2} \text{ s}^{-1}$; AOT40, accumulated exposure of ozone over a threshold of 40 ppb.

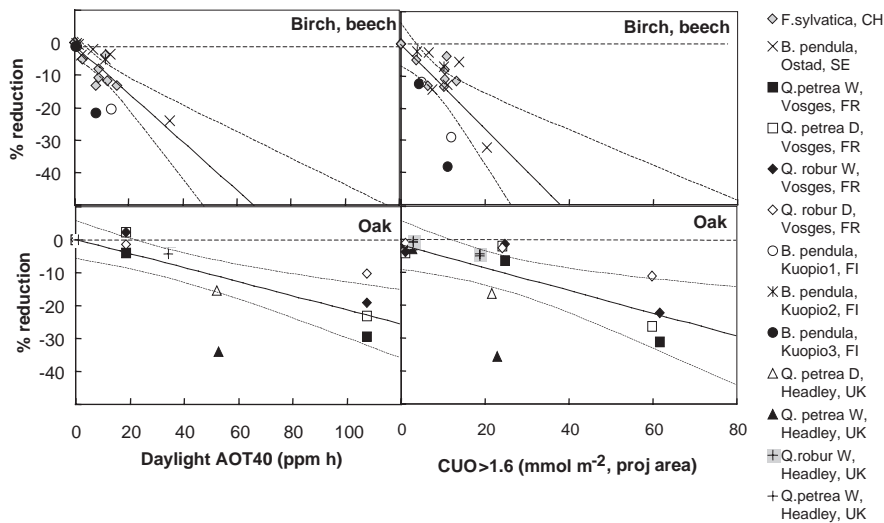


Fig. 2. The relationships between % reduction in biomass and ozone exposure, on an annual basis, for broadleaf tree species and experiments. Ozone exposure is expressed as AOT40 or as cumulative uptake of ozone above a threshold $1.6 \text{ nmol m}^{-2} \text{ s}^{-1}$. The relationships were analysed as linear regressions with 99% confidence intervals. The two upper panels include the sensitive broadleaf species (birch, beech) and the two lower panels show the less sensitive broadleaf species, represented by two species of oak: W, well watered and D, drought treatment.

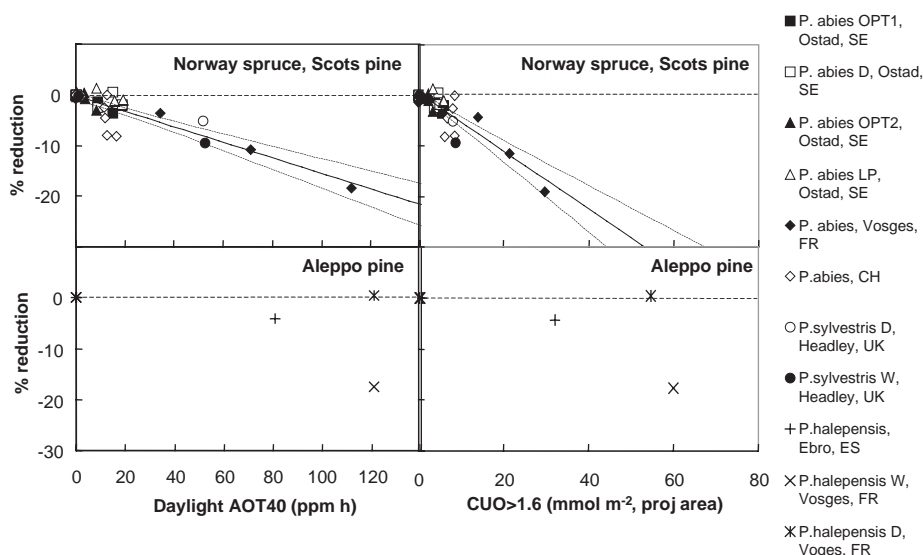


Fig. 3. The relationships between % reduction in biomass and ozone exposure, on an annual basis, for conifer tree species and experiments. Ozone exposure is expressed as AOT40 or as cumulative uptake of ozone above a threshold $1.6 \text{ nmol m}^{-2} \text{ s}^{-1}$. The relationships were analyzed as linear regressions with 99% confidence intervals. The two upper panels include the sensitive conifer species (Norway spruce, Scots pine) and the two lower panels show the less sensitive conifer species, represented by Aleppo pine. The regressions describing the exposure–response relationships for Aleppo pine were not significant and are therefore omitted: W, well watered and D, drought treatment. OPT1, optimal water and nutrient supply experiment 1; OPT2, optimal water and nutrient supply experiment 2; LP, low phosphorus supply.

CUO > 1.6 values of $2\text{--}3 \text{ mmol m}^{-2}$. The critical levels for the less sensitive broadleaf category (oak) were substantially higher, with values of 18 ppm h and 12 mmol m^{-2} proposed for AOT40 and CUO > 1.6, respectively. Table 5 also indicates the level of response, again expressed on a single growing season basis, which constitutes a significant effect in this analysis. For both broadleaf species categories, the critical level indicates a 3–6% reduction in biomass, while for sensitive conifer species, an approximate 1% reduction in biomass is implied.

