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Test of the short-term critical levels for acute ozone injury on plants—improvements by ozone uptake modelling and the use of an effect threshold

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Abstract

The current short-term critical levels for acute ozone injury on plants were evaluated based on 32 datasets from eastern Austria, Belgium and southern Sweden with subterranean clover (*Trifolium subterraneum* L., cv. Geraldton). Potential improvements using an exposure index related to ozone uptake (AF_{st}, Accumulated Stomatal Flux), a modified accumulated exposure over the threshold (mAOT) exposure index and the introduction of an effect threshold in the short-term critical level were investigated. The existing short-term critical levels did not accurately describe the effects in terms of observed visible injury. Using a mAOT based on solar radiation and vapour pressure deficit (VPD) improved the explanation of observed visible injury. However, using a simple stomatal conductance model, driven by solar radiation, air temperature, VPD and ozone uptake, the correlation between modelled and observed effects were considerably improved. The best performance was obtained when an ozone uptake rate threshold of $10\,\mathrm{nmol\,m^{-2}\,s^{-1}}$ ($AF_{st}10$, per unit total leaf area) was used. The results suggested the use of an effect threshold of 10% leaf injury in order to minimise the risk of erroneously recorded visible injury due to observation technique or other injuries hard to distinguish from ozone injury. A new, AF_{st} based exposure index was suggested, an ozone exposure of $AF_{st}10 = 75\,\mu\mathrm{mol\,m^{-2}}$ during an exposure period of eight days was estimated to prevent more than 10% visible injury of the leaves. This study strongly suggests that a simple model for ozone uptake much better explains observed effects, compared to the currently used exposure index AOT40. However, if a lower degree of complexity, data requirements and also a lower extent of explanation of observed effects are to be considered a new short-term critical level, based on a mAOT may be suggested: a mAOT30 of 160 ppb h during an exposure period of 8 days is estimated to protect the leaves from visible injury on more than 10% of the leaves.

Keywords: AOT40; Clover; *Trifolium subterraneum*; Visible injury; Ozone effects; Accumulative stomatal flux (AF_{st}); Modified AOT (mAOT)

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1. Introduction

One of the first effects by ground-level ozone to be observed in plants was visible leaf injury. Already around 1950 such effects were established in California (Middleton et al., 1950) and certain cultivars of Tobacco (*Nicotiana tabacum*), were used as bioindicators of

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phytotoxic ozone already in the 1950s in the US (Heggestad, 1991).

Later, starting mainly in the 1970s, visible leaf injury by ozone was also considered in Europe (e.g. Jacobsen, 1977; Ashmore et al., 1978). Within the Convention on Long-Range Transboundary Air Pollution (CLRTAP), the co-operative programme ICP-Vegetation was set up by the end of the 1980s. Soon after that, ozone sensitive clovers were used in the experimental programme of ICP-Vegetation over Europe. For a number of years, the clover species *Trifolium subterraneum* L., cv. Geraldton, was used as the core experimental bioindicator plant by ICP-Vegetation (Benton et al., 1995).

Based on the experiences obtained, mainly with *T. subterraneum,* within ICP-Vegetation, short-term critical levels for acute injury of ozone on plants were defined at the United Nations Economic Commission for Europe (UNECE) workshop in Kuopio in 1996 (Benton et al., 1996; Kärenlampi and Skärby, 1996) and were included in the Mapping Manual of the CLRTAP (Umweltbundesamt, 1996). These critical levels values were intended to assess the risk for acute ozone injury on sensitive plants. The only adjustment to different climatic conditions, which potentially have strong effects on ozone uptake via their influence on stomatal conductance, was the use of a cut-off level for air water vapour pressure deficit (VPD) as an average 09:30 until 16:30. The current short-term critical levels are:

> 500 nmol mol^{-1} h accumulated exposure over the threshold 40 mmol mol^{-1} ozone (AOT40) for 5 days with a mean VPD above 1.5 kPa (9:30–16:30),
> 200 nmol mol^{-1} h (AOT40) for 5 days with a mean VPD below 1.5 kPa (9:30–16:30).

Since visible ozone injury represents the most direct evidence of the harmfulness of presently occurring, elevated ozone concentrations, the short-term critical levels represent an important instrument for environmental monitoring and for the policy process. Although visible leaf injury in sensitive plants do not necessarily represent as serious economic damage to vegetation, similar to crop loss or impaired forest growth except for some horticultural crops, it consists of an impact category which can be easily demonstrated and thus used to highlight the problem of phytotoxic ozone to a broad audience (Klumpp et al., 2002).

