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Risk assessments for forest trees: The performance of the ozone flux versus the AOT concepts

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Ozone stomatal flux based indices were superior, as compared to AOT40, for explaining biomass reductions and leaf visible injury

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Abstract

Published ozone exposure–response relationships from experimental studies with young trees performed at different sites across Europe were re-analysed in order to test the performance of ozone exposure indices based on AOTX (Accumulated exposure Over a Threshold of X nmol mol^{-1}) and AF$_{st}$Y (Accumulated Stomatal Flux above a threshold of Y nmol m^{-2} s^{-1}). AF$_{st}$1.6 was superior, as compared to AOT40, for explaining biomass reductions, when ozone sensitive species with differing leaf morphology were included in the analysis, while this was not the case for less sensitive species. A re-analysis of data with young black cherry trees, subject to different irrigation regimes, indicated that leaf visible injuries were more strongly related to the estimated stomatal ozone uptake, as compared to the ozone concentration in the air. Experimental data with different clones of silver birch indicated that leaf thickness was also an important factor influencing the development of ozone induced leaf visible injury.

Keywords: Ozone; Forest trees; Ozone flux; AFstY; AOT40

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1. Introduction

It is generally accepted that the most severe ozone effects on plants are caused by the ozone that is taken up through the stomata into the leaf interior (Reich, 1987; Ashmore et al., 2004). However, differences in intrinsic ozone tolerance between species and proveniences due to various de-toxification processes are important and will also have to be taken into consideration to understand how ozone exposure relates to ozone impacts (e.g. Bussotti and Gerosa, 2002; Wieser et al., 2002).

There is consensus within the EU and the UNECE Convention on Long-Range Transboundary Air Pollution (LRTAP) that the exposure based on the rate of stomatal uptake (flux) of ozone represents the most appropriate approach for setting future ozone critical levels for forests trees (Karlsson et al., 2003a). However, uncertainties in the development and

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application of flux-based approaches, as they are available at their current stage, have been regarded as too large to justify their application as a standard risk assessment method for trees at a European scale. Therefore AOT40 (Accumulated exposure Over a Threshold of 40 nmol mol^{-1}) has been retained as the basis for the ozone critical levels for trees (Karlsson et al., 2003a). However, at a recent LRTAP workshop in Obergurgl, Austria (Wieser and Tausz, 206) it was suggested that an ozone flux based index, AF$_{st}$1.6 (Accumulated Flux through stomata above a threshold of 1.6 nmol m^{-2} s^{-1}), should be used for risk assessments for forests on the European scale for the integrated assessment modelling within the LRTAP convention.

In a previous analysis of the relationships between biomass reductions and ozone exposures using experimental data with young trees from different sites across Europe, AF$_{st}$1.6 was not found to provide better correlations with biomass reductions, as compared to AOT40 (Karlsson et al., 2004b). However, in that study the aim was to develop new ozone critical levels for the protection of the most sensitive receptor with the highest possible accuracy. Therefore broadleaved and coniferous tree species were analysed separately, each divided into two sensitivity categories.

The aim of this study was to further analyse published ozone exposure–response data in order to test the hypothesis that ozone impacts on young trees under experimental conditions are better explained by indices that are based on stomatal ozone flux, as compared to indices based on the ozone concentrations in the surrounding air.

2. Materials and methods

2.1. Analysis of exposure–response relationships for biomass reductions

2.1.1. Description of the data sets

The experimental data sets that were used in the present study for the statistical analyses of exposure–response relationships for ozone impacts on biomass were identical to those described in Karlsson et al. (2004b), with the exception of an additional data set for silver birch (*Betula pendula*) from Birmensdorf, Switzerland. This latter dataset has previously been described by Uddling et al. (2004). Some information about the different data sets is presented in Table 1. In brief, the experimental data originate from experimental studies conducted on young trees exposed in open-top chamber or open-release experiments at seven sites across Europe. The control treatments were either ambient or filtered air and the response parameters used for the exposure–response relationships were either total or above ground biomass. Ozone flux was estimated on the basis of the multiplicative stomatal conductance simulation model (Jarvis, 1976; Emberson et al., 2000a,b,2001; CLRTAP, 2004) which is a key component of the DO$_3$SE (Deposition of Ozone and Stomatal Exchange) model (e.g. Emberson et al., 2001). When available, site specific information was used to parameterise the model. For all other simulations, the DO$_3$SE models default values (as described most recently in Simpson et al., 2003) were assumed, with the exception of the temperature and soil moisture response functions (Karlsson et al., 2004b). Daylight AOT40 was calculated according to Kärenlampi and Skärby (1996) for the same periods as used for estimates of cumulative ozone uptake. The analysis in the present study was restricted to daylight AOT40 and AF$_{st}$ with a threshold of 1.6 nmol O$_3$ m^{-2} projected leaf area (PLA) s^{-1}.

Each data point included in the regression analysis represented a single value for each species, treatment and harvest occasion in each experiment. The number of harvested trees described by each data point is indicated in Table 1. The approach for response normalisation suggested by Fuhrer (1994) was applied to all data-sets, with exposure and flux calculated for all treatments including the control treatments. The reduction in biomass for each treatment was calculated relative to the hypothetical biomass at zero AOT40 or AF$_{st}$1.6. The experimental data included multi-year experiments from one to five growing seasons. In order to obtain a common time basis for the exposure–response relationships for all experiments, both the ozone exposure index and the effect parameter were recalculated as annual values.

