

Final report for contract 2152/2003 to the EC and UNECE ICP-Forests

¹Sue Benham, ¹Mark Broadmeadow, ²Marcus Schaub, ³Vicent Calatayud, ⁴Filippo Bussotti

¹Environmental and Human Sciences Division, Forest Research, Alice Holt Lodge, Farnham, Surrey, UK

²WSL, Birmensdorf, Zurich, Switzerland

³CEAM, Valencia, Spain

⁴University of Tuscia, Italy

AN EVALUATION OF THE USE OF COMMERCIAL TREE NURSERIES TO MONITOR VISIBLE OZONE INJURY AND ASSESS EXCEEDANCE OF THE OZONE CRITICAL LOAD AT LEVEL 1

Executive summary

A limited pilot study has investigated the extent of visible ozone injury in commercial tree nurseries across central and southern Europe. The principal objective of the project was to test whether such nurseries represent an appropriate approach to assessing spatial and temporal variation in the extent to which trees are affected by ozone pollution. Commercial nurseries were selected for the project because:

- Nursery stock is not generally subject to the same level of water stress as field grown plants; stomatal flux is therefore not water-limited (in most cases) and these sites should therefore represent a 'level 1' assessment of the area at risk;
- A wide range of species, known to be sensitive to ozone pollution, can be assessed at each site.
- Trees are generally small, addressing the problems associated with field measurements of ozone damage to mature forest trees.

Assessments of ozone injury were made at a total of 13 nurseries in northwest Europe (UK, five sites), central Europe (Switzerland; two sites) and Mediterranean Europe (Spain and Italy; three sites each). At each nursery, an assessment of visible injury was made towards the end of the growing season for all species present that are documented as ozone sensitive. At most sites, an earlier assessment was carried out but revealed little additional information in the context of this study.

The ozone climate of each site was characterised using a combination of passive and active samplers. For all sites where active samplers were not present, the passive samplers enabled corrections to be applied to data from the nearest active monitoring station so that realistic estimates of AOT40 could be made. Meteorological data from the nurseries or local meteorological stations, together with the corrected active monitor data were used to calculate cumulative ozone flux using the DO₃SE model (Emberson *et al.*, 2005).

Ozone induced injury was observed in all four countries, with damage most severe and extensive in Switzerland, particularly at the high elevation site at Lattecaldo. The UK sites showed the least extent of injury, with ozone damage only confirmed at the nursery in southern England and only at the second time of assessment in late summer. In Spain and Italy, there was a clear relationship between the extent of injury and altitude, reflecting the higher ozone concentrations at the higher elevation sites. In Spain, confirmed ozone injury was restricted to two species, possibly reflecting drought stress and reduced stomatal uptake.

Within the limitations of the project design, the extent of injury reflected ozone exposure expressed as both AOT40 and cumulative stomatal flux relatively well. Mean ozone concentration performed less well as a predictor of ozone-induced visible injury. Injury was not observed at any site with an AOT40 of less than 12.7 ppm h, with 13.7 mmol

m^{-2} the minimum cumulative ozone flux (above $1.6 \text{ nmol m}^{-2} \text{ S}^{-1}$) at which injury was observed. In both cases, these observations are compatible with the current critical load of 5 ppm h or 4 mmol m^{-2} in the sense that growth may be reduced below these levels, without visible injury. However, the study provides no evidence (on the basis of visible injury) that 5 ppm h is a more appropriate Critical Level than 10 ppm h or for the current provisional flux-based Critical Level of 4 mmol m^{-2} (UNECE, 2007).

The study demonstrates that the impacts of ozone are not restricted to southern and Central Europe where higher ozone concentrations are experienced. Longer day-length and greater moisture availability exposure in north west Europe compensate for the lower concentrations through enhanced stomatal uptake.

This approach represents a cost effective methodology for assessing spatial and temporal variation in ozone-mediated damage across Europe. However, if it is implemented on a wider scale, a number of recommendations come out of this pilot study:

- A small number of widely distributed and cultivated ozone sensitive species should be assessed rather than the wider range included in this pilot study;
- A minimum of twenty plants per species should be assessed; a single assessment in late August is likely to be sufficient to provide the extensive information that such a network would be expected to produce;
- Through implementing the above recommendations, a quantitative scoring system could be developed to enable valid comparisons to be made between years and regions;
- It is essential that an experienced phytopathologist provides quality assurance following an assessment for ozone injury; this does not need to be in the field, but the second assessment can be carried out subsequently in the laboratory if appropriate measures are taken to preserve the leaf samples;
- Interpretation of the output from such a network would be important – a lack of visible injury does not mean the absence of ozone impacts; across the majority of Europe it is likely that ozone is reducing productivity and affecting other physiological functions.

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Introduction

Ozone is a photo-oxidant pollutant that can affect the appearance, growth and physiological processes of plants. Its primary mode of action is through the stomata, causing degradation of cell membranes, particularly those associated with photosynthesis. Background concentrations of ozone prior to the industrial revolution were around 15 ppb. Ozone has therefore always represented a potential pollutant impact to plants, and therefore they have had to develop strategies to cope with a certain level of damage. However, from the onset of industrialisation, large quantities of precursors to the formation of ozone particularly NO_x , have been emitted into the atmosphere, with the result that summer ozone episodes increased in severity, peaking in the 1980s and 1990s. Recent NO_x emission control policies have been the main driver of a recent reduction in peak concentrations. However, against this fall in peak concentrations has been a steady rise in background concentrations. Model predictions are for this trend to continue, with background concentrations rising to, possibly, double the current value by the end of this century. Ozone is unique amongst air pollutants, in that current ambient levels across much of Europe can cause direct damage to plants. Current estimates suggest that ozone may at present be responsible for limiting the growth of European forest trees by about 10%, and may also contribute to the deterioration of forest condition in some species. Large areas of Europe currently exceed thresholds for effects on vegetation and human health.

Unlike many other pollutants ozone affects foliage directly, and there is no role for soil processes in mediating its harmful effects (with the exception of soil moisture limited stomatal conductance and thus exposure. There is some evidence linking ozone with degradation of surface waxes increasing water loss from the leaf surface, and also occluding the stomata. However, ozone principally affects the internal components of leaves, gaining access through the stomata. Microscopic analysis reveals degradation of internal membranes, particularly those associated with the chloroplasts. Indeed, microscopic analysis is used as a diagnostic tool for assessing ozone pollution where other symptoms are not apparent. This physical disruption of the photosynthetic apparatus often results in reduced levels of chlorophyll, a lower photosynthetic capacity and advanced leaf senescence. The degradation of chlorophyll is manifested as generalised, diffuse chlorosis, or yellowing of the foliage. The lower photosynthetic capacity, and also, the continuing costs (in terms of energy) of repairing damage to the cell membranes often results in reduced growth. In conifer species, advanced leaf senescence appears as reduced needle retention, with fewer cohorts of needles retained in areas experiencing ozone pollution.

