Ozone exposure thresholds and foliar injury on forest plants in Switzerland

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“Capsule”: Ozone injury symptoms on forest species in Switzerland were identified, verified and related to the current European ozone standard.

Abstract

Canton Ticino in southern Switzerland is exposed to some of the highest concentrations of tropospheric ozone in Europe. During recent field surveys in Canton Ticino, foliar symptoms identical to those caused by ozone have been documented on native tree and shrub species. In Europe, the critical ozone level for forest trees has been defined at an AOT40 of 10 ppm.h O\textsubscript{3} (10 ppm.h accumulated exposure of ozone over a threshold of 40 ppb) during daylight hours over a six-month growing season. The objective of this study was to determine the amount of ambient ozone required to induce visible foliar symptoms on various forest plant species in southern Switzerland. Species were grown within eight open-top chambers and four open plots at the Vivaio Lattecaldo Cantonal Forest Nursery in Ticino, Switzerland. Species differed significantly in terms of the ppb.h exposures needed to cause visible symptoms. The most to least symptomatic species grown within open-plots in this study rank as \textit{Prunus serotina}, \textit{Salix viminalis}, \textit{Viburnum lantana}, \textit{Rhamnus cathartica}, \textit{Betula pendula}, \textit{Rumex obtusifolius}, \textit{Sambucus racemosa}, \textit{Morus nigra}, \textit{Prunus avium}, \textit{Fraxinus excelsior}, \textit{Rhamnus frangula}, \textit{Alnus viridis}, \textit{Fagus sylvatica} and \textit{Acer pseudoplatanus}. Similar rankings were obtained in the non-filtered chamber plots. The ranking of species sensitivity closely follows AOT values for the occurrence of initial symptoms and symptom progression across the remainder of the exposure season. Species that first showed evidence of foliar injury also demonstrated the most sensitivity throughout the growing season, with symptoms rapidly advancing over ca. 25–30\% of the total plant leaf surfaces by the end of the observation period. Conversely, those species that developed symptoms later in the season had far less total injury to plant foliage by the end of the observation period (1.5 to <5\% total leaf area injured). The current European ambient ozone standard may be insufficient to protect native plant species from visible foliar injury, and many more native species may be sensitive to ozone-induced foliar injury than are currently known. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Ozone; Foliar symptoms; Plant injury; AOT40; Critical levels; Switzerland

1. Introduction

Many internal and external factors affect the sensitivity of an individual plant leaf to ozone. External phenomena affecting ozone sensitivity include both a range of factors that influence gaseous uptake rates in the leaf and the characteristics of the ozone regime. Nutrition, water availability, temperature, atmospheric humidity, wind speed, and incident light levels are all known to affect uptake (Sandermann et al., 1997). These factors interact in a complex fashion to determine whether or not the leaf will develop symptoms of injury, making the experimental simulation of ozone exposure extremely difficult. For example, the application of a simulated ozone regime typical of a high-altitude site to plants growing in chambers at a lowland site is of questionable...
value because of differing climatic and soil conditions. Instead, the development of ozone-induced injury needs to be examined in situ if the short- and/or long-term ozone concentrations that will induce foliar injury are to be established. In Europe, an opportunity to undertake such studies is provided by the high ozone concentrations that occur in southern Switzerland.

Bacci et al. (1990) and Wunderli and Gehrig (1990) have previously described patterns of tropospheric ozone exposures across the sub-Alpine regions of southern Switzerland (Canton Ticino) and northern Italy, with the upper Po Plain industrial area and the city of Milan, Italy, being identified as major contributors of the pollutant precursors. Staffelbach et al. (1997) have further described high ozone exposures in the insubric region of Sotto Ceneri in Canton Ticino in southern Switzerland. Solar radiation levels are high, as most of the moisture is associated with short-term, intense precipitation (Frei and Schär, 1998). The Alps form an arc around the plain of the Po Valley, acting as a barrier against central European weather; the high Alps also trap northerly moving air masses from the Po Plain of northern Italy, directly influencing ambient ozone concentrations in southern regions of Switzerland (Angelino et al., 1997; Staffelbach and Neftel, 1997). The Level I critical level for forest trees, AOT40 10 ppm.h (10 ppm.h accumulated exposure of O3 over a threshold of 40 ppb), is exceeded annually at many monitoring stations throughout Switzerland (NABEL, 1995). Mean hourly ambient ozone concentrations have attained 166 ppbv near Chiasso (Staffelbach et al., 1997).

