

## OZONE – A RISK FACTOR FOR TREES AND FORESTS IN EUROPE?

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(Received 25 September 1998; accepted 25 February 1999)

**Abstract:** Tropospheric ozone (O<sub>3</sub>) may adversely affect tree growth, with critical levels for O<sub>3</sub> being exceeded in many parts of Europe. However, unequivocal evidence for O<sub>3</sub>-induced foliar injury on woody species under field conditions has only been found in a few places. Visible O<sub>3</sub> injury appears to occur mainly in the Mediterranean Basin, which is also the area where the least amount of information is available on O<sub>3</sub> exposure as well as the sensitivity of individual species. Overall, the quantitative risk assessment of O<sub>3</sub> impacts on mature trees and forests is vague at the European scale, as most knowledge is derived from controlled O<sub>3</sub> fumigations of young trees, grown in isolation in exposure chambers. Research suggests that risks exist, but these need to be validated for stand conditions. O<sub>3</sub>-induced changes in resource allocation rather than productivity appear to be crucial as they affect competitiveness and predisposition to parasite attack and may eventually lead to the loss of genetic diversity. 'Free-air' O<sub>3</sub> fumigations in forest canopies may reveal processes that are susceptible to O<sub>3</sub> stress under field conditions and provide a scientific basis towards quantitative risk assessment and realistic definitions of critical levels for O<sub>3</sub> in forest ecosystems.

**Keywords:** Tropospheric ozone, Forests, Ecological risk analysis, Critical levels, Free-air fumigation, Visible injury, European Alps

### 1. Introduction

Of the various different types of air pollutants currently impacting forests in Europe, the greatest concern surrounds ozone (O<sub>3</sub>). In contrast to pollutants associated with heavy industry, such as sulphur dioxide (SO<sub>2</sub>), O<sub>3</sub> concentrations do not appear to have declined in recent years, and concentrations in some areas may even be increasing (Stachelin *et al.*, 1994; Dollard *et al.*, 1995). Current levels are clearly greater than pre-industrial concentrations (Volz and Kley, 1988; Anfossi *et al.*, 1991), but current trends in O<sub>3</sub> concentrations are uncertain, as systematic monitoring in Europe was only started in the late 1980s. In addition, many measurement stations are primarily aimed at assessing O<sub>3</sub> levels in relation to human health risks and many monitoring stations are therefore located in urban and suburban areas. These stations have little relevance to O<sub>3</sub> concentrations in rural situations because of scavenging by nitrogen oxides (NO<sub>x</sub>).

The following account concentrates on rural environments as these are of relevance for examining the risks that O<sub>3</sub> may impose to forest trees and forest ecosystems. After providing an overview of O<sub>3</sub> regimes in the major regions in Europe, visible injury in woody plants is examined as an indicator of O<sub>3</sub> impact and in relation to current definitions of 'Critical Levels for Ozone'. Evidence for O<sub>3</sub> impacts at the ecosystem level is examined, as is the information required for judging the level of risk to ecosystems. With respect to trees, risks inferred from young plants under controlled exposure conditions must be the basis for scenarios of O<sub>3</sub> effects on forest stands. The importance of tree ontogeny is highlighted and linked to potential mechanisms of O<sub>3</sub>

impact on mature trees and forest ecosystems. Finally, approaches towards a mechanistic understanding of O<sub>3</sub> impact under stand conditions are examined and, through this, possible methods for a quantitative risk assessment of forest development are proposed.

In contrast to the recent, excellent review by Skärby *et al.* (1998), we concentrate on risk assessment and its uncertainties for mature trees and forests as related to the European O<sub>3</sub> scenarios. Mechanisms of O<sub>3</sub> impact and their interactions with other factors in young plants, as well as the 'Critical Level' concept, are addressed only as a 'reference' for approaching risk assessment in the field. However, whole-plant allocation is viewed as a determinant of O<sub>3</sub> impacts on competitiveness and the predisposition of trees to multiple stress and thus as a key factor in determining resource fluxes and the responsiveness of forest ecosystems to O<sub>3</sub> stress.