The aims of the present investigation were: (1) to test the current short-term critical levels using data from experiments with *T. subterraneum* from three different countries in Europe, (2) to test if the performance of the short-term critical levels can be improved by using a simple stomatal conductance model to estimate leaf ozone uptake, (3) to test the performance of a modified AOT (mAOT) approach and (4) to investigate the relevance of including an effect threshold in the short-term critical level, which is lacking in the currently used concept.

2. Materials and methods

2.1. Clover experiments

This paper is based on experiments performed with subterranean clover, *T. subterraneum* cv. Geraldton, in eastern Austria, Belgium and southern Sweden. In total 32 data sets from experiments conducted between 1991 and 1995 were used. All experiments were performed within the ICP Vegetation and seeds were provided by the coordinating centre. All countries used the same experimental protocol where growth and harvest methods were described in detail. Basic data from the experiments are presented in Table 1. As can be inferred from the table, the temperatures and VPD values were on average higher in Austria, but also in Belgium, compared to Sweden. The range of observed visible ozone injury on the clover leaves was large in all three countries. Visible injury was assessed manually at harvest by classing the leaves as injured or not injured. The percent injured leaves of total number of leaves for the different experiments are presented in Table 1. Ozone concentrations were monitored nearby the experiments. For Sweden, a number of open-top chamber studies with *T. subterraneum* were included. The results from the Swedish experiments in 1991 and 1992 have been published earlier in a different context (Pihl Karlsson et al., 1995). Ozone concentration and wind speeds were observed in different heights in the different sites. All ozone concentrations and wind speeds were recalculated to 1 m level based on principles suggested by Pleijel (1998) and Tuovinen (2000). The values of ozone concentrations and wind speeds in Table 1 have been recalculated.

2.2. Ozone exposure indices

The following ozone exposure indices were tested: AOT (accumulated exposure over a threshold concentration based on hourly averages of the ozone concentration, Kärenlampi and Skärby, 1996) calculated as nmol mol^{-1} h, using concentration cut-offs from 0 nmol mol^{-1} up to 60 nmol mol^{-1} with 10 nmol mol^{-1} steps, accumulated stomatal flux (AF$_{st}$) in μmol m^{-2} per unit total leaf area using 12 different cut-offs: 0, 0.5, 1, 2, 3, 4, 6, 8, 9, 10, 11, 12, 13 and 14 nmol m^{-2} s^{-1} and modified AOT (mAOT) where mainly the influence of solar radiation and air water VPD were considered. Also the duration of the relevant exposure period (range: 1–21 days) and the effect of including a lag period (range: 0–7 days) between the end of the exposure period and the day of observation of visible injury were tested.

Table 1
Average levels of visible injury at harvest (injured leaves in percent of total number of leaves), ozone concentrations (recalculated to 1 m height) and climate variables (daylight hours): wind speed (recalculated to 1 m height), PAR, Temperature, Water VPD for the different experiments. The averages represents the period from 1 week after emergence until analysis

Experiment	Visible injury (%)	Ozone (nmol mol^{-1})	Wind speed (m s^{-1})	PAR (μmol m^{-2} s^{-1})	Temperature (°C)	VPD (kPa)
Sweden:1991:1:AA	22.40	39	1.30	608	18.9	0.71
Sweden:1991:2:AA	7.90	33	1.47	551	16.9	0.54
Sweden:1992:1:AA	9.47	30	1.68	632	19.6	0.83
Sweden:1994:1:AA	8.37	34	1.35	692	17.3	0.73
Sweden:1994:2:AA	45.54	47	1.15	821	23.6	1.42
Sweden:1995:1:AA	1.78	30	1.25	670	17.5	0.51
Sweden:1995:2:AA	18.51	31	1.13	841	21.0	0.79
Sweden:1995:3:AA	5.48	27	1.60	521	16.0	0.52
Austria:1994:1:AA	0.05	25	1.27	1022	22.5	1.25
Austria:1994:2:AA	7.41	33	0.85	1030	24.8	1.60
Austria:1994:3:AA	1.10	32	0.93	795	21.7	1.03
Austria:1995:1:AA	0.20	28	0.97	951	20.6	0.96
Austria:1995:2:AA	22.87	36	1.10	1032	26.2	1.71
Austria:1995:3:AA	5.06	30	0.92	877	23.0	1.29
Austria:1995:4:AA	1.32	35	1.02	1058	26.0	1.81
Austria:1995:5:AA	10.22	26	1.08	677	19.6	0.85
Belgium:1992:1:AA	1.47	33	2.75	742	23.3	1.21
Belgium:1992:2:AA	14.09	20	2.49	599	20.4	0.71
Belgium:1992:3:AA	2.67	14	3.08	517	14.9	0.51
Belgium:1993:1:AA	9.64	24	2.44	574	20.0	0.97
Belgium:1993:2:AA	0.00	22	2.71	538	20.1	1.06
Belgium:1994:3:AA	0.20	15	3.32	442	18.8	0.74
Belgium:1995:1:AA	14.67	38	2.40	1011	22.4	0.90
Belgium:1995:2:AA	29.24	49	2.44	946	24.9	1.34
Sweden:1991:3:OTC	1.60	9	—	466	19.3	0.57
Sweden:1991:4:OTC	11.50	24	—	466	19.3	0.57
Sweden:1992:2:OTC	9.68	9	—	531	20.5	0.82
Sweden:1992:3:OTC	32.99	17	—	531	20.5	0.82
Sweden:1992:4:OTC	4.49	9	—	399	17.1	0.37
Sweden:1992:7:OTC	6.93	23	—	399	17.1	0.37
Sweden:1992:10:OTC	28.17	36	—	399	17.1	0.37
Sweden:1992:13:OTC	46.52	48	—	399	17.1	0.37