Little information is available on the effects of ozone on the multitude of native forest plants species throughout Europe (Fuhrer et al., 1997; Reiling and Davison, 1992; Davison and Barnes, 1998). Forest and nursery surveys in Canton Ticino in southern Switzerland have recorded ozone-like symptoms on numerous native tree, shrub, and forb species (Innes et al., 1996; Skelly et al., 1998, 2000). Open-top chamber studies (filtration studies) and research using Continuously Stirred Tank Reactors (CSTRs) (fumigation studies) have confirmed that ozone is the cause of the visible injury seen on seedlings of a variety of forest species (Skelly et al., 1998).

Innes and Skelly (1996) found that seedlings varied markedly in symptom severity and development both within and among species. Several species were found to develop ozone symptoms at exposures below the current short- and long-term European air quality standards. Of the tree species, Prunus serotina Ehrh. (black cherry), an introduced species, was found to be the most sensitive to ozone (Skelly et al., 1998). There has been much discussion about the optimal way to characterize ozone exposures. Increasingly, there is a concern that any such characterization should be biologically meaningful, but there is no requirement that the standard protects vegetation of economic importance from significant loss. In Europe, exposures are assessed in relation to the type of vegetation under consideration. Exposures are based on the AOT40 statistic. It is important to emphasize that Kärenlampi and Skärby (1996) developed the AOT thresholds for forests in Europe based on significant (10%) reductions in annual biomass increment of a forest tree species (Fagus sylvatica, one of the least sensitive species assessed in the current study), and not in relation to the occurrence of foliar injury. This threshold very much reflects both past concerns about the impacts of ozone on the productivity of agricultural crops and the lack of adequately documented evidence of ozone-induced foliar injury to tree and shrub species in central and northern Europe. No short-term Level I threshold has been defined for forest trees, but seminatural vegetation (which in the UNECE discussions excludes forest tree species and primarily refers to herb species growing in agricultural areas) is assumed to have the same sensitivity as agricultural crops, and critical levels of 500 ppb.h over 5 days when vapor pressure deficit (09.30–16.30) exceeds 1.5 kPa and 200 ppb.h over 5 days when vapor pressure deficit (09.30–16.30) is below 1.5 kPa has been adopted (Kärenlampi and Skärby, 1996). In this study, we examine long-term, cumulative, ozone concentrations in relation to the onset and development of visible foliar injury on several forest, shrub and herb species in southern Switzerland.

2. Materials and methods

The research site was located at the Vivaio Lattecaldo Cantonal Forest Nursery (9° 3’E, 45° 51’ N, 600 m asl) in the Muggio Valley of Canton Ticino, within the sub-Alpine region of southern Switzerland (Fig. 1). An open-top chamber (Heagle et al., 1973) research facility was established in 1995 within the nursery, and was used for 2 years with six plots prior to this study (Innes and Skelly, 1996; Skelly et al., 1998). During the spring of 1997, six additional plots were established to allow for more replications. The 16 species utilized within this study were selected from those previously observed with ozone-like foliar symptoms during the above surveys. Species were grown within eight open-top chambers and four open plots at the nursery site during the 1997 and 1998 growing seasons (Table 1, Fig. 2) (VanderHeyden, 1999). At the end of the 1997 growing season, Rumex obtusifolius and Sambucus racemosa were removed and replaced by Alnus viridis, Frangula alnus, and Salix viminalis. These two species were removed because both Rumex obtusifolius and Sambucus racemosa showed definite ozone injury with injury thresholds clearly confirmed, and because the large size of individuals of these species threatened to crowd and/or shade other species remaining in the chambers for the second year of study.
All seedlings were evaluated daily until symptoms first appeared and then twice weekly thereafter. Dates of first foliar injury appearance were recorded. Evaluations of foliar injury took place from 9 May to 18 August in 1997, and from 13 May to 20 August in 1998. Leaf maturity after bud break was estimated to be 1 April for all but one species (Morus nigra). Visible injury typically progressed as chlorotic spotting, adaxial pigmented stippling, reddening, and premature leaf senescence. Chlorosis was not recorded as injury but was noted when preceding and accompanying other foliar symptoms. Comparisons of the plants within non-filtered and filtered chambers were made to confirm ozone as the cause of the visible injury. Visible foliar symptoms in filtered chambers (50% ambient ozone) were expected to occur later in the growing season but with less severity. Further development of the foliar symptoms was recorded every 3–5 days using a modified Horsfall–Barratt scale (0, 1, 3, 6, 12, 25, 50, 75, 88, 94, 97, 99, 100%) for recording percent leaf area injury (Horsfall and Barratt, 1945), and a 5% scale (0, 5, 10, 15, 20...100%) for