Changes in carbon allocation resulting from diversion to ozone damage repair has been shown to lead to reduced root biomass, and reduced root exploration (shorter roots). Ozone exposure is thus likely to lead an exacerbation of drought conditions. Furthermore, ozone impairs the functioning of the stomata with the result that stomatal closure in response to drought is compromised, thus compounding the effects of water

shortage. Increased mortality and susceptibility to extended periods of drought would therefore be expected.

A number of statistical analyses have revealed links between ozone exposure and both productivity and condition, while exposure experiments in open-top chambers and other controlled environment facilities have also demonstrated clear relationships. However, these relationships can be difficult to quantify because of limited replication in these experiments, the short time-frame of the experiments relative to the life cycle of trees, and variability in site conditions. These problems are particularly relevant to field investigations of the effects of ozone pollution. In contrast, herbaceous species provide opportunities for quantitative investigations. The reduction in annual biomass production for two genotypes of clover (*Trifolium repens*) with contrasting sensitivities to ozone has been assessed across Europe in an experimental programme operated by the International co-operative programme on effects of air pollution on natural vegetation and crops (ICP Vegetation). The productivity of the ozone-sensitive relative to the ozone-resistant genotype has been compared from 1996 at 31 sites across Europe, and suggests a 40% reduction in productivity of the sensitive genotype at the most polluted of the sites.

Although the reduction in biomass production is the most important of the observed effects of ozone pollution and is generally used as a quantitative indicator, a range of other effects are apparent. Ozone pollution results in characteristic visible symptoms in some species. These symptoms are distinct to those associated with senescence, although extreme care needs to be taken not to confuse the two processes. An important point to note is that although a species may be symptomatic at relatively low ozone exposure, this may not result in poor health or yield loss. The converse is also true – non-symptomatic plants may suffer reduced growth and increased susceptibility to biotic and abiotic damage.

These 'visible symptoms' vary greatly between species, and for each species, these symptoms need to be validated by comparing symptomatic foliage from fumigated trees, with foliage grown in unpolluted air. In some species, particularly conifers such as Aleppo pine (*Pinus halepensis*) generalised, diffuse chlorosis of one year old needles has been shown to be associated with ozone pollution. Some broadleaf species such as beech (*Fagus sylvatica*) and ash (*Fraxinus excelsior*) also show interveinal chlorosis initially, followed by 'stippling', or bronzing. Other species, including many shrub species (eg *Prunus spinosa* and *Viburnum lantana*), show characteristic reddening, although care has to be taken in distinguishing between this response to ozone pollution and the natural response of some plant species to produce red pigmentation (anthocyanins) in response to high light and other stresses.

Ozone injury is monitored as part of the UN-ECE/ICP Forests Intensive Forest Health Monitoring network. Across Europe, the observation of ozone-related damage has largely been restricted to southern and central regions, where ozone levels are higher as a result of climate, altitude and photochemical precursor formation in industrial areas. However, canopy access in mature trees presents difficulties in the assessment of ozone-related injury, and the reported extent of visible injury may therefore underestimate the problem. An assessment of injury in young plants presents an alternative approach in

which all leaves can be assessed on a larger number of individuals. These symptoms are also more readily expressed in young trees. Furthermore, if the assessment is carried out in commercial tree nurseries, a wider range of forest and amenity tree species (including non-native) known to be sensitive to ozone can be assessed than in current intensive forest health monitoring network. Commercial nurseries have the added advantage of irrigation being available, thus minimising the masking effect of soil moisture limitation.

The primary objective of this project was thus to demonstrate a framework for mapping ozone injury to sensitive tree species under theoretically optimal site conditions for ozone uptake across Europe. By implementing such a framework it would be possible to map the exposure of ozone below which harmful effects to sensitive elements of an ecosystem do not occur, based on observations of visible injury rather than inferred injury through modelling studies. An approach such as this based on physical damage would be effective in terms of communicating the importance of ozone pollution and in driving forward control strategies to reduce the emissions of precursors to its formation.

Methods

Four countries (United Kingdom, Switzerland, Italy, and Spain) participated in the project, representing northwest/oceanic, continental and Mediterranean climates. In each participating country, up to five commercial tree nurseries specialising in a wide range of tree species known to be sensitive to ozone pollution were selected (see Table 1 and Figure 1 for further details). Where possible these nurseries covered the geographical spread of the country. Agreement was obtained from each nursery to permit access to the site for assessment purposes. The two Swiss sites were not irrigated as rainfall is sufficient for conventional forest nursery practice.

Table 1 Details of the thirteen commercial tree nurseries involved in the project.

Name	Altitude	Latitude	Longitude	Annual Temp (°C)	Annual rain (mm)	Irrigation	Active O ₃ (km)
UNITED KINGDOM							
Hilliers	70	51° 02' N	0° 54' W	9.9	867	Y	
Bellwood	65	56° 38' N	3° 10' W	9.5	785	Y	
Deepdale	50	52° 08' N	0° 12' W	9.6	793	Y	
Palmstead	30	51° 11' N	0° 56' E	10	748	Y	
Huntley	60	51° 53' N	2° 25' W	9.6	854	Y	
SWITZERLAND							
Lattecaldo	600	45° 51' N	09° 03' E	11.1	1333	N	On-site
WSL	553	47° 21' N	08° 27' E	9.1	1135	N	On-site
ITALY							
Curno	250	45° 41' N	9° 37' E	13.6	1100	Y	On-site
Cespevi	60	43° 56' N	10° 54' E	14.3	1250	Y	2
Casentino	450	43° 44' N	11° 45' E	12.3	1100	Y	30
SPAIN							
Font Roja	1015	38° 40' N	0° 32'	13.8	552	Y	
Quart	83	39° 29' N	0° 31'	16.5	462	Y	On-site
Bunyol	320	39° 24' N	0° 47'	13.6	516	Y	

Ozone monitoring

At each site, monitoring was carried out to determine ambient ozone concentrations. Passive samplers (molecular diffusion of a pollutant to a specific absorbent formula) were used on the nurseries (April-September) to provide a measure of ozone exposure. Each country was free to choose its own type of sampler, within the bounds of existing tested technology. At each site three tubes were exposed plus one blank, these were changed on a fortnightly basis (Annex 2). These passive samplers were cross-calibrated with ozone data available from the closest continuous automatic monitoring station. The exception to this was Switzerland where automatic analysers were installed at the nurseries providing continuous monitoring data. Basic meteorological data was provided by the nurseries or where unavailable, were obtained from the closest meteorological station within the national monitoring network.