## 2. Characteristics of O<sub>3</sub> regimes in Europe

Knowledge of the characteristics of O<sub>3</sub> regimes over the European region is at best patchy. In the alpine region (Switzerland, Austria, and southern Germany), the majority of stations show no clear trends (Smidt, 1998). For example, in Switzerland, annual average concentrations, April-September 1000-1700 concentrations, peak hourly concentrations and the numbers of hours above 60 ppb all show no clear trends over the period 1982-1996 (BUWAL, 1997). This contrasts with longer-term data obtained for the upper troposphere, where a definite increase in O<sub>3</sub> has been recorded in the period 1969-1989 (Staehelin and Schmid, 1991) and with data from other countries, such as the United Kingdom (Dollard *et al.*, 1995), where increasing concentrations have also been documented.

In central and northern Europe, O<sub>3</sub> episodes primarily occur downwind of industrialised areas (Proyou *et al.*, 1991; Grennfelt and Beck, 1994). Episodes are typically associated with periods of high pressure, when atmospheric conditions are relatively stable. O<sub>3</sub> episodes can also occur in spring (e.g. Davies and Schuepbach, 1994; Staehelin *et al.*, 1994; Laurila and Tuovinen, 1996; Skärby and Karlsson, 1996), although the causes are uncertain, with incursions of stratospheric O<sub>3</sub> being a likely contributing factor (UKPORG, 1987; Ebel *et al.*, 1991; Davies and Schuepbach, 1994). Tropospheric O<sub>3</sub> formation is dependent on sufficient concentrations of NO<sub>x</sub> and reactive volatile organic compounds (VOCs). In urban areas, concentrations of VOCs tend to limit the formation of O<sub>3</sub>, whereas in rural areas, O<sub>3</sub> production tends to be limited by NO<sub>x</sub> concentrations (Chameides *et al.*, 1992). The known emissions of various pollutants, combined with the available monitoring data, has enabled maps to be prepared of the distribution of O<sub>3</sub> in Europe (Hettelingh *et al.*, 1996, 1997). One such map, prepared in relation to the accumulated hourly O<sub>3</sub> exposure above 40 ppb (AOT40) for forests, is shown in Figure 1. This map is based on 50 km x 50 km grid squares, and therefore the variation in O<sub>3</sub> concentrations that occurs with altitude (e.g. Reiter *et al.*, 1987; Proyou *et al.*, 1991; Loibl and Smidt, 1996) is inadequately addressed.

Reliable estimates of O<sub>3</sub> concentrations are extremely difficult to obtain from eastern Europe, although concentrations in areas as far east as the Ukraine are known to

be sufficient to cause visible injury to Bel-W3 tobacco, and O<sub>3</sub>-like symptoms have been recorded on sensitive species such as red-berried elder (*Sambucus racemosa*) and wild hop (*Humulus lupulus*) (Blum *et al.*, 1997). Potentially phytotoxic concentrations have also been reported in Poland (Bytnerowicz *et al.*, 1993). Other information is very limited, however modelling studies suggest that AOT40 thresholds are regularly exceeded in the region (Dollard *et al.*, 1995).