The proposed critical levels (Table 5) indicate that the current AOT40 critical level for forest trees of 10 ppm h (Kärenlampi and Skärby, 1996), is too high to protect the most sensitive receptors under the most sensitive conditions. This analysis indicates that new critical levels of 5 ppm h AOT40 and 4 mmol m^{-2} CUO > 1.6 would protect sensitive European broadleaf and conifer tree species with a reasonable degree of confidence (UNECE, 2003).

3.4. Validity of the approach adopted in this study

The approach and analysis presented in this study, together with the range of experimental approaches included raises some critical questions. However, these issues must be viewed in the context of the objectives of the analysis presented here, which is to provide

appropriate critical levels for integrated assessment modelling. The current policy is for emission abatement strategies in Europe, within the UNECE CLRTAP, to be effect-based. Critical levels that are used to drive abatement strategies should thus be based on the most robust exposure–response relationships that can be provided on the basis of current knowledge. However, numerous or very specific critical levels cannot be used in integrated assessment modelling at the European scale. The role of the scientific community is therefore to provide a limited number of critical levels which protect the majority of vegetation with a reasonable degree of confidence. It should also be appreciated that the current status for ground-level ozone is that the critical level for forest trees is based on a level I approach, which aims to identify areas where ozone exposure is above the level at which sensitive receptors may be at risk of damage (Führer, 2000; Karlsson et al., 2003c).

3.5. Relative response calculations

The derivation of estimated biomass at zero ozone exposure based on extrapolation of linear exposure–response relationship constitutes a key assumption in this study. The largest extrapolations were for the birch data sets from Sweden and Finland, where the ozone exposure (AOT40 and CUO > 1.6) in the ambient air, ‘control’ treatments were up to approximately 50% of

the highest exposure. Detoxification capacity is considered an important issue in studies of ozone impacts and has the potential to introduce non-linearity into dose–response relationships, thus confounding the fundamental requirement for linear relationships. However, detoxification has been accounted for in this study through the analysis of varying thresholds for hourly ozone exposure values.

3.6. Differences in experimental approaches

The wide range of experiments included in this study raises the question of whether fundamental differences in dose–response relationships can arise as a result of differences in experimental conditions, thus precluding the use of combined data sets in regression analysis.

The main differences between the experiments presented in this study (Tables 1 and 2) are exposure system (open-top chambers and open-release systems), exposure duration (several months up to 5 years), plant age (from seedlings to approximately 10-year-old trees), growth medium and water supply. Of the factors listed above, we suggest that the exposure system has no fundamental influence on the dose–response relationships (Musselman and Hale, 1997). The number of data sets available was insufficient to enable an analysis of pot effects and the influence of the growth medium. A difference in the relative response between the well watered and drought stressed oak trees is indicated in Fig. 2, although the difference was not reflected in the estimated CUO. The modelling of stomatal responses to reduced water availability in different growth media is complex and remains a challenge for future research.

Since this study deals exclusively with seedlings and young trees, plant age and exposure duration are closely linked, particularly for the beech and birch data-sets, where the initial age of the plants was not more than one year (Table 2). A detailed analysis of the relationships between the relative response to a defined ozone dose and exposure duration, as well as plant age, was made for the beech/birch and spruce/pine categories. Regression analysis indicated a logarithmic relationship between the relative response to a defined ozone dose and experiment duration for the beech/birch category when $CUO > 1.6$ was used as the ozone exposure index ($p = 0.04$). In essence, a shorter exposure period resulted in a larger relative response. However, this analysis was hampered by the limited number of observations for the longer duration experiments compared to the many observations for experiments lasting two years or less. The relationship between relative response and exposure duration was not significant for the beech/birch category when AOT40 was used as the exposure index or for the spruce/pine category with either AOT40 or CUO. The effect of excluding data-sets from all experiments in the beech/birch category lasting less than two year was

investigated, and found to have minimal impact on both the linear regression analyses (which were still highly significant) and the derived critical levels.

The data sets included in this study therefore fulfil the general requirements for inclusion in common regression analysis. Although limited confounding effects of experimental duration and plant age on the dose–response relationships were evident, it is unlikely that they had a significant impact on the derived critical levels. Further discussion of these relationships is beyond the scope of this paper.

3.7. Further developments

The analysis presented here represents the first step in the derivation of critical levels for forest trees that can be used for quantitative assessments of the impact of ozone on forests. Further information is needed on the relative sensitivity to ozone of young and mature trees of different species, while further analysis and reworking of existing experiments can provide additional data sets to improve on the dose–response relationships presented here. The parameterisations of the multiplicative stomatal conductance model adopted in this study can also be improved upon through analysis of existing information in the published literature.

Although there are uncertainties associated with the proposed critical levels, particularly those based on CUO as outlined above, they can provide a focus for the development of ozone impact assessment. The use of dose–response as opposed to exposure–response relationships will improve the physiological basis for these assessments, enabling both environmental limitation of ozone effects and the impacts of low, but chronic, levels of ozone in some regions to be accounted for.

Acknowledgements

The contribution by Per Erik Karlsson to this study was financially supported by the ASTA project, financed by the Foundation for Strategic Environmental Research (MISTRA).

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