Figure 1 Location of the commercial trees involved in the project.

Ozone injury assessment

Assessments concentrated on field-grown trees and shrubs although, in some cases, container grown augmented the species list. Species chosen were, in the main, selected from the European list of sensitive species (UNECE, 2005). In some cases other species

common to the area were also assessed. A list of species assessed by each country is given in Annex 1.

The national field teams were trained in visible symptom identification, quantification of foliar injury symptoms and sampling. At least one member of the field teams had attended the inter-calibration courses on visible ozone damage assessment, and were tested in data collection procedures. The inter-calibration course played a secondary, but equally important role in ensuring that visible ozone injury was discriminated from mimicking symptoms attributable to drought, senescence or insect pest/disease outbreak (Schaub, 2002).

The extent of ozone injury was determined by site visits during which assessments were carried out on leaves of the upper fully sun exposed crown. Photographs were taken of all ozone-like symptoms (see Annex 6-9). Voucher leaf samples were also taken for the validation of the visible ozone injury symptoms observed in the field. Symptoms were validated microscopically to confirm alteration in the palisade parenchyma, characteristic of oxidative stress, according to Günthardt-Goerg and Vollenweider (2006).

Visible injury was assessed on a minimum of 30 plants per species, where available. For each species, the percentage of trees affected was recorded. For each individual plant, ozone symptoms were assessed using the following classes:

Table 1 Visible injury classes for (a) proportion of symptomatic leaves and (b) proportion of leaf area affected.

Percentage of symptomatic leaves	
0	No injury, none of the leaves injured
1	1%-5% of the leaves show ozone symptoms
2	6%-50% of the leaves show ozone symptoms
3	51%-100% of the leaves show ozone symptoms

Percentage of affected leaf area	
0	No injury present
1	1%-5% of the surface is affected
2	6%-50% of the surface is affected
3	51%-100% of the surface is affected

Calculating ozone exposure

Ozone exposure was calculated on the basis of AOT40, mean ozone concentration and cumulative stomatal ozone flux over a threshold of $1.6 \text{ nmol m}^{-2} \text{ s}^{-1}$ (AF_{st}) during the

assessment period. AOT40 was calculated for daylight hours (global radiation $>50 \text{ W m}^{-2}$). Cumulative stomatal flux was calculated using the DO₃SE multiplicative model developed by Emberson *et al.*, (2001) and further elaborated in Karlsson *et al.*, (2007). Physiological parameterisation was for a 'generic broadleaf' species based on published characteristics of beech.

Gap filling and data manipulation:

Valid between site comparisons of AOT40 of AF_{st} requires continuous hourly data-sets. As is often the case with monitoring data, there were gaps in both the ozone concentration data and the meteorological data used as driving variables in the DO₃SE stomatal uptake model.

Gaps of a single record were filled using the average of the two surrounding records. For larger gaps where data from an adjacent site was available a linear regression was fitted to the data and used to correct the second site's records to fill the gaps. Where this was not possible, the monthly diurnal mean for the site was used to fill the missing records.

Meteorological data:

The DO₃SE model requires hourly meteorological data to drive it but, in some cases, the only data source was an agro-met station recording data manually on a daily basis. The following section describes the approach adopted.

'Sunshine hours' (ie the duration of direct solar radiation) were converted to hourly global radiation in a two-stage process. The relationship between theoretical extra-terrestrial radiation and surface radiation and sunshine hour and day length has been demonstrated by Angstrom (1924) and Collingbourne (1976). Daily values of sunshine and measured radiation for the Alice Holt site for 1995 and 1996 have been used by Randle (1997) in parameterising the relationship:

$$G/G_0 = a + b(S/S_0) + c(S/S_0)^2 + d(S/S_0)^3$$

Where: G is terrestrial radiation; G₀, theoretical extra terrestrial radiation; S sunshine hours; and S₀, day length. a, b, c and d are regression coefficients.

Once the daily radiation was calculated, a sinusoidal curve was fitted with positive values between dawn and dusk, such that the total integral equals the daily radiation, with a peak at 12 noon. The curve is then integrated on an hourly basis giving estimates of hourly radiation. It should be noted that this methodology will not reflect natural hour-to-hour variability

Daily Max-Min temperature records have been manipulated in a similar way at some sites to derive hourly data. Temperature at any time of the day is calculated by fitting a sinusoidal relationship between the recorded maximum and minimum daily temperature. The method employed modifies the sinusoidal wavelength (both positive and negative amplitudes), such that the positive amplitude occurs with a maximum at 14:00, and the negative amplitude having a minimum one hour before dawn. In turn, dawn is estimated from the daily declination of the sun and the latitude of the site.

Standard 'agro-met' weather stations record humidity (wet and dry bulb temperature) at 9 am. It is assumed that the vapour pressure stays largely unchanged though the day, with relative humidity (RH) changing through the course of the day according to the relationships given below:

$$SVP = 6.1078 e^{\frac{(17.269T)}{(237.3+T)}}$$

and the VP (for example at 9 am) is given by:

$$VP_{d9} = SVP_{wet,9} - 0.66(T_{d9} - T_{w9})$$

where $SVP_{wet,9}$ is the saturated vapour pressure at 9 am; T_{d9} is the air temperature at 9am, and T_{w9} , the wet-bulb temperature at 9 am.

$$RH = \frac{VP_{d9}}{SVP_{dry}} \times 100$$

Clearly, a number of transformations have been introduced which could affect estimates of stomatal uptake. However, it is considered that the approach is appropriate for describing the gross differences in stomatal uptake between regions.

Results: United Kingdom

Ozone climate

Ozone was monitored using passive diffusion tubes at four of the sites. The fifth site (Huntley) failed to return any diffusion tubes and therefore their results are based solely on active monitoring data from the nearest rural automatic monitoring network site. Mean concentrations were similar across the four sites, with the exception of Bellwood. Here the limited data-set available suggested that concentrations were significantly higher than at the other sites. It is likely to be a function of Bellwood being a relatively clean air site (located in Central Scotland), with high night-time concentrations maintained. At the other sites, higher NO_x concentrations and an enhanced ozone creation/destruction cycle as a consequence is likely to result in much lower night-time concentrations, as confirmed by the active monitoring data. This highlights the benefit of using either AOT40 or stomatal flux estimates as indicators of ozone exposure over mean concentration.