AA = Ambient Air; OTC = Open-Top Chamber

2.3. Stomatal conductance model

A limited number ($n = 110$) of stomatal conductance measurements were available from a Swedish experiment performed in 2000 which included different ozone levels in open-top chambers (Pihl Karlsson et al., 2002), as well as from additional measurements in the ambient air from 2001, to parameterise a simplified, multiplicative, Jarvis-type stomatal conductance model (Jarvis, 1976). The stomatal conductance measurements were made using a Li-Cor 6200 portable exchanges system (Pihl Karlsson et al., 2002). As a basis for the parameterisation the general concept suggested by Emberson et al. (2000) was used. Values for maximum ($g_{\max}$) and minimum ($g_{\min}$) conductance per unit total leaf area were extracted from the data set, which included night-time measurements. The parameterisation of the stomatal conductance model is presented in Fig. 1.

The model assumed that the stomatal conductance could be described by multiplicative functions of different factors acting independently. The factors in the model are expressed in relative terms and vary between 1 and 0. If the factors are 1 they do not influence the conductance, and if they are <1 they modify $g_{\max}$ negatively.

The stomatal conductance g_s, was simulated with the following factors:

$$g_s = g_{\max}\, g_{\text{pot}}\, g_{\text{rel}}, \tag{1}$$

$$g_{\text{rel}}, = (g_{\text{temp}}\, g_{\text{light}}\, g_{\text{ozone}}\, g_{\text{VPD}}) \text{ if } g_{\text{rel}} > g_{\min}$$
$$\text{otherwise } g_{\text{rel}} = g_{\min}, \tag{2}$$