**Table 1**

<table>
<thead>
<tr>
<th>Scientific name</th>
<th>Common name</th>
<th>Years grown</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Alnus viridis</em> (Chaix.) DC.</td>
<td>Sitka Alder</td>
<td>1998</td>
</tr>
<tr>
<td><em>Cornus sanguinea</em> L.</td>
<td>Common Dogwood</td>
<td>1997, 1998</td>
</tr>
<tr>
<td><em>Cornus avellana</em> L.</td>
<td>European Filbert</td>
<td>1997, 1998</td>
</tr>
<tr>
<td><em>Fagus sylvatica</em> L.</td>
<td>European Beech</td>
<td>1997, 1998</td>
</tr>
<tr>
<td><em>Frangula alnus</em> Miller</td>
<td>Glossy Buckthorn</td>
<td>1998</td>
</tr>
<tr>
<td><em>Prausnitzia</em> avium L.</td>
<td>Sweet Cherry, Bird Cherry</td>
<td>1997, 1998</td>
</tr>
<tr>
<td><em>Rumex obatusfolius</em> L.</td>
<td>Bitter Dock</td>
<td>1997</td>
</tr>
<tr>
<td><em>Salix viminalis</em> L.</td>
<td>Silky Osier</td>
<td>1998</td>
</tr>
<tr>
<td><em>Sambucus racemosa</em> L.</td>
<td>Alpine Elder</td>
<td>1997</td>
</tr>
<tr>
<td><em>Viburnum lantana</em> L.</td>
<td>Wayfaring Tree</td>
<td>1997, 1998</td>
</tr>
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Fig. 1. Location of the Lattecaldo Cantonal Forestry Nursery, Morbio Superiore, Ticino, Switzerland.
estimating the percent of the entire plant with injured leaves. Training and eye calibration for repeatability of visual injury estimation were facilitated using the Forest Health Expert Advisory System (Nash et al., 1992a,b). Injury initiation was observed using a 10× hand lens.

Ozone concentrations were monitored from 3 March to 10 October in 1997 and from 13 March to 24 August in 1998. Ozone monitoring therefore began before leaf flush. Two-minute air samples were taken for ambient air at plot 5, and for each individual chamber on a 20-min interval period of sampling. A Monitor Labs Model 8810 Ozone Analyzer was used to determine ozone exposures within each of the respective treatment chambers and within one of the four open plots (ambient). Filtered chambers reduced ozone exposures by ca. 50% while non-filtered chambers delivered 96% of ambient ozone.

Cumulative parts per billion hours (ppb.h) expressed as AOT40 ozone exposures were examined against the development of foliar symptoms for each species for the 1997 and 1998 observations. Analysis of variance among species was performed to test for significant differences in sensitivity to ozone injury (Steel et al., 1997). Regression analysis was performed for AOT40 ozone thresholds and percent area injured for all species in 1997 and 1998. Statistical analysis was performed using SAS® 6.12 for Windows (SAS Institute Inc., 1985)

3. Results

3.1. Onset of foliar injury

The European air quality standard for tropospheric ozone in forests is set at an AOT40 value of 10 ppm.h during daytime hours over a 6-month growing season. The AOT40 standard was exceeded at the research site by 10 June 1997 and by 7 June 1998; cumulative ozone exposures (AOT 40) were higher in 1998 than in 1997 (Fig. 3).