2.1.2. Statistical methods

Regression models are based on some general assumptions (Underwood, 1997). In the data sets used in the present study, there were no repeated measurements on the same plant individuals, the sum of the residuals was close to zero and did not vary systematically with the index used. However, the assumption that the residuals should be evenly distributed along the *x*-axis was to some extent violated (see e.g. Fig. 1). This could have been caused by the method used to relate the biomass of all treatments, including the controls, to the hypothetical biomass at zero exposure. As some of the experiments used in this study had only two ozone treatments, with low ozone exposure values for the control treatment, this might have caused some artificially low variation in the lower part of the ozone exposure scale. On the other hand, a higher variation may also be expected at higher ozone exposure treatments due to the variation in ozone responses often found between tree individuals.

The relationships between biomass responses and exposure indices were fitted using two different linear regression analysis methods. In method 1, the relationships between biomass responses and exposure indices were fitted using simple linear regression, using the data from all ozone treatments including the controls ($n = 78$ for the sensitive category and $n = 26$ for the less sensitive category), and not forcing the linear regression through zero. This regression analysis was performed using Statgraphics Plus for Windows 3.1 software (StatPoint Inc., VA, USA). For method 2, the data for the control ozone treatments were excluded ($n = 46$ for the sensitive category and $n = 15$ for the less sensitive category) and the linear regressions were forced though zero. Method 2 used a regression method that corrected for heteroscedasticity, i.e. non-homogeneous variance and was performed using SAS software, PROC REG, option ACOV and SYSTAT, module TSLS.

2.2. Analysis of the relationship between relative ozone sensitivity and leaf thickness for silver birch

The relation between ozone sensitivity and leaf thickness for different clones of Silver birch, *B. pendula* and *B. pubescens*, was analysed based on data which were previously published by Pääkkönen et al. (1997). Young birch trees were exposed to ambient and elevated ozone concentrations during two growing seasons in Finland. Ozone sensitivity was assessed with regard to above ground biomass, leaf biomass, visible leaf injury and pre-mature leaf senescence (yellowing). For each response parameter, the clones were divided into four different sensitivity classes (scored 1–4, where 1 stands for most sensitive). Except for biomass, scores are given as average values for both years. For above ground and leaf biomass and for leaf yellowing, the score was based on the level of significance for a negative ozone effect, where 1 was significant at the 0.1% level, 2 at the 1% level, 3 at the 5% level and 4 not significant. Regarding visible leaf injury, score 1 was set when 75–100% of the total number of leaves showed visible injury, score 2 when 35–74%, score 3 when 1–34% and score 4 when <1% of the leaves showed visible injury.

2.3. Analysis of the relationship between leaf visible injury and the ozone dose for black cherry

The amount of leaf visible injury on black cherry (*Prunus serotina*), grown under two different irrigation treatments, within open-top chambers was correlated with two different ozone indices. These were the 7 h mean ozone concentration during day-time and an ozone uptake index calculated as the product of seasonal average stomatal conductance (calculated on a projected leaf area basis) and seasonal cumulative ozone exposure (SUM0). The experimental data are described in detail by Schaub et al. (2003).