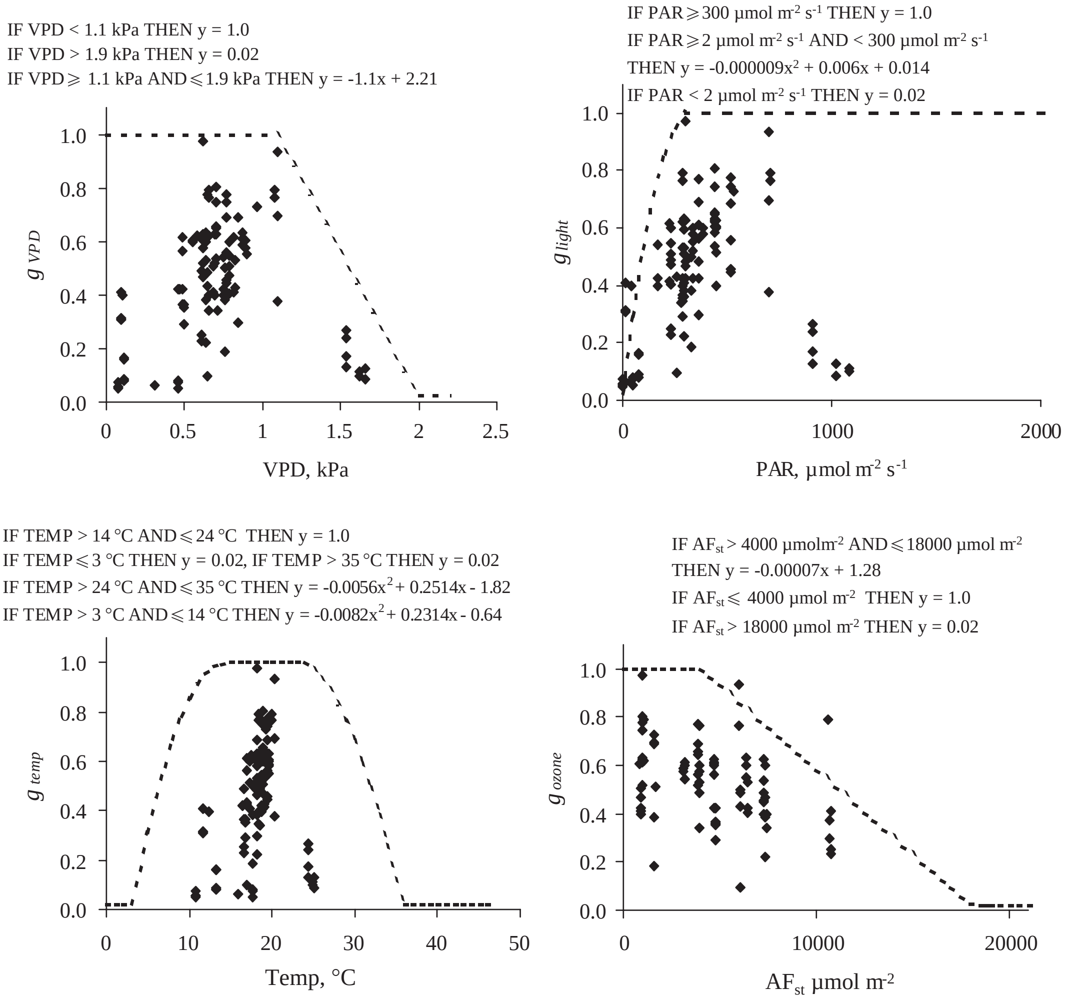

Fig. 1. Boundary lines including equations and stomatal conductance measurements in relation to the four stomatal conductance modifying factors included in the simple Jarvis type multiplicative model used for *T. subterraneum*: VPD (air water VPD), light (based on PAR, photosythetically active radiation), air temperature and accumulated stomatal flux of ozone AF_{st}.

where g_s is the stomatal conductance in mmol $H_2O\,m^{-2}$ (total leaf area) s^{-1}, g_{pot} represents phenological changes during leaf life span, g_{temp} represents the influence by air temperature (°C), g_{VPD} by water VPD of the air (kPa), g_{light} represents the effect by solar radiation expressed as the photosynthetically active radiation (PAR) ($\mu mol\,m^{-2}\,s^{-1}$) and g_{ozone} modifies g_{max} by ozone described by the AF_{st} ($\mu mol\,m^{-2}$) calculated from one week after emergence of the first trifoliate leaf until 1 h before the hour that is being calculated.

The uptake of ozone into the leaf was calculated from the ozone concentration and the stomatal conductance but also by the leaf boundary layer conductance. The value of the boundary layer conductance to heat transfer ($mm\,s^{-1}$) was given by (Jones, 1992)

$$g_{aH} = 6.62\,(u/d)0.5 \tag{3}$$

and was recalculated for ozone. In Eq. (3) d is the characteristic dimension (m) and u is the wind velocity ($m\,s^{-1}$). In this study d was taken to be 0.01 m for the clover leaves. The conductance for water vapour was converted to conductance for ozone by dividing with the factor 1.65. This factor takes into account the differences in molecular diffusivity between water and ozone.

The mAOT was calculated each hour by multiplying the ozone concentration with the VPD-factor (g_{VPD}) developed for the AF_{st} model. Then the mAOT was accumulated for the different exposure periods