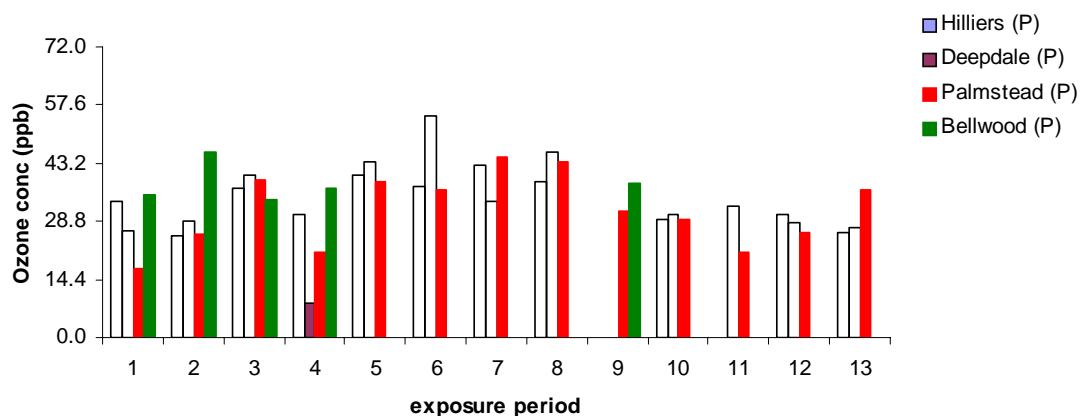


Figure 2 Mean ozone concentrations measured with passive samplers in the UK. See Annex 2 for details of sampling periods. Missing data are the result of data quality issues with the passive samplers or difficulties with site operatives.

Visible injury

Five sites were selected (see Annex 3) with a focus on southern England (three sites), where past monitoring has revealed ozone exposure to be highest. One site in central Scotland and one site on the England/Wales border provided further spatial coverage. In the past there had been no evidence of ozone induced injury on nursery trees in the UK. A single assessment was therefore conducted late in the growing season on four of the sites. The fifth site in the South east of England was assessed twice (end July) and (September) as it was considered on past experience to be the site most likely to show ozone induced-injury. During the course of the assessments many tree species presented non-specific ozone-like symptoms (Annex 4), at all five nurseries:

Deepdale

The early July assessment revealed inter-veinal bronzing on *Alnus incana*, *Carpinus betula*, *Prunus avium* and *Corylus avellana* (combination of juvenile leaf material and conventional light response). Stippling was seen on *Populus nigra* and *Liriodendron tulipifera*. The stippling on *Liriodendron* was, however, clearly visible on both sides of the leaf indicating that this was unlikely to be associated with ozone-induced injury (pathogen-induced), while it is likely that damage to *P. nigra* represented early symptoms of rust infection. *Pseudotsuga menziesii* (combination of senescence and aphid damage) showed some chlorotic mottling on older needles whilst *Quercus robur* showed severe interveinal chlorosis on one tree (early symptoms of mildew), with fewer symptoms visible on the shaded leaves.

Huntley (late August)

Interveinal bronzing was observed on *Alnus viridus*, *Carpinus betulus*, *Viburnum lantana*, *Viburnum opulus* (senescence/light responses). *Populus alba* had stippling on the younger growth but, as suspected, this was confirmed to be fungal in nature.

Palmstead (early September)

Interveinal reddening was seen on *Alnus area*, *Prunus avium* and *Viburnum lantana*. *Populus spp.* found in the hedges displayed severe stippling on older leaves but this was identified as an outbreak of *Pollaccia*.

Bellwood (mid August)

Alnus incana showed reddening on 30% of the older leaves. *Liriodendron tulipifera* had stippling on 20% of the older leaves. Bronzing was seen on occasional needles in *Picea abies* and occasional leaves of *Juglans regia*.

Hilliers

In July *Tilia cordata*, *Carpinus betulus*, *Alnus incana*, *Corylus avellana* and *Fagus sylvatica* all showed varying degrees of interveinal bronzing. Stippling was seen on a few leaves of *Liriodendron tulipifera*. *Pinus nigra subsp. austriacalis* had minor mottling on some needles which was worse on the sun exposed side however the trees were all retaining 3 years needles. *Pyrus malus subsp. malus* displayed extreme examples of red coloration on the lower surface of the older leaves with 10% of leaf area effected. *Sorbus aria* also had leaf reddening confined to the outer edges of the leaf. In all these examples ozone damage was ruled out. The red pigment seen on some species *Viburnum lantana*, *Viburnum opulus*, *Alnus incana*, *Alnus area*, *Prunus avium* and *Pyrus malus* was due to natural build up of pigments as the season progressed but no evidence is available as to the usual timing of this event. Stippling observed on *Liriodendron tulipifera* at Bellwood and Hilliers was caused by tissue necrosis but was not thought to be ozone induced.

At the second inspection at Hilliers in late September, yellowing was seen on the older leaves of *Buxus sempervirens*, and *Betula pendula*; this was confirmed as natural senescence. However this is earlier than would normally be expected possibly as a result of the high temperatures and prolonged early drought. Stippling was observed on 50% of the *Liriodendron tulipifera* trees inspected, to some extent, with 10% of these displaying extreme symptoms (Figure 3). The symptoms on older leaves in full sun were confirmed, by microscopy, to be ozone-induced, but with no evidence of damage on younger leaves. *Fagus sylvatica* showed bronzing on 80% of the older leaves on exposed branches, with 80% of the trees affected to some extent. Many leaves also displayed a classical 'shading response' (see Annex 4). Microscopical examination again confirmed tissue damage typical of ozone injury. Photo-microscopy of undamaged and damaged sections are shown (Annex 4) with damaged cells in the palisade parenchyma detected as positive evidence of ozone damage.

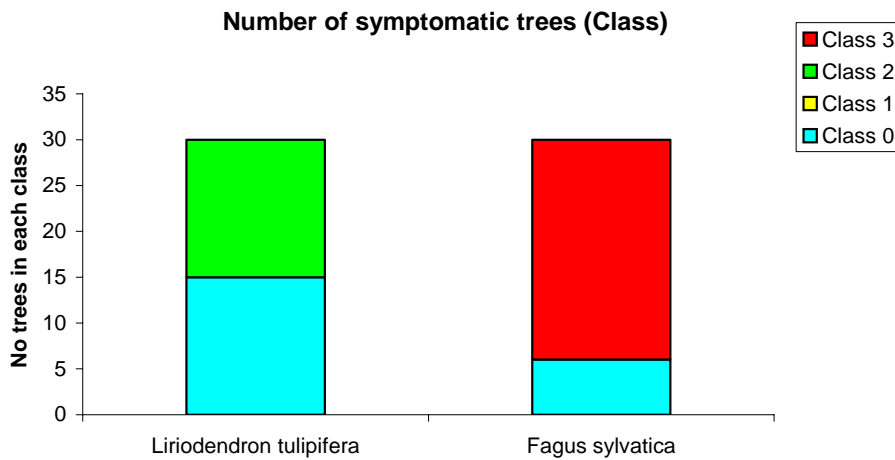


Figure 3 Percentage of symptomatic leaves for Liriodendron and Fagus at Hilliers (UK) in late August.