Table 1
Description of the data sets

Data set	Sensitivity category	Site	Reference[b]	Exposure system	Control treatments[c]	Ozone treatments[c]	Combination treatments	Experiment duration (years)	Species	Age (years)[d]	Response parameter	Number of plants per data point
CH_FS	Sensitive	S/Z[a]	1	OTC	CF	NF	–	1–3	*Fagus sylvatica*	<1	Total biomass	15
FI_BP	Sensitive	Kuopio	2	Open-field	AA	AA+	–	1–5	*Betula pendula*	1	Perennial biomass	10–100
SE_BP	Sensitive	Östad	3	OTC	NF	NF+	–	2	*Betula pendula*	<1	Perennial biomass	24
CH_BP_OPT	Sensitive	Birmensdorf	4	OTC	CF	CF+, CF++, CF+++	–	1	*Betula pendula*	Cuttings	Total biomass	20
CH_BP_LF	Sensitive	Birmensdorf	4	OTC	CF	CF+, CF++, CF+++	Low fertilisation	1	*Betula pendula*	Cuttings	Total biomass	20
SE_PA_OPT1	Sensitive	Östad	5	OTC	CF	NF+	–	4	*Picea abies*	3	Total biomass	12–36
SE_PA_D	Sensitive	Östad	5	OTC	CF	NF+	Drought	4	*Picea abies*	3	Total biomass	12–36
SE_PA_OPT2	Sensitive	Östad	5	OTC	CF	NF, NF+	–	4	*Picea abies*	3	Total biomass	12–36
SE_PA_LP	Sensitive	Östad	5	OTC	CF	NF+	Low phosphorous supply	4	*Picea abies*	3	Total biomass	12–36
F_PA	Sensitive	Vosges	6	OTC	CF	NF, NF+, NF++	–	5	*Picea abies*	5	Above ground biomass	12
CH_PA	Sensitive	S/ Z[a]	1	OTC	CF	NF	–	1–3	*Picea abies*	<1	Total biomass	14–30
UK_PS_D	Sensitive	Headley	7	OTC	NF	NF+	Drought	2	*Pinus sylvestris*	1	Above ground biomass	24
UK_PS_OPT	Sensitive	Headley	7	OTC	NF	NF+	–	2	*Pinus sylvestris*	1	Above ground biomass	24
F_QP_OPT	Less sensitive	Vosges	6	OTC	CF	NF, NF+, NF++	–	2	*Quercus petraea*	2	Total biomass	16
F_QP_D	Less sensitive	Vosges	6	OTC	CF	NF, NF+, NF++	Drought	2	*Quercus petraea*	2	Total biomass	16
F_QR_OPT	Less sensitive	Vosges	6	OTC	CF	NF, NF+, NF++	–	2	*Quercus robur*	2	Total biomass	16
F_QR_D	Less sensitive	Vosges	6	OTC	CF	NF, NF+, NF++	Drought	2	*Quercus robur*	2	Total biomass	16
UK_QP_D	Less sensitive	Headley	7	OTC	NF	NF+	Drought	2–3	*Quercus petraea*	1	Above ground biomass	40
UK_QP_OPT1	Less sensitive	Headley	7	OTC	NF	NF+	–	2–3	*Quercus petraea*	1	Above ground biomass	40
UK_QP_OPT2	Less sensitive	Headley	7	OTC	NF	NF+	–	2–3	*Quercus petraea*	1	Above ground biomass	40
UK_QR_OPT	Less sensitive	Headley	7	OTC	NF	NF+	–	2–3	*Quercus robur*	1	Above ground biomass	40
ES_PH	Less sensitive	Ebro	7	OTC	CF	NF, NF+	–	3	*Pinus halepensis*	2	Total biomass	18
F_PH_OPT	Less sensitive	Vosges	6	OTC	CF	NF, NF+	–	2	*Pinus halepensis*	2	Total biomass	32
F_PH_D	Less sensitive	Vosges	6	OTC	CF	NF, NF+	Drought	2	*Pinus halepensis*	2	Total biomass	32

[a] S/Z, Schönenbuch/ Zugerberg.

[b] References: 1, Braun and Flückiger, 1995; 2, Oksanen, 2003; 3, Karlsson et al., 2003b; 4, Uddling et al., 2004; 5, Ottosson et al., 2003; Karlsson et al., 2004a: 6, Dixon et al., 1998; 7, Medlyn et al., 1999; Broadmeadow and Jackson, 2000.

[c] AA, ambient air; AA+, ambient air with extra ozone added; CF, charcoal filtered air; CF+, charcoal filtered air with extra ozone added; NF, non-filtered air; NF+, non-filtered air with extra ozone added.

[d] Age refers to the age of the plant material at the start of the experiment.