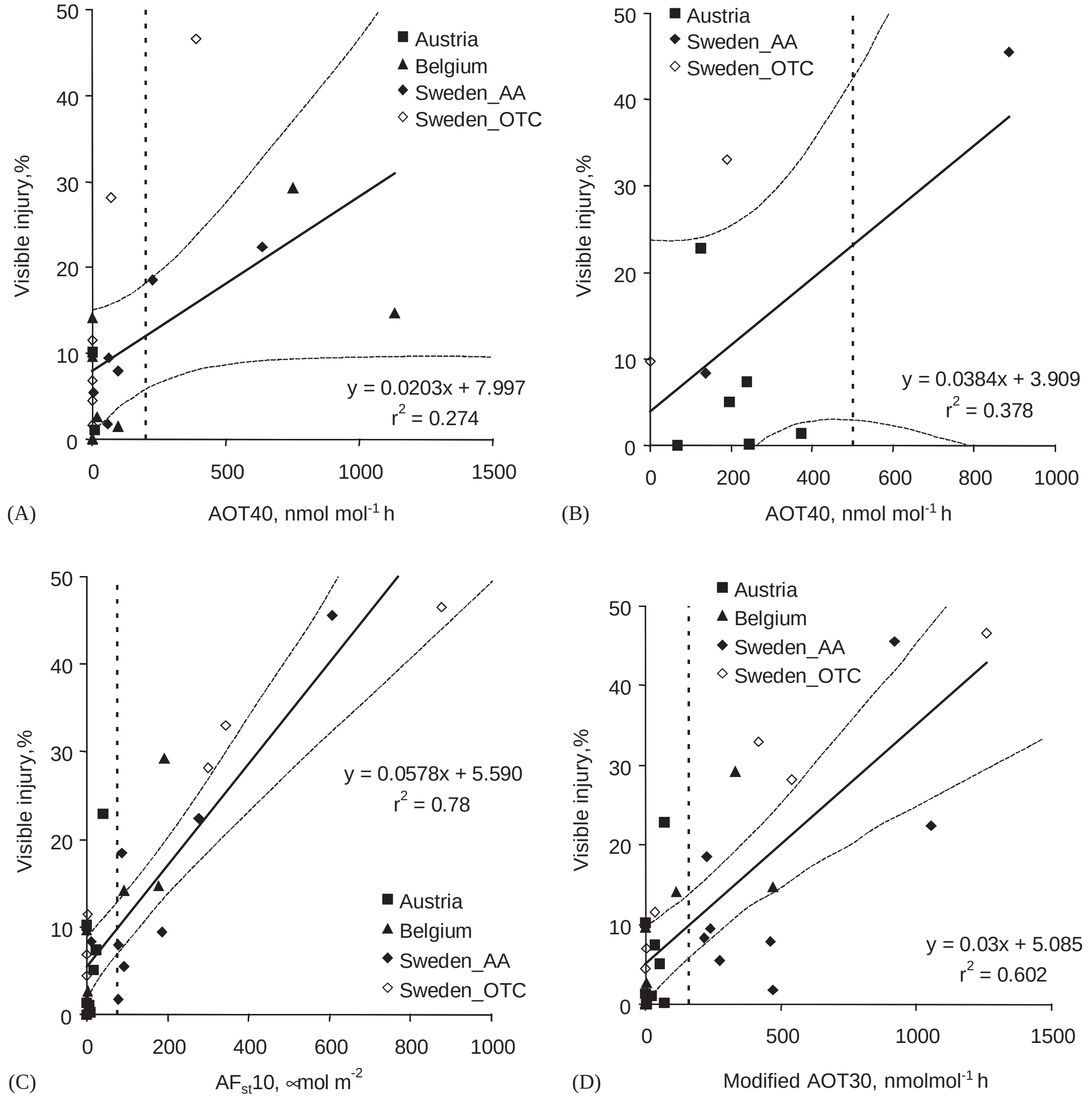

Fig. 2. The currently used short-term critical level, AOT40 accumulated over 5 days with VPD $<1.5\,\text{kPa}$ (09:30–16:30), 22 datasets (A). The currently used short-term critical level, AOT40 accumulated over 5 days with VPD $>1.5\,\text{kPa}$ (09:30–16:30), 10 datasets (B). The extent of visible injury plotted versus the best flux-based index $\text{AF}_{\text{st}}10$ (C). The AF_{st} period was 8 days before observation of visible injury. The extent of visible injury plotted versus mAOT30 (D). The accumulation period was 8 days before observation of visible injury. The period of 8 days represents the exposure period that performed best in the statistical analysis and the period of 5 days represent the current short-term critical level. The accumulation was made during daylight hours (Global radiation $>50\,\text{W}\,\text{m}^{-2}$). In all figures, the 99% confidence interval is included. The dotted vertical line in the A and B represents the two current short-term critical levels. The dotted vertical line in C represents the suggested new short-term critical level for AF_{st} and the dotted vertical line in D represents the suggested new short-term critical level for mAOT30.

with two different considerations of solar radiation: firstly as the current short-term critical levels where global radiation $>50\,\text{W}\,\text{m}^{-2}$ and secondly by multiplying the VPD-modified ozone concentrations each hour by the light-factor (g_{light}) from the AF_{st} model.

3. Results

3.1. Stomatal conductance model

The parameterisation of the stomatal conductance model regarding the multiplicative factors g_{VPD}, g_{light},

g_{temp} and g_{ozone} is presented in Fig. 1. For the temperature factor it was not possible to strictly use the boundary line technique because the most stomatal conductance measurements were performed in a narrow range of temperatures. Therefore, a broader function was used. The highest measured stomatal conductance value was 585 mmol $H_2O\,m^{-2}\,s^{-1}$. In line with this $g_{\max}$ was set to 600 mmol $H_2O\,m^{-2}\,s^{-1}$. The lowest observed conductance values were approximately 2% of the maximum values and consequently $g_{\min}$ was set to 0.02. The g_{pot} factor was set to 0.4 the first week after emergence of the first trifoliate leaf and at later stages to 1.0, based on the observation that newly developed leaves initially had substantially lower (approximately 50%) of the conductance of fully developed leaves. In practice, this factor had little influence since the best correlations between effect and ozone exposure was obtained for relatively short exposure periods before observation of leaf injury, not including the period during which many leaves were less than one week old. AF_{st}, using no cut-off, calculated for the period after the first week (after emergence of the first trifoliate leaf) until the hour before the plants were checked for visible injury, was used to define g_{ozone}. The g_{ozone} factor was developed based on stomatal conductance measurements in the open-top chamber experiment in Sweden, 2001.