AOT40f (1990) over forests

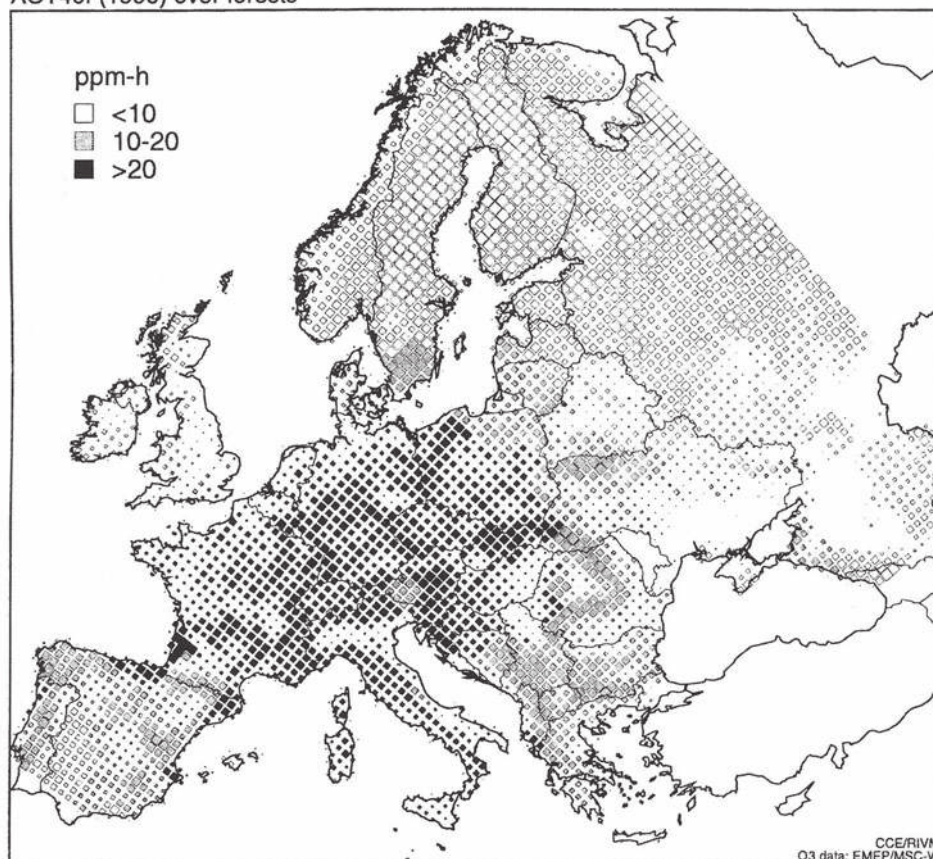


Fig. 1. AOT40 values for forests in Europe in 1990. Areas with <10 ppm.h fall below the critical level identified as potentially causing growth losses in forests. Map prepared by M. Posch of the UN-ECE Co-ordination Center for Effects (Working Group on Effects, Convention on Long-Range Transboundary Air Pollution) using ozone data from EMEP/MSC-W.

Special conditions surround the occurrence of O<sub>3</sub> in the Mediterranean region. The region is dominated by two major weather systems: the Azores anticyclone in the west and a low-pressure system in the east which extends from the Middle East to southwest Asia (Sanz and Millán, 1998). The boundary between the two is located over Italy, and

changes in the relative importance of the two systems have a marked impact on air quality in the central Mediterranean. This large-scale pattern is modified by local conditions. Much of the Mediterranean is surrounded by mountains 1500 m or more in altitude. The orography of the region results in site-specific meteorological conditions influenced by sea breezes and up-slope winds, which can have marked impacts on patterns of local air quality (e.g. Lalas *et al.*, 1983). Consequently, in the Mediterranean region, the maps produced by EMEP (Figure 1) appear to be of less value than elsewhere in Europe, as meso-scale processes result in significant diurnal fluctuations that are not taken into account in the EMEP model (Sanz and Millán, 1998).

In the western Mediterranean, the build-up of O<sub>3</sub> concentrations occurs through the enhancement of sea breezes by up-slope winds in the coastal region (where there are substantial NO<sub>x</sub> sources) and the subsequent seaward return of these air masses at high altitudes. This causes the formation of stacked layers, 2-3 km deep and more than 300 km wide, with the oldest air masses near the sea (Millán *et al.*, 1996, 1997). Such circulation cells result in the build-up of high O<sub>3</sub> concentrations, with the usual model of increasing concentrations downwind of NO<sub>x</sub> being sources being inapplicable. Similar processes probably occur over the Italian Peninsula, but detailed information is lacking (Millán *et al.*, 1997).