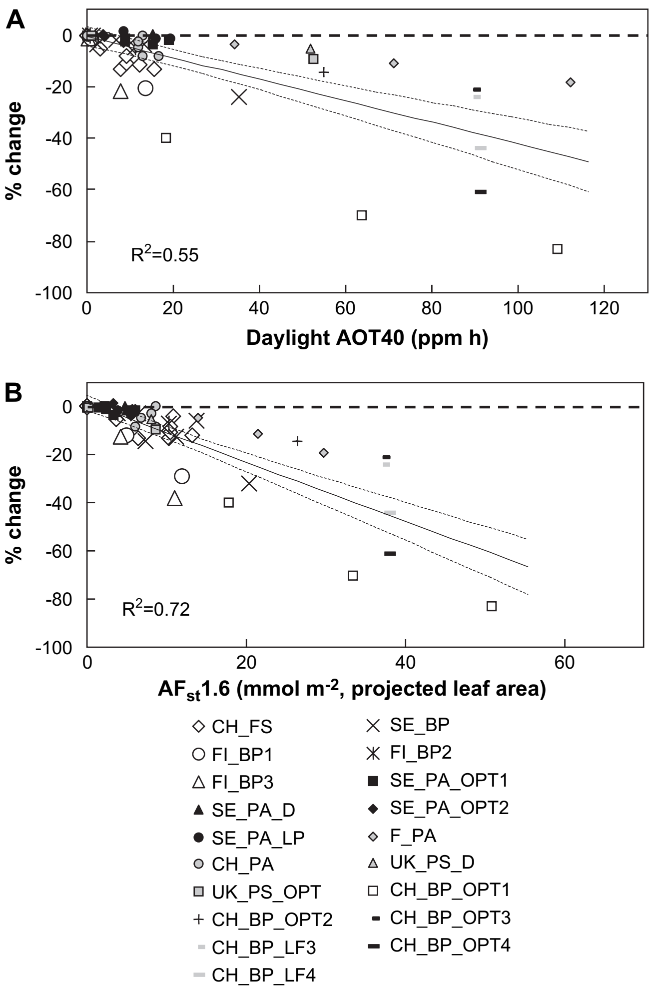

Fig. 1. Relationships between the annual biomass reduction and the annual ozone exposure expressed as daylight AOT40 (A) and accumulated stomatal flux of ozone (AF$_{st}$1.6) on a projected leaf area basis (B). The analysis was made using simple linear regression analysis including all data points and not forcing the regression through zero. The included species are those that were categorised as broadleaved sensitive (open symbols, cross and points) and coniferous sensitive (closed symbols with black or grey) (Karlsson et al., 2004b), with additional data sets with *Betula pendula* from experiments performed at Birmensdorf. For further explanations, see Section 2. The coefficient of determination (R^2) from linear regression analysis and the 99% confidence limits are shown in the figure. The legend for the different data sets can be found in Table 1. The detailed information on the regression analysis was for figure (A) $y = -0.417x - 0.471$, $R^2 = 0.55$, p slope <0.0001 and for figure (B) $y = -1.228x + 1.193$, $R^2 = 0.72$, p slope <0.0001.

3. Results

3.1. Comparison of AF$_{st}$1.6 and AOT40-based exposure indices for explaining biomass reductions in young trees

The results from the linear regression analyses of the exposure–response data sets with broadleaved and coniferous tree species, which were rated as ozone sensitive and less sensitive, respectively (Table 1; Karlsson et al., 2004b), are shown in Table 2 and Figs. 1–4. Both regression methods, outlined in Section 2, indicated that AF$_{st}$1.6 was substantially better than AOT40 in explaining the reductions in biomass for sensitive broadleaved and sensitive coniferous tree species (Table 2 and Figs. 1 and 3). The coefficients of determination were 0.72 and 0.55 for AF$_{st}$1.6 and AOT40, respectively, for the simple regression analysis (method 1) and 0.78 and 0.64, respectively for the method taking the non-homogeneous variance into account (method 2). There were no significant intercepts indicated by method 1. A similar analysis made in the present study on broadleaved and coniferous tree species which were rated as less sensitive, indicated that AF$_{st}$1.6 was not superior to the AOT40 in this case (Table 2; Figs. 2 and 4). However, the values for the coefficients of determination were more similar (0.40 and 0.44 for AF$_{st}$1.6 and AOT40, respectively, method 1, and 0.62 and 0.62 for AF$_{st}$1.6 and AOT40, respectively, method 2), as compared to the previous analysis of the less sensitive broadleaf category (coefficients of determination were 0.49 and 0.65 for AF$_{st}$1.6 and AOT40, respectively, Karlsson et al., 2004b). Again, there were no significant intercepts indicated by method 1.

3.2. Comparison of flux-based exposure indices taking leaf morphology into account

It was tested to modify the estimated accumulated ozone flux with different factors that might account for differences in leaf de-toxification capacity. The annual cumulated AF$_{st}$1.6 values were either modified based on leaf thickness or based on the specific leaf area (SLA). Neither one of these modifications resulted in substantial improvements in the linear regression analyses, using method 1 (data not shown).

Evidence for the significance of leaf morphology for ozone sensitivity was derived from a re-analysis of the data from Pääkkönen et al. (1997), were the difference in ozone sensitivity between different birch clones were investigated. A significant correlation (R^2, 0.51; p slope, <0.01) between leaf thickness and sensitivity score for leaf ozone injury indicated that the more ozone sensitive clones had thinner leaves (Fig. 5).

3.3. Evidence for the importance of ozone flux for the development of visible leaf injury

Schaub et al. (2003) examined the occurrence of leaf visible injury on black cherry (*Prunus serotina*) seedlings grown within open-top chambers and kept under two different irrigation regimes. They found that increased irrigation increased the stomatal uptake of ozone leading to more severe ozone induced leaf visible injury. Furthermore, the amount of leaf injury was better described by an ozone uptake index as compared to daylight, 7-h mean ozone concentrations (Fig. 6).

4. Discussion

When an analysis was made including several different broadleaved and coniferous tree species that were all rated as ozone sensitive, but with considerably different leaf morphology, then the stomatal AF$_{st}$ Y concept was indeed superior to the concentration based AOTX concept. The results were

Table 2
Statistical information from the linear regression analyses

	n	AFst1.6 (mmol m^{-2} PLA)				AOT40 (ppm h)			
		Slope	SE slope	*p* slope	r^2	Slope	SE slope	*p* slope	r^2
Method 1									
Sensitive	78	−1.228	0.089	<0.001	0.72	−0.417	0.043	<0.001	0.55
Tolerant	26	−0.286	0.071	<0.001	0.40	−0.149	0.034	<0.001	0.44
Method 2									
Sensitive	46	−1.172	0.156	<0.001	0.78	−0.425	0.084	<0.001	0.64
Tolerant	15	−0.317	0.061	<0.001	0.62	−0.162	0.039	<0.001	0.62

The exposure indices, AFst1.6 and AOT40, were expressed as annual values. The response parameter was annual % biomass reduction. PLA, projected leaf area. Method 1. Simple linear regression analysis, all data included, regression not forced through zero. Method 2. Linear regression analysis compensating for the non-homogenous variance (heteroscedasicity), no control datapoints included, regression forced through zero. There were no significant intercepts in any case.