3.2. *Test of the current critical levels*

The result of the test of the current short-term critical levels is presented in Figs. 2A and B. For the case with VPD <1.5 kPa, a weak relationship between visible injury and AOT40 during 5 days was obtained (Fig. 2A). When situations with VPD >1.5 kPa were considered (Fig. 2B) a weak relationship was obtained between visible injury and AOT40.

3.3. *Test of alternative exposure indices*

The exposure indices tested, which provided the best fit with the observed extent of visible injury (% injured leaves) were $AF_{st}10$ using an exposure period of 8 days and no lag period. The correlation coefficient was 0.78. The relationship between visible injury and $AF_{st}10$, during 8 days before observation of visible injury is shown in Fig. 2C. Different non-linear relationships were tested but the correlation coefficient for those analyses were <0.78. Table 2 presents the different correlation coefficients and the percent injury at index = 0 with the different thresholds for AF_{st}, current AOT critical levels and mAOT30 and mAOT40.

The best-performing AOT-index was one of the AOT30 indices: AOT30 during 5 days before observation of visible injury (VPD <1.5 kPa, ozone exposure accumulated during daylight hours) having a correlation coefficient of 0.58. Generally, the use of a flux threshold or cut-off concentration for the AF_{st} and AOT indices, improved the correlation with observed effects up to approximately 9–10 nmol $m^{-2}\,s^{-1}$ and 30 nmol mol^{-1}, respectively. At higher ozone uptake thresholds or cut-offs for the AOT indices the correlation between observed visible injury and ozone exposure declined.

Table 2
Correlation coefficients and the percent injury at index = 0 for AF_{st} with the different thresholds, current AOT critical levels and mAOT30 and mAOT40

Index (length of period)	r^2	% Observed injury at index = 0
$AF_{st}0$ (8 days)	0.33	—
$AF_{st}0.5$ (8 days)	0.34	—
$AF_{st}1$ (8 days)	0.36	—
$AF_{st}2$ (8 days)	0.39	—
$AF_{st}3$ (8 days)	0.44	—
$AF_{st}4$ (8 days)	0.50	4
$AF_{st}6$ (8 days)	0.63	4
$AF_{st}8$ (8 days)	0.73	10
$AF_{st}9$ (8 days)	0.77	10
$AF_{st}10$ (8 days)	0.78	10
$AF_{st}11$ (8 days)	0.76	12
$AF_{st}12$ (8 days)	0.70	12
$AF_{st}13$ (8 days)	0.63	23
$AF_{st}14$ (8 days)	0.57	23
$AF_{st}15$ (8 days)	0.53	28
$AF_{st}16$ (8 days)	0.51	29
AOT 40 VPD < 1.5 kPa	0.27	14
AOT 40 VPD > 1.5 kPa	0.38	10
MAOT0	0.25	No observations
MAOT30	0.60	10
MAOT40	0.64	23

The mAOT30 index explained 60% of the variation of the observed extent of visible injury (% injured leaves) accumulated during solar radiation $>50\,W\,m^{-2}$ using an exposure period of 8 days. The relationship between visible injury and mAOT30 during 8 days before observation of visible injury is shown in Fig. 2D. The relationship between visible injury and accumulated mAOT30 using the g_{light} factor, resulted in almost exactly the same relationship as between visible injury and accumulated mAOT30 using solar radiation $>50\,W\,m^{-2}$. The correlation coefficients for mAOT30 and mAOT40 are presented in Table 2. For the mAOT40 index, the distribution of data along the x-axis was very uneven, with a cluster of points at zero x, and, as a result, a very high degree of injury (23% according to the regression) associated with zero exposure.

Fig. 3 shows the relationship between the estimated ozone uptake (hourly values) above the threshold 10 nmol $m^{-2}\,s^{-1}$ and the ozone concentrations in the air, recalculated to 1 m height. Concentrations below

31.4 nmol mol^{-1} did not contribute to the index. Above this concentration, contributions will be obtained to the index AF$_{st}$10 depending on the estimated stomatal conductance. Quite generally, like in Figs. 2A and B, the use of a VPD cut-off in the different AOT indices, i.e. excluding the experiments with high average VPD, improved the correlation between visible injury and ozone exposure. Furthermore, it can be seen that the ozone uptake at similar concentrations was lower in the OTCs, as compared to ambient air. This was probably due to the fact that the boundary layer conductance were lower and thus restricted the uptake of ozone inside the OTCs than outside in the ambient air.