Results: Italy

Ozone climate

High ozone concentrations were recorded at all three sites during the early part of the growing season, with much lower concentrations after period 5 (see Annex 2 for details of sampling periods).

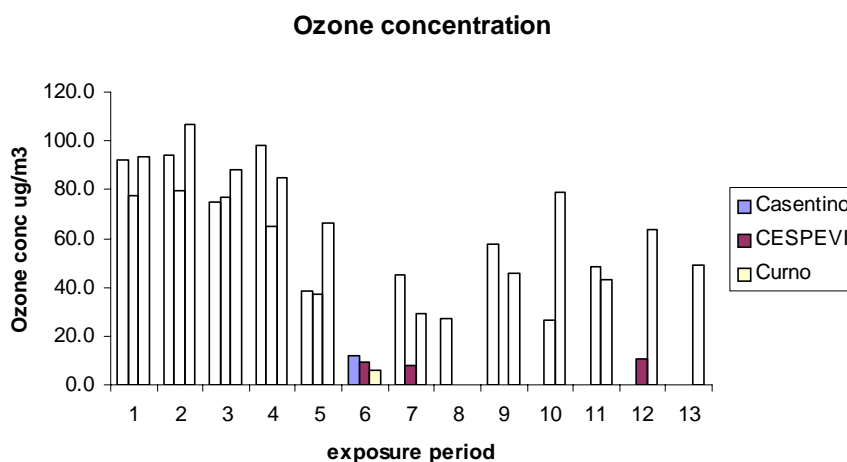


Figure 4 Mean ozone concentrations ($\mu\text{g m}^{-3}$) measured with passive samplers at the different exposure times.

Visible injury

In Italy assessments were carried out in late June and again in late August (see Annex 5). At the first assessment (late June) many tree species presented non-specific ozone-like symptoms at all three nurseries. In most of these species, the observed symptoms were interpreted as natural accumulation of anthocyanins in response to the high irradiance typical of the Mediterranean climate during the summer. Nevertheless, these symptoms appeared earlier than expected on the basis of past observations.

Cespevi first assessment: reddening on *Prunus serotina*, *Crataegus oxyacantha*, *Viburnum opulus*, *Cornus mas* and *Cornus sanguinea*; bronzing on *Corylus avellana*.

Casentino first assessment: reddening of *Cornus mas* and *Prunus serotina*; bronzing on *Populus nigra*;

Curno first assessment: reddening on *Viburnum lantana* and *Viburnum opulus*.

At the second assessment (late August), some of the plants that were symptomatic at the previous assessment, had lost their leaves prematurely. This applied to *Prunus serotina* (Cespevi and Casentino), *Prunus oxyacantha* (Cespevi) and *Populus nigra* (Casentino). For other species, the leaf reddening observed at the first assessment, increased in intensity and in the proportion of plants affected. Examples of species that exhibited this response include *Viburnum lantana* (Cespevi and Curno), *Viburnum opulus* (Cespevi and Curno), *Cornus mas* (Cespevi) and *Prunus avium* (Casentino and Curno).

In late summer, the observed 'symptoms' are typical responses of these particular species to the combination of drought and high irradiance and cannot be considered as indicating ozone impact without further evidence. However, the early onset of these 'symptoms' suggests the presence of a response to ozone pollution. Difficulty in interpretation is further compounded by the loss of symptomatic leaves prior to the second assessment.

Another symptom characteristic of ozone damage, but also a response to high irradiance, is leaf bronzing. This was observed in *Fagus sylvatica* and *Corylus avellana* at Cespevi. The same is true of the discolouration and diffuse chlorosis that was observed for many species. In other cases non-specific leaf necrosis were observed.

At other sites, pest and disease outbreaks prevented a valid second assessment being carried out. This was true for *Quercus robur* (*Oidium*) at Curno, *Labrum anagyroides* (fungi and aphids) at Casentino and *Acer pseudoplatanus* (*Rhytisma acerinum*) at Cespevi. Of the species selected for ozone assessment, the more reliable symptoms were observed at Cespevi (*Populus nigra* and *Fraxinus excelsior*: Annex 7) and Curno (*Populus nigra*). In all cases, class 2 damage was observed (Figure 5).

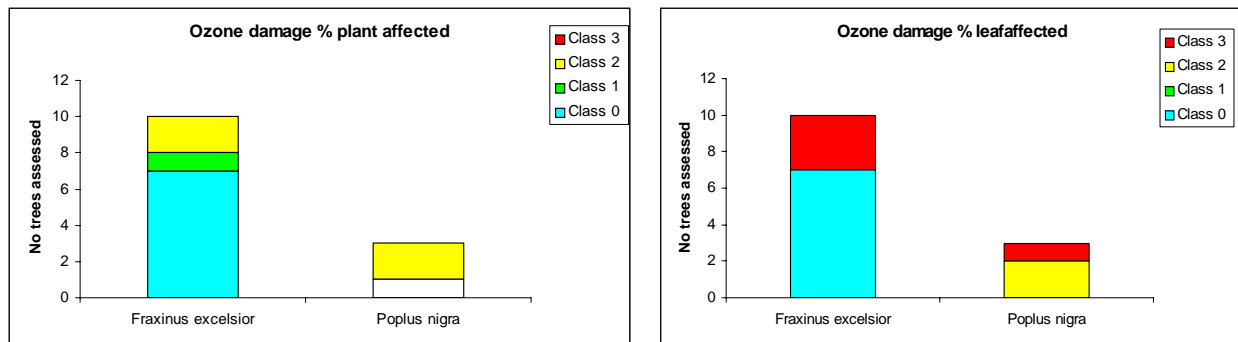


Figure 5 The percentage of symptomatic leaves and affected leaf area (in classes) at Cespevi.

In order to clarify these findings, a more comprehensive survey was carried out at Curno, and symptoms were assessed in plants throughout the whole nursery. The results are summarised in Table 2:

Table 2 Results of a detailed survey of ozone-like symptoms at the Curno nursery in Italy.