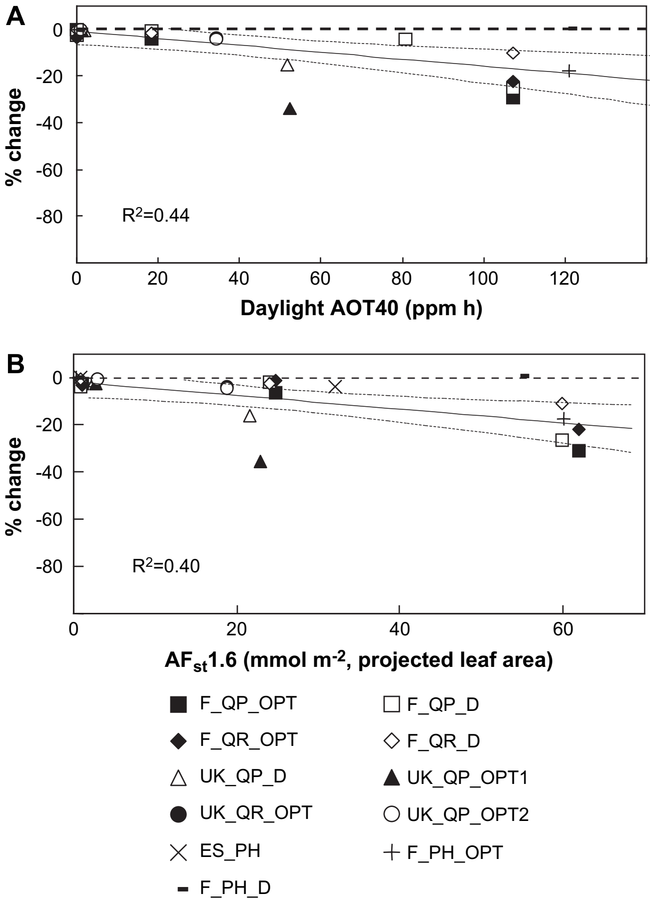

Fig. 2. Relationships between the annual biomass reduction and the annual ozone exposure expressed as daylight AOT40 (A) and accumulated stomatal flux of ozone ($AF_{st}1.6$) on a projected leaf area basis (B). The analysis was made using simple linear regression analysis including all data points and not forcing the regression through zero. The included species are those that were categorised as conifer less sensitive (crosses and points) and broadleaved less sensitive (open and closed symbols) (Karlsson et al., 2004b). For further explanations, see Section 2. The coefficient of determination (R^2) from linear regression analysis and the 99% confidence limits are shown in the figure. The legend for the different data sets can be found in Table 1. The detailed information on the regression analysis was for figure (A) $y = -0.149x - 1.248$, $R^2 = 0.44$, p slope $= 0.0002$ and for figure (B) $y = -0.286x + 1.429$, $R^2 = 0.40$, p slope $= 0.0005$.

similar for both linear regression analysis methods that were applied. It is evident that this result depended to a large extent on the differences in the values for maximum conductance used when simulating the ozone flux. The values for maximum conductances used were 155 mmol H_2O m^{-2} PLA s^{-1} for Norway spruce and Scots pine and 220 mmol H_2O m^{-2} PLA s^{-1} for beech and birch (Karlsson et al., 2004b). The result found in the present study is in line with what was suggested by Reich (1987), almost 20 years ago. He stated that differences in ozone sensitivity between species could be predicted to a large extent based on differences in the leaf conductance. The analysis made in this study showed that the flux concept was not superior to the AOTX concept for broadleaf and coniferous tree species which were rated less ozone sensitive. That the flux- and concentration-based methods gave about the same results for non-sensitive species is perhaps to be expected since the sensitivity of these species may be more strongly determined by the internal detoxification than the exposure.

As mentioned in the section about statistical methods, there was a potential problem with the statistical method 1, that the variance in the residuals was not evenly distributed along the *x*-axis. Furthermore, there were a few data points to the far right of the *x*-axis. The influence of this problem on the main results of the regression analysis was, to some extent, tested by removing the two data sets with the highest exposure values in the sensitive species data set. The data sets that were removed from the analysis was the one with *Betula pendula* from Birmensdorf (CH_BP, see Table 1) and the one with *Picea abies* from France (F_PA). The results from this new regression analysis of the sensitive species data set, using method 1, were essentially the same as for the original analysis, even though the R^2 values decreased somewhat for both regressions. The R^2 values for this new analysis were 0.53 when $AF_{st}1.6$ was used as the ozone exposure index and 0.23 when AOT40 was used. Thus, removing the experimental data with the highest ozone exposure values did not change the main conclusion, that $AF_{st}1.6$ was superior to the AOT40 concept for explaining ozone impacts on the biomass response of sensitive tree species.