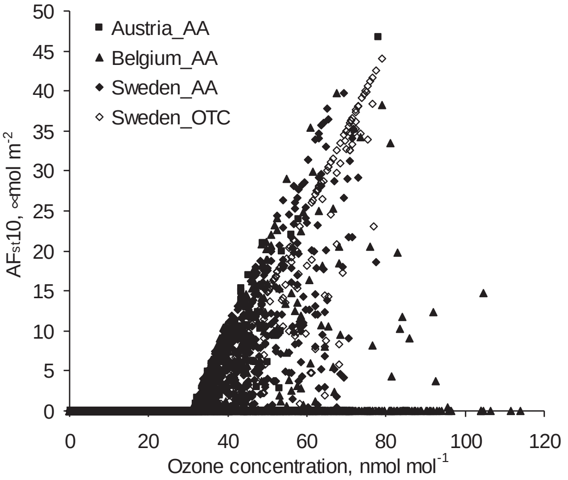

Fig. 3. Hourly AF$_{st}$10 values, µmol m^{-2}, in relation to hourly ozone concentrations (nmol mol^{-1}) for all 32 datasets included in the study. The ozone concentrations were recalculated to 1 m height.

Table 3 shows the percentage contribution to AF$_{st}$10 in different ozone concentration intervals for the different countries, for the AA and OTC treatments in Sweden and for all experiments. In Austria high ozone concentrations were associated with low conductance due to high VPD and temperature and most of the AF$_{st}$10 was accumulated in the ozone concentration range from 35 to 40 nmol mol^{-1}. High ozone concentrations were to a lesser extent linked to low conductance in Belgium compared to Austria. Here, ozone concentrations in the range from 60 to 90 nmol mol^{-1} contributed substantially to AF$_{st}$10. Despite substantially lower ozone concentrations, concentrations in range above 60 nmol mol^{-1} were more important for AF$_{st}$10 in the Swedish AA experiments compared to Austria, because of the more favourable conditions for stomatal opening. The large contribution to AF$_{st}$10 in the range of 71–80 nmol mol^{-1} in the Swedish OTC experiments was related to the addition of ozone in some of the chamber experiments.

3.4. Effect thresholds

As evident from the Figs. 2A and C, and to some extent also Fig. 2B, the range of observed visible injury at zero exposure was approximately from 0% up to 10% visible injury. This pattern did not depend on the particular choice of AF$_{st}$Y or AOTX as exposure indices, although at very low flux rate thresholds or cut-off concentrations, some of the observed injury levels below 10% were associated with a certain amount of ozone exposure. In these cases, however, the

Table 3
Percentage contribution to AF$_{st}$10 (8 days before observations of visible injury) and the current short-term critical level AOT40 with VPD below or above 1.5 kPa (5 days before observations of visible injury) in different ozone concentration intervals for the different countries, for all AA experiments included in the study

	Ozone concentration (nmol mol^{-1}) intervals										
	<30	30–35	36–40	41–45	46–50	51–60	61–70	71–80	81–90	91–100	>100
Austria (AF$_{st}$10) (8 exp)	0	13	60	18	9	0	0	0	0	0	0
Belgium (AF$_{st}$10) (8 exp)	0	0	9	14	19	18	14	9	16	0	0
Sweden (AF$_{st}$10) (8 exp)	0	4	11	10	12	28	26	9	0	0	0
All (AF$_{st}$10) (24 exp)	0	4	13	11	14	24	22	9	4	0	0
Austria (AOT40; VPD$<$1.5 kPa) (2 exp)	0	0	0	100	0	0	0	0	0	0	0
Belgium (AOT40; VPD$<$1.5 kPa) (8 exp)	0	0	0	1	4	13	8	14	41	19	0
Sweden (AOT40; VPD$<$1.5 kPa) (6exp)	0	0	0	11	19	43	24	3	0	0	0
All (AOT40; VPD$<$1.5 kPa) (16 exp)	0	0	0	5	9	24	15	9	26	12	0
Austria (AOT40; VPD$>$1.5 kPa) (6 exp)	0	0	1	18	36	31	14	0	0	0	0
Sweden (AOT40; VPD$>$1.5 kPa) (2exp)	0	0	0	11	12	22	41	12	0	0	0
All (AOT40; VPD$>$1.5 kPa) (8 exp)	0	0	0	15	25	27	27	6	0	0	0

correlation between observed effect and exposure was much weaker than in Fig. 2C. In Fig. 2, the different confidence intervals are shown and for both $AF_{st}10$ and mAOT30 the upper confidence limit at index = 0 was just below or at 10%.