Species	Ozone symptoms		Observations
	Leaves affected	Leaf area affected	
<i>Viburnum opulus</i>	3	3	Reddening
<i>Viburnum lantana</i>	3	3	Reddening
<i>Cornus sanguinea</i>	3	3	Reddening
<i>Morus nigra</i>	3	2	Interveinal brown stipples (ozone typical)
<i>Fraxinus excelsior</i>	1	3	Interveinal brown stipples (ozone very typical)
<i>Fraxinus ornus</i>	1	2	Bronzing
<i>Prunus avium</i>	3	2	Reddening
<i>Acer pseudoplatanus</i>	2	3	Interveinal brown stipples (ozone very typical)
<i>Acer platanoides</i>	1	1	Interveinal brown stipples (ozone-like)
<i>Acer campestre</i>	3	2	Interveinal brown stipples (ozone-like)
<i>Rhamnus catharticus</i>	3	2	Interveinal brown stipples (ozone very typical)
<i>Ulmus campestris</i>	2	2	Reddening
<i>Populus nigra</i>	3	1	Interveinal brown stipples (ozone very typical)
<i>Ligustrum vulgare</i>	2	2	Bronzing

The symptomatic *Populus nigra* at Cespevi and at Curno, together with *Fraxinus excelsior* at Cespevi were validated by microscopy. Leaves displayed the characteristic collapse of the palisade tissue cells, indicating a Hypersensitive Response (HR). The HR of the palisade cells is considered the clearest manifestation of the stress induced by ozone.

The same microscopic symptoms were also observed in several other species at the Curno nursery besides *Fraxinus excelsior* and *Populus nigra* - particularly *Acer pseudoplatanus*, *Rhamnus catharticus*. In other cases (*Viburnum lantana* and *Viburnum opulus* at Cespevi and Curno; *Cornus* sp. at all sites) the microscopic symptoms consisted of non-specific accumulation of anthocyanins both in the mesophyll and in the epidermis. In conclusion, ozone was confirmed as the causative agent of the ozone-like

symptoms observed on *Populus nigra*, *Fraxinus excelsior*, *Acer pseudoplatanus* and *Rhamnus catharticus*.

Results: Spain

Ozone climate

The highest ozone levels occurred in June and July. Ozone exposure also varied with altitude, with the largest concentrations experienced at the highest elevation site (up to 50 ppb for a 2-week period recorded at the highest altitude site, Font Roja), while the lowest altitude site (Quart), experienced the lowest values (32 ppb for a 2-week period: Fig 6).

In Spain plants were assessed every two weeks, except at the Font Roja nursery, where assessments were conducted every 4 weeks between April and September (see Annex 2).

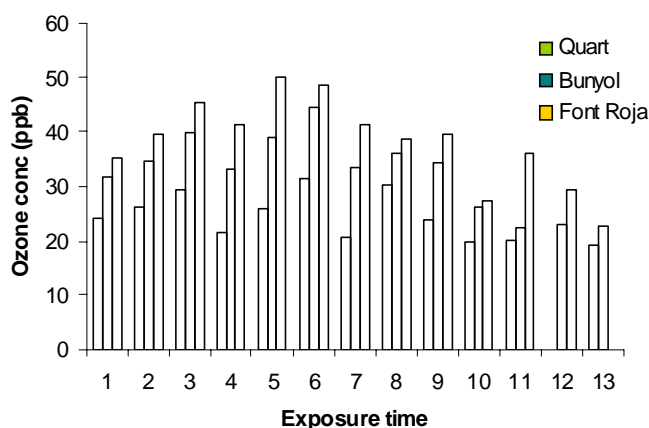


Figure 6 Mean ozone concentrations (ppb) measured with passive samplers at the three tree nurseries in Spain. [Note: due to technical problems, two data points are missing].

Visible injury

At Quarts no visible ozone-induced injury was observed on any of the species assessed. At the Font Roja nursery *Prunus spinosa* and *Fraxinus ornus* showed visible injury, while at the Buryol nursery, injury was observed on *Viburnum lantana*.

Ozone-induced symptoms observed in fumigation experiments at the La Peira Open Top Chamber facility (2005-2006) were compared with ozone-like symptoms observed in the field to indicate ozone as a causative factor. In all cases symptoms first appeared in July and increased in severity during August. Symptoms were validated microscopically to confirm that ozone was likely to be the causative agent. The ozone-like symptoms recorded on *Fraxinus ornus* were not attributed to ozone as microscopy revealed that the

palisade parenchyma was not affected. Ozone damage was confirmed in *Viburnum lantana* and *Prunus spinosa* (Annex 5).

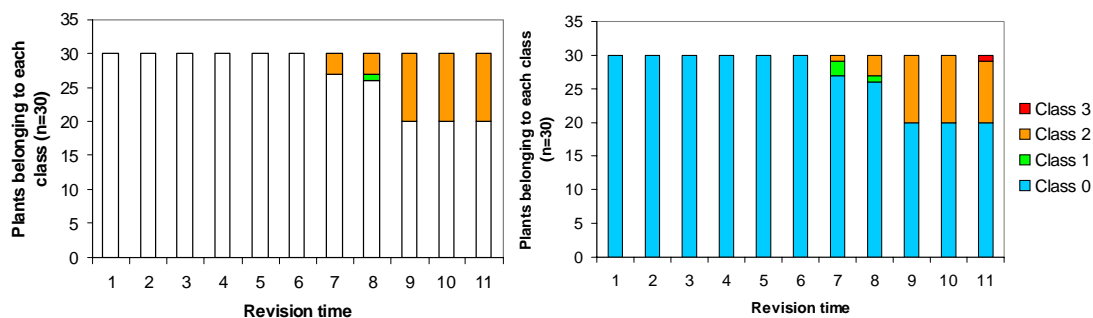


Figure 7 Evolution of the percentage of symptomatic leaves and affected leaf area (in classes) for *Viburnum lantana*. [Note: assessment was not possible in periods 12 and 13 due to natural senescence confounding the observations.]

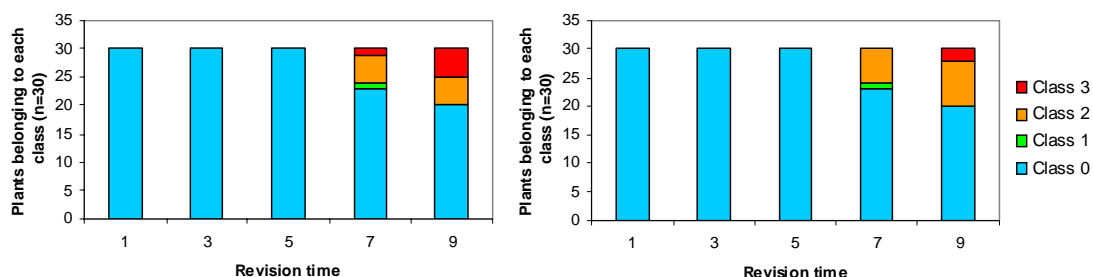


Figure 8 Evolution of the percentage of symptomatic leaves and affected leaf area (in classes) for *Prunus spinosa*. [Note: assessment was not possible after period 9 due to natural senescence and leaf fall confounding the observations.]