Based on this as well as on other published studies, four different cases may be distinguished for the comparison of

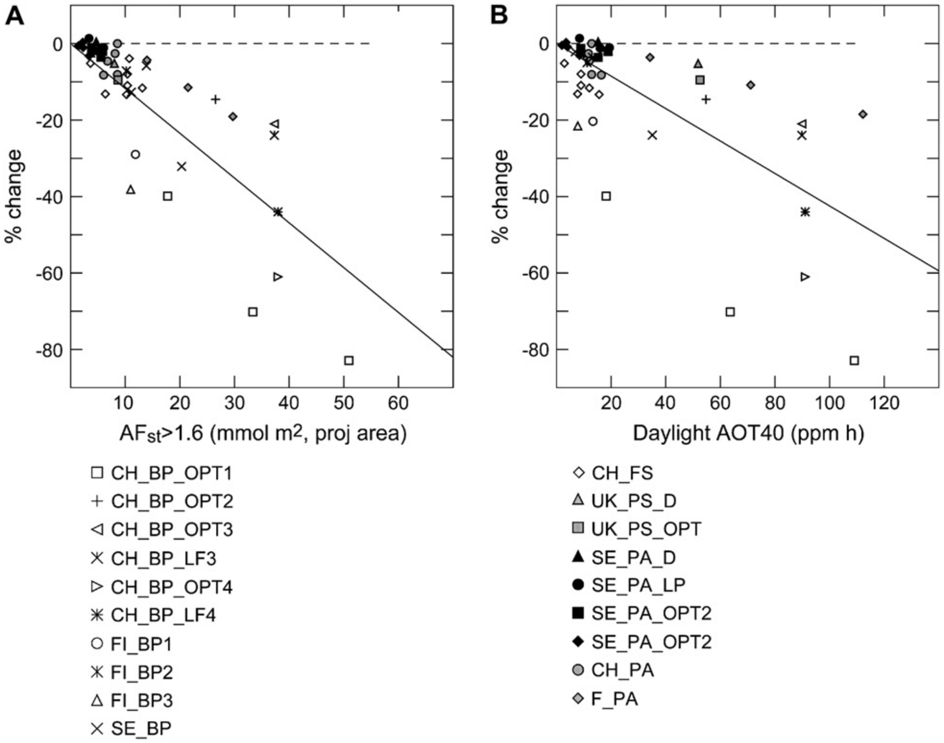

Fig. 3. Relationships between the annual biomass reduction and the accumulated stomatal flux of ozone ($AF_{st}1.6$) on a projected leaf area basis (A) and the annual ozone exposure expressed as daylight AOT40 (B). The analysis was made using a linear regression analysis that corrected for the non-homogenous variance, excluding all data points from the control treatments and forcing the regression through zero. The included species are those that were categorised as broadleaved sensitive (open symbols, cross and points) and coniferous sensitive (closed symbols with black or grey) (Karlsson et al., 2004b), with additional data sets with *Betula pendula* from experiments performed at Birmensdorf. For further explanations, see Section 2. The legend for the different data sets can be found in Table 1. The detailed information on the regression analysis was for figure (A) $y = -1.172x$, $R^2 = 0.78$, p slope <0.001 and for figure (B) $y = -0.425x$, $R^2 = 0.64$, p slope <0.001.

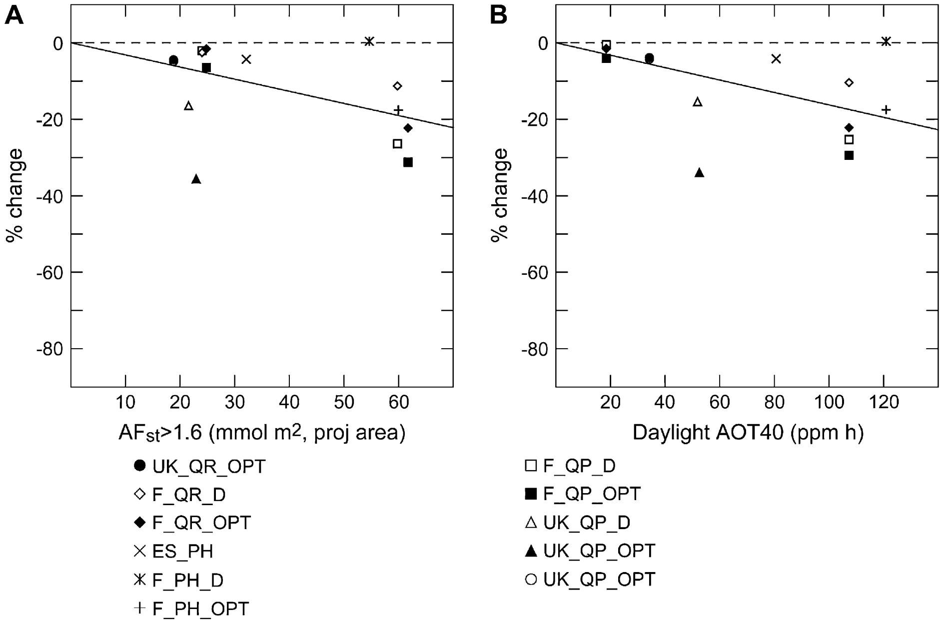

Fig. 4. Relationships between the annual biomass reduction and the accumulated stomatal flux of ozone ($AF_{st}1.6$) on a projected leaf area basis (A) and the annual ozone exposure expressed as daylight AOT40 (B). The analysis was made using a linear regression analysis that corrected for the non-homogenous variance, excluding all data points from the control treatments and forcing the regression through zero. The included species are those that were categorised as conifer less sensitive (crosses and points) and broadleaved less sensitive (open and closed symbols) (Karlsson et al., 2004b). For further explanations, see Section 2. The legend for the different data sets can be found in Table 1. The detailed information on the regression analysis was for figure (A) $y = -0.317x$, $R^2 = 0.62$, p slope <0.001 and for figure (B) $y = -0.162x$, $R^2 = 0.62$, p slope <0.001.