4. Discussion

The purpose of the short-term critical levels is to ensure protection of all plants to acute ozone injury. The currently used short-term critical level refers to the occurrence of visible leaf injury during ozone episodes. The current critical levels is based on the ozone exposure of the plants during daylight hours expressed as AOT40.

The results of the present study clearly indicated that the current short-term critical levels do not ensure protection of all plants to acute ozone injury. There was no effect of harvest number on the results, i.e. the sensitivity of the plants did not change during the season. The correlation between observed visible injury and ozone exposure was weak for both cases with low and high VPD. This reflects the fact that the stepwise procedure using one AOT40 value below and another, 2.5 times larger value, above the VPD threshold does not accurately describe the exposure situation. When only Swedish data were used (Pihl Karlsson et al., 2003), AOT30, and to a lesser extent also AOT40, performed relatively well, at least for situations with VPD <1.5 kPa. This was however not the case when the data from three countries were combined, which reflects the influence of the more fundamental variation in climate from south Scandinavia with mainly relatively cool summers, over Belgium with a warmer, but still relatively humid climate of western Europe to eastern Austria with a much more arid, continental climate. The fact that AOT30 worked well in Sweden can probably be attributed to the fact the climatic variation was small compared to the climatic variation between the three countries. It is worth to note that the threshold $10\,\mathrm{nmol\,m^{-2}\,s^{-1}}$ in $AF_{st}10$ corresponds to ozone exposure starting to be added at a concentration slightly above $30\,\mathrm{nmol\,mol^{-1}}$ ozone. Thus, when visible ozone injury in subterranean clover is considered, a relation between AOT30 and $AF_{st}10$ exists, but if the stomatal limitation to ozone uptake is strong the contribution to $AF_{st}10$ will be very small or absent also in the concentration range immediately above $30\,\mathrm{nmol\,mol^{-1}}$.

A disadvantage of the current concept for the short-term critical level is the stepwise, discontinuous procedure around the threshold VPD value. One advantage in using an ozone uptake approach is that no such threshold is needed. The results are explained by one and the same model without non-realistic discontinuities. In many plants 1.5 kPa VPD, and according to the present study *T. subterraneum* is one of them, is just where the sensitivity of the stomatal conductance to VPD is largest. This was the background to the choice of this threshold, but the strong sensitivity of stomatal conductance to VPD values of this magnitude is also a reason why a strong discontinuity in the definition of the current short-term critical levels fails to explain observed effects. The importance of VPD for the explanation of observed effects in terms of visible injury was confirmed by Ribas and Peñuelas (2003). They also concluded that the VPD-factor are especially important in the Mediterranean region.

Ideally, a critical level should have a relatively simple definition to make mapping easy. The definition also needs to be sufficiently robust to apply to sensitive vegetation growing in a diverse range of climates. The latter is hard to achieve if not the stomatal limitation of ozone uptake is quantitatively taken into account. The larger complexity and larger data requirements of the AF_{st} approach have to be balanced against the closer correlation with observed effects.

It seems relevant to use a visible injury threshold in a new definition of the short-term critical level. This was shown already in the investigation by Pihl Karlsson et al. (1995) and was confirmed by Pihl Karlsson et al. (2003). The present investigation indicated that a 10% injury level will minimise the risk of erroneously concluding that ozone injury is present, due to observation technique or other injuries not caused by ozone but hard to distinguish from ozone injury.

Visible injury by ozone represents an important impact category which may easily be demonstrated and communicated (Klumpp et al., 2002). This supports the continued use, but also the revision, of the current short-term critical level. Based on the present study a new short term critical level, based on a simple model for stomatal conductance and visible ozone injury in *T. subterraneum* can be suggested: a $AF_{st}10$ of $75\,\mathrm{\mu mol\,m^{-2}}$ during an exposure period of 8 days is estimated to protect the leaves from visible injury on more than 10% of the leaves. The result of the study strongly suggests that an ozone uptake based exposure index for phytotoxic ozone much better explains observed effects of visible injury (% injured leaves) than the concentration based and currently used exposure index AOT40 in combination with a cut-off value for VPD. However, if a lower degree of complexity and data requirements are to be considered a relatively simple modification of AOT by considering solar radiation and by using a VPD-factor may be used. But it also has to be taken under consideration that the correlation with observed effects was about 20% lower when using the mAOT-approach compared to if the AF_{st}-approach was used. A new short-term critical level, based on such a modified AOT may be suggested: a mAOT30 of 160 ppb h during an exposure period of 8 days is estimated to protect the leaves from visible injury on more than 10% of the leaves.

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