For several previously untested species (Acer pseudo-platanus, Alnus viridis, Fraxinus excelsior, Frangula alnus, Morus nigra, Prunus avium, Rhamnus cathartica, Rumex obtusifolius, Salix viminalis, Sambucus racemosa and Viburnum lantana), the results confirmed that the ozone-like visible symptoms previously found during field surveys were indeed caused by ozone. Within open plots and non-filtered chambers, symptoms were expressed primarily as adaxial stipple. The stipple coloration was identical for the respective species to that observed earlier within the field surveys and in fumigation studies undertaken in CSTRs at the Pennsylvania State University (Innes et al., 1996). Chlorotic spotting, general leaf reddening and early leaf senescence comparable to former survey observations (Skelly et al., 2000) were also observed and recorded (VanderHeyden, 1999). As might be expected from the recorded season-long ozone concentrations, symptoms also occurred within the filtered chambers but with a much lower incidence and severity and little progression during the remainder of the growing season.

Species differed significantly in terms of the date of development of visible symptoms. The ppm.h AOT40 exposures that had accumulated prior to the onset of these first visible symptoms are presented in Figs. 4 and 5 in relation to date. The relationships between total AOT40 cumulative ozone exposures and injury onset for non-filtered and open plots are given in Fig. 6.
Injury progression differed among species but displayed similar trends. *Cornus sanguinea* and *Corylus avellana* did not show ozone-induced injury during the course of the experiment.

The onset of symptoms in the non-filtered chamber plots normally occurred earlier in the year (for most symptomatic species), and after lower cumulative ozone exposures (for all symptomatic species, with the exception of *Alnus viridis* in 1998) than in the open plots.

### 3.2. Development of foliar injury

Examples of injury progression for selected species during season long exposures of 1997 and 1998 are presented in Figs. 7–12. Injury progression for other species may be found in VanderHeyden (1999). The seasonal pattern of injury development followed by apparent recovery recorded for *Prunus serotina* in 1995 (Ghosh et al., 1998) was also observed for this species in both 1997
and 1998. The pattern can be attributed to the progressive loss of symptomatic leaves and replacement by new, uninjured leaves, and is apparent in the other indeterminate species used in the study.

Of those species showing significant foliar injury, *Prunus serotina*, *Rhamnus cathartica* and *Viburnum lantana* exhibited a faster rate of symptom development and ended with greater severity expressed as total leaf area injured during the 1998 growing season when compared to 1997 (Figs. 7–9). Likewise, for *Morus nigra*, there was significantly more injury in 1998 than in 1997. There was a comparable rate of development and total amount of leaf injury for *Prunus avium* during both years of the investigation (Figure 10). By the end of the observation season, foliar injury for the more sensitive species was ca. 20–30% of the total leaf area per plant. As with observations elsewhere (e.g. Skelly et al., 1987; Innes et al., 1996), foliar injury was most prominent on the older and, therefore, longest-exposed leaves. Seasonal patterns of increasing symptom development were observed for all other species examined within this investigation but the total percent of plant leaf area injured was considerably lower (i.e. < 5%; Figs. 11, 12).

### 3.3. Relative sensitivity of species

Based upon the development of the initial foliar symptoms in response to the cumulative AOT40 exposures, the most to least symptomatic species grown within open-plots in this study rank as *Prunus serotina*, *Viburnum lantana*, *Salix viminalis*, *Rhamnus cathartica*, *Betula pendula*, *Prunus avium*, *Fraxinus excelsior*, *Morus nigra*, *Alnus viridis*, *Frangula alnus*, *Fagus sylvatica* and *Acer pseudoplatanus*. Similar rankings were obtained in