Results: Switzerland

Ozone climate

Ozone was monitored using continuous automatic analysers. The highest ozone concentration of 165 ppb was recorded at the highest altitude site (Lattecaldo), while the lowest altitude site (WSL), experienced a maximum concentration of 104 ppb. AOT40 recorded for the two sites was 56.3 ppm h at Lattecaldo and 24.1 ppm h at WSL. Lattecaldo is situated in the mountains above the plain of Milan and is known to have high ozone concentrations with damage frequently seen at this site.

Visible injury

In Switzerland, plants were assessed twice at WSL and three times at the Lattecaldo nursery (see Annex 2). At both sites, ozone-induced injury was observed on many of the species assessed (see Annex 6 for further details).

At WSL *Betula pendula*, *Robinia pseudoacacia*, *Corylus avellana*, *Rhamnus cartharticus*, *Ulmus glabra*, *Prunus serotina*, *Fagus sylvatica* and *Populus tremula* all showed signs of ozone injury. However, the appearance of symptoms was restricted to the September assessment, with no symptoms observed during the August assessment. The most seriously affected species was *Prunus serotina*, confirming its position as a highly sensitive bio-indicator of ozone pollution.

At Lattecaldo, damage was observed on *Prunus mahaleb*, *Populus nigra*, *Salix daphnoides*, *Fagus sylvatica*, *Carpinus betulus*, *Ligustrum vulgare*, *Sambucus nigra* and *Viburnum opulus*. At Lattecaldo no symptoms were seen at the July assessment with symptoms first appearing in early August, increasing in severity over the following two months. Symptoms were validated by comparison of symptoms with photographic evidence from literature (Innes *et al.*, 2001) and with previous findings from OTC experiments.

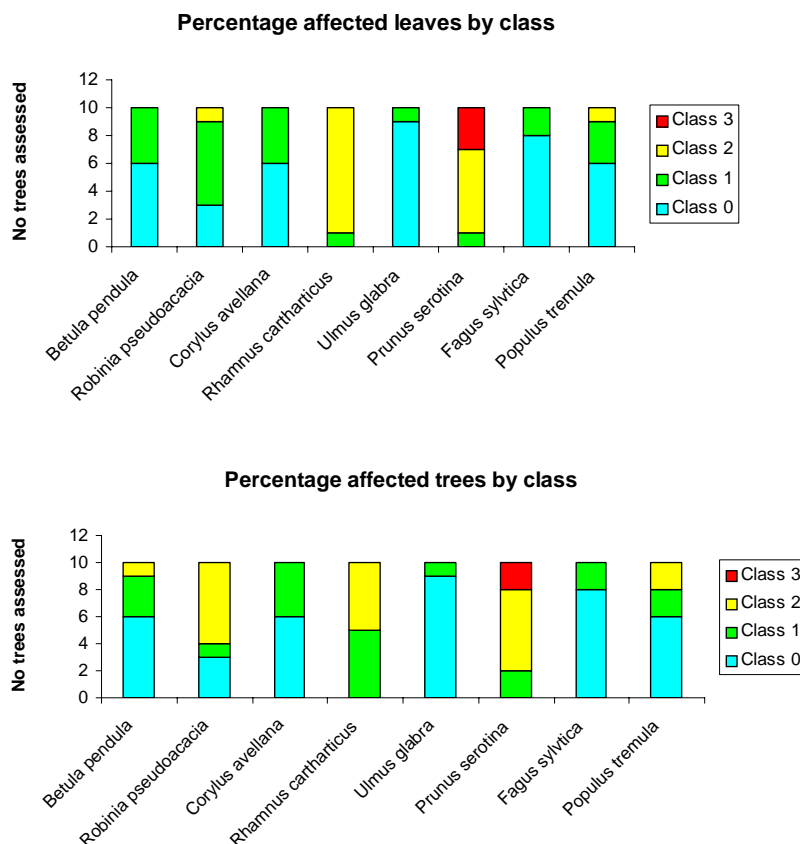


Figure 9 Proportion of symptomatic leaves (top) and trees (bottom) denoted by damage class observed at WSL in September.

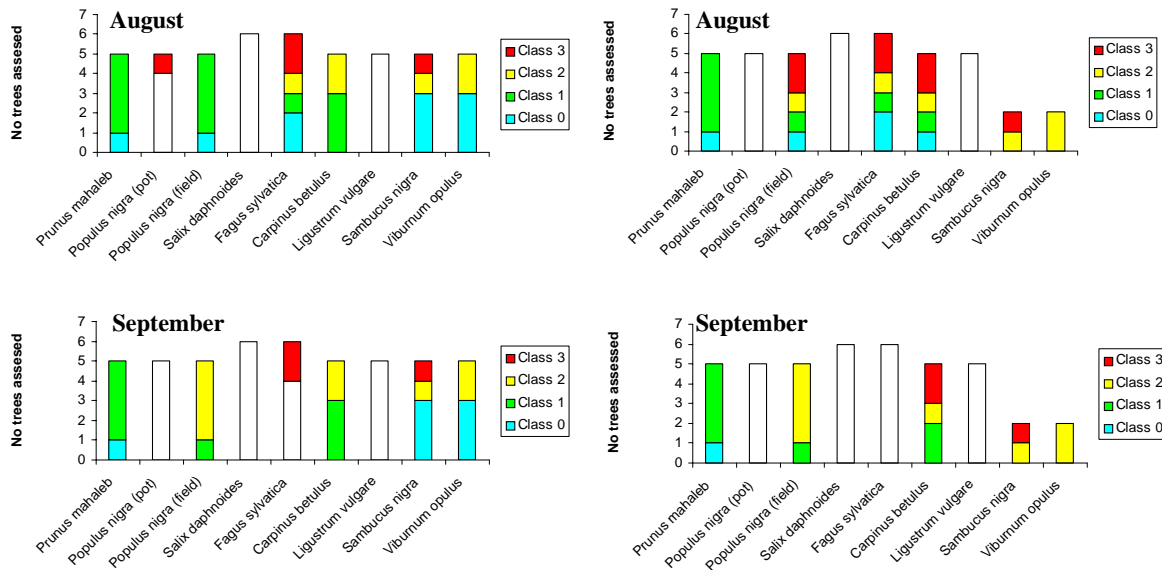


Figure 10 Evolution of ozone-induced damage at Lattecaldo, expressed as proportion of leaves (left) and trees (right) affected in August (top) and September (bottom) and broken down by damage class.