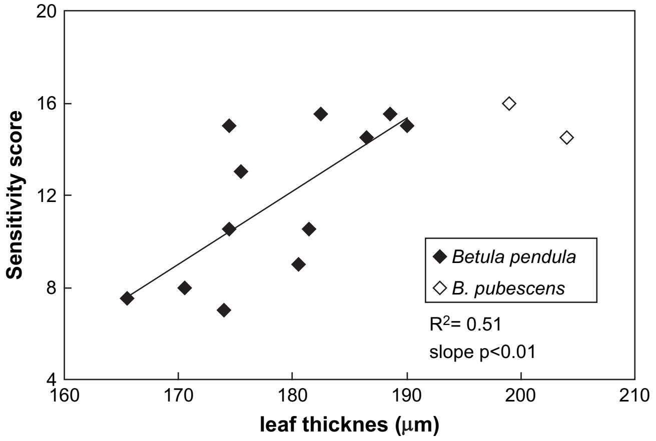

Fig. 5. The relation between ozone sensitivity and leaf thickness in different clones of *Betula pendula* and *B. pubescens* exposed to ambient and elevated ozone concentrations during two growing seasons in Finland. Ozone sensitivity was assessed with regard to above ground biomass, leaf biomass, visible leaf injury and pre-mature leaf senescence (yellowing). For further explanations, see Section 2. The regression analysis was made for the *B. pendula* values only. The detailed information on the regression analysis was $y = 0.319x - 45.2$, $R^2 = 0.51$, p slope $= 0.009$.

flux- and AOTX-based ozone indices to describe effects on biomass under experimental conditions (Table 3). When analyses were made separately on one single tree species, or on one category with similar species, and when one single value was used for the maximum conductance within each species, then the flux concept was not superior to the AOTX concept. This was the case even when data from different experimental sites across Europe were included (Karlsson et al., 2004b). Several experiments were included in this latter analysis where different ozone treatments were applied in combination with severe drought stress conditions. This indicated that variations in weather conditions and soil water availability between different growing seasons and/or sites, were not large enough to make $AF_{st}Y$ superior to AOTX, at least not for the experimental data sets that were available for this study. Alternatively, in the case of *Pinus halepensis* and *Quercus* sp., some problems regarding the parameterisation of the stomatal conductance model may remain since a relatively similar ozone uptake was estimated for the well-watered and drought-stressed saplings of these species (Karlsson et al., 2004b). When an analysis was conducted on one single tree species (*Betula pendula*) with experiment and site specific values for $g_{\max}$ and g_{dark} (g_{dark} was used as the minimum conductance measured in darkness), the flux concept was superior to the AOTX concept (Uddling et al., 2004). These findings indicated that the variation in $g_{\max}$ between different growth conditions and/or provenances is an important factor to be considered. The analysis made in the present study showed that the stomatal ozone flux concept was indeed superior to the AOTX concept for ozone sensitive species with considerably different leaf morphology. However, an analysis of broadleaf and coniferous tree species which were considered less ozone sensitive, showed that the flux concept was again not superior to the AOTX concept, most likely due to differences in de-toxification capacity.

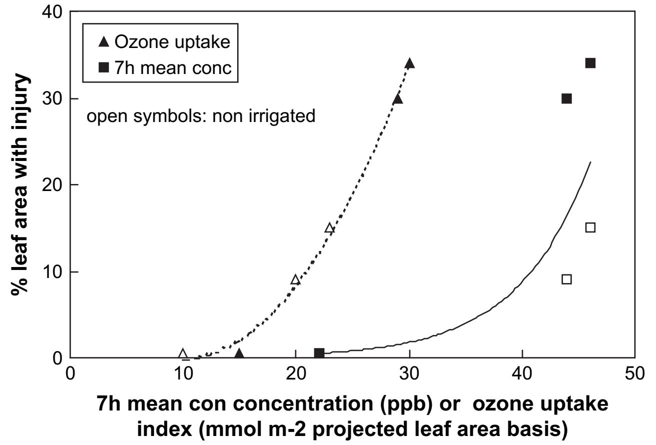

Fig. 6. The amount of ozone induced visible leaf injury on *Prunus serotina* (black cherry), grown under two different irrigation regimes. The amount of injury was correlated with two different ozone indices, the 7-h mean ozone concentration during day-time (squares) and an ozone uptake index calculated as the product of seasonal average stomatal conductance (projected leaf area basis) and seasonal cumulative ozone exposure (SUM0) (triangles). Closed symbols represent high irrigation, open symbols low (ambient) irrigation. Trendlines are included for illustrative purposes only. Data are recalculated from Schaub et al. (2003).