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**Fig. 6**. AOT40 threshold to show injury for each treatment 1997 vs. 1998. Daytime 7.00–18.59, Lattecaldo Cantonal Forestry Nursery, Morbio Superiore, Switzerland. Lower case indicates a species was not present that year. Species ordered by 1998 open plot thresholds.
the non-filtered chamber plots (Fig. 6). No obvious symptoms developed on *Corylus avallana* during the period of observations although a very fine upper surface stipple was evident during the close-down of the site in mid-September. The timing and general appearance of leaf reddening as a possible ozone-induced symptom on *Cornus sanguinea* was indistinguishable across the plants in the various chambered and open-plots. Some shifting of the 1998 sensitivity rankings were in evidence in the 1997 season but *Prunus serotina*, *Viburnum lantana*, *Salix viminalis*, *Rhamnus cathartica*, and *Morus nigra* were still among the most sensitive (Fig. 6). *Rumex obtusifolius* and *Sambucus racemosus* would rank within this group of most sensitive species as based upon their initiation of symptoms to AOT40 exposures during the 1997 season. These two species were replaced in early spring 1998 due to their large size in overcrowding the surrounding species.

The ranking of species sensitivity closely follows AOT values for the occurrence of initial symptoms and symptom progression across the remainder of the exposure season (Figs. 4–6). Species that first showed evidence of foliar injury (e.g. *Prunus serotina*, *Viburnum lantana*, and *Rhamnus cathartica*) also demonstrated the most sensitivity throughout the growing season, with symptoms rapidly advancing over ca. 25–30% of the total plant leaf surfaces by the end of the observation period (Figs. 5–7). Conversely, those species that developed symptoms later in the season (e.g. *Rumex obtusifolius*, *Fagus sylvatica*, *Alnus viridis*, and *Rhamnus*...
frangula) had far less total injury to plant foliage by the end of the observation period (1.5 to <5% total leaf area injured).

4. Discussion and conclusions

Symptoms of ozone-induced foliar injury were observed on a variety of species within the open and non-filtered chamber plots. Symptoms were comparable to typical symptoms observed for the respective species under natural conditions and ambient ozone exposures at many sites in Switzerland (Skelly et al., 1998; 2000). More species showed injury in 1998 than in 1997. This was to be expected because of the stresses encountered during the first year of planting (1997) and the more fully developed seedlings that were present during the 1998 season. Additionally, AOT40 values (Fig. 3) and peak ozone episodes (VanderHeyden, 1999) were higher in 1998 than in 1997. Continuing ozone exposures were related to the rapid advancement of symptoms for several ozone-sensitive species grown within the open plots at the Ticino site across two seasons of ambient ozone exposures. The use of open-top chambers and reduced ozone exposures in the filtered air supplied chambers (50% of ambient) resulted in no symptom development and/or, more commonly, delayed and far less severe symptoms. Thus ambient ozone exposures were shown to be responsible for the symptoms observed within this study and the previous surveys of reported symptoms for many of these species.
In this study, by making repeated, detailed observations of a large number of plants, the onset of first symptoms was successfully determined and these symptoms were subsequently tracked across whole leaf surfaces. Foliar symptoms often developed earlier in the year and at lower cumulative ozone exposures in the non-filtered chambers than in the open plots. We did not make any measurements at the time that would explain this observation. However, we believe that the differences are most likely due to microclimatic differences (temperature, water availability, radiation, air turbulence) between the open plots and chambers. In addition, the data presented here do not enable the determination of the relative importance of short- and long-term ozone exposures in the onset and development of foliar injury. However, several commonly occurring species (Prunus serotina, Rhamnus cathartica, Viburnum lantana, Salix viminalis and Betula pendula) proved sensitive in open plots at thresholds below the current long-term AOT40 standard. Consequently, achievement of this standard will not necessarily protect some tree, shrub and herb species from ozone-induced injury.