Discussion

The results indicate that although ozone induced symptoms are not serious and extensive across commercial tree nurseries in Europe, the three geographic regions assessed in this study have all returned records of visible ozone injury, indicating that it is a widespread phenomenon. Additionally, there were many reports of ozone-like symptoms that were not confirmed by microscopic analysis or were rejected on the grounds of confounding insect pest or disease damage or natural senescence. It is also noteworthy that 2006 was not an 'extreme ozone year' and thus that the extent of visible injury would be expected to be greater in such years

Table 3 Ozone exposure for 2006 for the thirteen sites assessed. Exposure is reported as AOT40, stomatal flux above a threshold of $1.6 \text{ nmol m}^{-2} \text{ s}^{-1}$ and mean concentration.

Country	Nursery	Accumulated Stomatal ozone flux (AF_{st})	AOT40	Mean Ozone Concentration	Sampling period
		mmol m^{-2}	Ppm h	Ppb	
United Kingdom	Hilliers	13.0	15.4	32.6	1/4/06-30/9/06
	Palmstead	8.8	5.4	28	1/4/06-30/9/06
	Deepdale	10.4	1.9	28.9	1/4/06-30/9/06
	Bellwood	11.3	4.6	33.6	1/4/06-30/9/06
	Huntley	16.0	9.3	34.5	1/4/06-30/9/06
Switzerland	Lattecaldo	24.9	38.1	49.8	1/4/06-30/9/06
	WSL	14.0	17.4	36.0	1/4/06-30/9/06
Spain	Font Roja	24.9	20.0	41.6	1/4/06-30/9/06
	Quarts	7.8	1.2	24.1	1/4/06-27/9/06
	Bunyal	15.7	12.7	32.6	1/4/06-27/9/07
Italy	Curno	23.6	36.4	40.5	11/4/06-30/9/06
	CESPEVI	13.4	24.1	35.2	1/4/06-30/9/06
	Casentino	18.4	18.1	43.3	1/4/06-13/9/06

Injury was most extensive at the Lattecaldo site in Switzerland, where the maximum mean ozone concentrations were experienced. This was also the site with the highest ozone exposure when expressed as AOT40 (see Table 3). Ozone-induced symptoms were expressed to the least extent in the UK, an observation again compatible with recorded exposure using all three indices. However, it is noteworthy that the Spanish sites also expressed relatively low levels of ozone-induced injury although exposure was significantly higher than in the UK. This corroborates the assertion of Karlsson *et al.*, (2007) that the extent of ozone damage to vegetation in Europe is greater than would be expected on the basis of the original critical level for ozone of 10 ppm h.

Ozone induced injury was observed in all four countries, with damage most severe and extensive in Switzerland, particularly at the high elevation site at Lattecaldo. The UK sites showed the least extent of injury, with ozone damage only confirmed at the Hilliers nursery in southern England and only at the second time of assessment in late summer. In Spain and Italy, there was a clear relationship between the extent of injury and altitude, reflecting the higher ozone concentrations at the higher elevation sites. In Spain, confirmed ozone injury was restricted to two species, possibly reflecting drought stress and reduced stomatal uptake. Furthermore, injury was not observed at Quarts, where ozone exposure measured as mean concentration, AOT40 or AF_{st} were all significantly lower than at the other two sites.

Within the limitations of the project design, the extent of injury reflected ozone exposure expressed as both AOT40 and cumulative stomatal flux relatively well. Mean ozone concentration performed less well as a predictor of ozone-induced visible injury. Injury was not observed at any site with an AOT40 of less than 12.7 ppm h (Bunyal, Spain), with 13.7 mmol m^{-2} (Hilliers, UK) the minimum cumulative ozone flux (above 1.6 nmol

$\text{m}^{-2} \text{S}^{-1}$) at which injury was observed. In both cases, these observations are compatible with the current critical load of 5 ppm h or 4 mmol m^{-2} in the sense that growth may be reduced below these levels, without visible injury. However, the study provides no evidence (on the basis of visible injury) that 5 ppm h is a more appropriate Critical Level than 10 ppm h or for the current provisional flux-based Critical Level of 4 mmol m^{-2} (UNECE, 2007). In terms of visible injury, and it must be stressed not necessarily growth or other impacts of ozone, the evidence presented here suggests that the two exposure-based Critical Levels are precautionary and may overestimate the geographical extent of ozone impacts.

The study therefore demonstrates that the impacts of ozone are not restricted to southern and Central Europe, where higher ozone concentrations are experienced. Longer day-length and greater moisture availability exposure in north west Europe compensate for the lower concentrations through enhanced stomatal uptake. Through implementing a comprehensive assessment of ozone-induced injury in commercial tree nurseries, hard evidence for ozone-induced damage would be made available, highlighting the importance of ozone pollution. Such evidence may have a role to play in communicating the impacts of ozone pollution, leading to acceptance of the need for, and the implementation of, emissions control policies.

Recommendations

Commercial tree nurseries have potential to map spatial and temporal variability in the extent and severity of ozone induced damage to trees and represent a cost-effective approach. If such a scheme is rolled out more widely as a demonstration of the extent of damage, the following recommendations should be considered:

- A small number of highly sensitive widely distributed and grown species should be selected;
- A single assessment in mid to late August is likely to be sufficient to provide the extensive information that such a network would be expected to produce;
- A minimum of twenty plants of each species should be assessed at each site;
- Through implementing the above recommendations, a quantitative scoring system could be developed to enable valid comparisons to be made between years and regions.
- It is essential that an experienced phytopathologist provides quality assurance following an assessment for ozone injury; this does not need to be in the field, but the second assessment can be carried out subsequently in the laboratory if appropriate measures are taken to preserve the leaf samples;
- Interpretation of the output from such a network would be important – a lack of visible injury does not mean the absence of ozone impacts; across the majority of Europe it is likely that ozone is reducing productivity and affecting other physiological functions.

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Annexes

Annex 1: List of species assessed at each site

Annex 2: Assessments dates

Annex 3: Photographs of UK Nurseries

Annex 4: Photographic evidence – UK

Annex 5: Photographic evidence – Spain

Annex 6: Photographic evidence – Switzerland

Annex 7: Photographic evidence – Italy