It has been suggested that the expression of the ozone uptake could, to some extent, be improved by taking into account the de-toxification capacity of the leaves (Musselman et al., 2006). A greater ratio of mesophyll tissue to surface area may result in reduced ozone sensitivity (e.g. Wieser et al., 2002). Attempts to modify the accumulated ozone uptake with factors reflecting the de-toxification capacity, such as leaf thickness or SLA, were not successful in the present study. However, a re-analysis of previously published data on the ozone impacts on different clones of silver birch (Pääkkönen et al., 1997) showed that the more ozone sensitive clones of silver birch had thinner leaves, as also reported for aspen (*Populus tremuloides*) (Oksanen et al., 2001). On the other hand, Gerosa et al. (2003) found a greater extent of ozone-induced leaf visible injury in European ash (*Fraxinus excelsior*) as compared to European beech (*Fagus sylvatica*), even though ash had thicker leaves. They explained the greater ozone sensitivity of ash with its larger stomatal conductance in combination with a lower leaf density resulting in a more efficient gas exchange, as compared to beech.

The studies investigating the development of leaf visible injury induced by ambient ozone exposures provide additional support to the superiority of flux-based ozone indices to explain ozone impacts on young trees under experimental conditions. Schaub et al. (2003) found that increased irrigation increased the stomatal uptake of ozone as well as the amount of ozone induced leaf visible injury. Furthermore, the amount of leaf injury could be better correlated with an ozone uptake index as compared to the daylight, 7-h mean ozone concentrations (Fig. 6). Similar results have been reported by Gerosa et al. (2006).

All experimental data that were used in the present study were derived from experiments with young, juvenile trees while the objective for the ozone critical levels used within

Table 3
Different cases for the comparison of $AF_{st}Y$- and AOTX-based ozone indices to be used to describe effects on trees under experimental conditions

Case	Species investigated/reference	$AF_{st}Y$–AOTX comparison
One single tree species, or species category, a standard value for g_{max}	Norway spruce, Karlsson et al., 2004a,b; Braun et al., 2003 Silver birch, Uddling et al., 2004 *Quercus coccifera*, Elvira et al., 2004	$AF_{st}Y$ not superior to AOTX
One single tree species, experiment and site specific values for g_{max} and g_{dark}	Silver birch, Uddling et al., 2004	$AF_{st}Y$ superior to AOTX
Sensitive broadleaved and coniferous tree species analysed together, standard value for g_{max} within each species	Norway spruce, Scots pine, silver birch, European beech. Table 2	$AF_{st}Y$ superior to AOTX
Less sensitive broadleaved and coniferous tree species, standard value for g_{max} within each species	Aleppo pine, Oak sp. Table 2	$AF_{st}Y$ not superior to AOTX

the LRTAP convention is to protect mature forest trees against the risks for negative ozone impacts. The relative ozone sensitivity between juvenile and mature trees remains a controversial issue. There are no fundamental changes in the foliar defensive mechanisms against oxidant injury during tree maturation (e.g. Kolb and Matyssek, 2001). Leaves of mature trees may have lower ozone uptake per unit leaf area than leaves of juveniles under some environmental conditions, but they may also have greater ozone uptake under other conditions. Thus, the relative leaf responses to ozone, comparing mature and juvenile trees, may vary between species (Kolb and Matyssek, 2001). It has been demonstrated that experimentally elevated ozone concentrations at the Kranzberg Forest in southern Germany can affect mature trees of beech and Norway spruce at the cellular, leaf and whole-tree level (Nunn et al., 2005). Furthermore, in the Kranzberg experimental study, a statistically significant, negative influence of the elevated ozone concentrations on the stem increment growth of mature Norway spruce trees was also demonstrated (Wipfler et al., 2005). Similarly, Karlsson et al. (2006) found that differences in ambient ozone exposure between years could be correlated with reductions of stem increment growth of mature Norway spruce trees in southern Sweden. At the same site, ozone specific symptoms were demonstrated on the needles of mature Norway spruce trees using light and electron microscopy methods (Kivimäenpää et al., 2004). Also, negative effects of ambient ozone exposure on the growth of mature beech trees have been demonstrated statistically (Braun et al., 1999). In the case of Scots pine, slightly elevated ozone concentrations have been shown to considerably reduce the rate of photosynthesis of 30-year-old trees after 3 years of exposure in open-top chambers in Finland (Kellomäki and Wang, 1997). Hence, there exists a considerable amount of evidence in the literature that mature trees can be negatively affected by ambient or slightly elevated ozone concentrations in Europe.

5. Conclusions

The results presented in this study support the hypothesis that impacts on biomass, as well as on leaf visible injuries, could be better explained by the accumulated stomatal ozone flux, $AF_{st}1.6$, as compared to the concentration based index, i.e. AOT40, for young trees of species that were considered as ozone sensitive and that were exposed under experimental conditions. Regarding young trees of species, which are considered to be less ozone sensitive, the flux concept was not superior to AOT40 for explaining biomass reductions. Leaf thickness could be one factor that contributed to the differences in the ozone sensitivity between different species and varieties, as indicated by studies of different Silver birch clones. Incomplete understanding of the internal exposure–response relationships, as well as perhaps some remaining problems regarding the current parameterisation of the stomatal conductance model, do not as yet allow the flux concept to be applied to all forest tree species across Europe.

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