In this paper, the timing and development of ozone-induced foliar injury for a number of species have been identified. Information has also been presented on the relative sensitivity of the species involved in the study. It is important to point out that these results are dependent of the material used in the study. All plants were obtained from seed sources in the canton of Ticino and, in some cases (e.g. with Morus nigra and Prunus serotina),
the seed was obtained from one or more symptomatic trees. Given the within-species variability in sensitivity that has been encountered with _Prunus serotina_ (Lee et al., 1999), it is likely that a different set of seed sources would have yielded rather different results. Consequently, care should be taken in any extrapolation of these results to the entire range of the species involved. This is particularly so given that the incidence of ozone-induced injury to plants in southern Switzerland may be the result of the particular combination of high exposure, high radiation levels and high soil-moisture availability (associated with the insubric climate). However, it is interesting to note that _Prunus serotina_ is generally recognized as an ozone-sensitive species in the USA and Mexico (Skelly et al., 1987, 1997) — despite the presence of highly tolerant genotypes (Lee et al., 1999) — and that several of the genera involved (e.g. _Fraxinus_, _Sambucus_) have previously been documented as containing sensitive species (e.g. Davis and Coppolino, 1976; Steiner and Davis, 1979).

At the workshop “Critical Levels for Ozone in Europe: Testing and Finalizing the Concepts”, held in Kuopio, Finland, on 15–17 April 1996, experiments made with young _Fagus sylvatica_ and _Picea abies_ were used to define a Level I critical level. Level I is defined as setting a single critical load, designed to protect the most sensitive known receptor under the most sensitive environmental conditions. This is consistent with the critical loads concept, which is concerned with any adverse effects on the most sensitive components of an ecosystem (Nilsson and Grennfelt, 1988). The workshop identified deciduous trees as the most sensitive receptors, and the standard was based on three open-top chamber studies (Küppers et al., 1994, Braun and Flückiger, 1995; Steinrüver et al., 1995). The number of suitable studies for the standard was very limited, and the limitations of the standard have been acknowledged (Fuhrer et al., 1997; Skärby et al., 1998). A value of 40 ppb was identified as the cut-off level for calculating a biologically meaningful index of ozone exposure. The response parameter chosen was percentage reduction in annual biomass increment. The AOT40 index was to be calculated for daylight (<50 W m⁻²) hours only, and for the 6-month period starting on 1 April. Although it was realised that this might be inappropriate for some Mediterranean countries, the 1 April date was accepted. The critical level of 10 ppm.h over 40 ppb was accepted.

The AOT40 Level I threshold does not address the problem of whether ozone is adversely affecting the most sensitive components of the forest ecosystem. As shown in this paper, the most sensitive components are not necessarily the dominant tree species, nor is the most sensitive indicator necessarily a reduction in above-ground biomass increment. Consequently, the current Level I threshold that is currently used fails to achieve its goal, namely to protect the most sensitive receptor under the most sensitive environmental conditions. There is, therefore, an urgent need to examine indicators and species that are currently known to be more sensitive than those used in the current assessments.

The current long-term standard is based on the ozone exposure believed to induce a “direct adverse effect” (UNECE, 1988). For ozone, this has been interpreted as a significant (i.e. 10%) reduction in the annual biomass increment of trees, the justification being that biomass increment is the end result of many internal and external processes and because it has clear practical and economic value (Fuhrer et al., 1997). However, the interpretation of growth data is notoriously difficult [see, for example, the difficulties surrounding the explanation of the documented (Spiecker et al. 1996) increases in tree growth over much of Europe]. We have not linked the onset and development of foliar injury to biomass; this was outside the scope of the study and our sampling design was not intended for this purpose. However, at a time when less and less emphasis is being placed on the role of European forests in timber production, we consider that foliar injury in itself is an important diagnostic, that its use is entirely consistent with the 1988 UNECE definition of a critical level and that the use of production-related criteria alone for assessing impacts on trees and forests is inappropriate. Fuhrer et al. (1997) and Skärby et al. (1998) have also expressed concern about the use of an index based on biomass increment when looking at ozone impacts on forests. While production-related indices remain crucial for agriculture, they are of less importance within the context of European forests in the 21st century, and we consider that the use of indices such as foliar injury should receive more attention when assessing the impacts of pollutants such as ozone on forest vegetation.

References