O<sub>3</sub> concentrations are extremely difficult to characterise in a way that is meaningful for biological impact studies. For example, there is still considerable debate over the relative importance of short-term peak concentrations versus longer-term accumulated concentrations (Lefohn 1992). For forests, the latter approach has been deemed the most suitable, and the AOT40 index has been devised as a critical level. This is based on the accumulated hourly exposure to O<sub>3</sub> above 40 ppb during daylight hours (>50 W m<sup>-2</sup>) between April and September (Kärenlampi and Skärby, 1996). The critical threshold of 10 ppm.h relates to an estimated 10% reduction in the growth of young (0-6 year old) beech (*Fagus sylvatica*) trees exposed to O<sub>3</sub> for between one and three seasons, based on five studies of above-ground growth and three studies of above-ground biomass increment (see Fuhrer *et al.* (1997) for further details of these studies). As these figures relate to above-ground responses only, the AOT40 index fails to take into account the substantial effects that are expected to occur below-ground as indicated from experiments with young trees grown under controlled conditions (Matyssek *et al.*, 1995a).

The AOT40 studies suggest that substantial areas of forest may be at risk and, although the objective of the UN-ECE Level I O<sub>3</sub> standards is simply to indicate where adverse effects of O<sub>3</sub> might occur (Skärby *et al.*, 1998), an O<sub>3</sub>-induced loss in forest productivity of 10% has been estimated for Europe (Broadmeadow, 1998). There seems to be very little basis for such a statement, which is similar to the scenarios developed in the 1980s for growth losses induced by acid rain and later shown to be without foundation. AOT40 values are widely exceeded in the European Alps (Figure 2), where the presence of suitable precursors combines with altitudinal effects to result in widespread exceedances (Smidt, 1998). At a European scale, a substantial proportion of the forested area appears to be at risk from O<sub>3</sub>, as judged by the AOT40 standard (Figure 3). Consequently, attention on the impacts of gaseous pollution on forests has increasingly concentrated on O<sub>3</sub> as the most important risk facing European forests today.



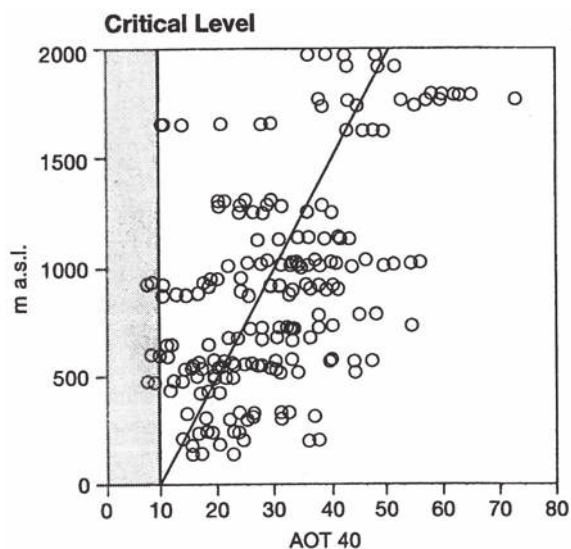


Fig. 2. Measured AOT40 values (1991-1996) for a variety of sites located near forests in the Austrian, German and Swiss Alps. From Smidt (1998), with permission.

### 3. Evidence of visible O<sub>3</sub> injury in woody species

In the USA, O<sub>3</sub> is widely recognized as a pollutant that can induce visual injury of foliage. Research in the San Bernardino Mountains of California has shown that O<sub>3</sub> is responsible for the development of visible injury in ponderosa pine (*Pinus ponderosa*) and Jeffrey pine (*Pinus jeffreyi*) (Miller *et al.*, 1963; Miller and Millecan, 1971; Miller and McBride 1999). Subsequent work has indicated visible injury to many species growing in forest environments, not only in California, but also in other parts of the USA (e.g., Davis and Skelly, 1992; Neufeld *et al.*, 1992; Simini *et al.*, 1992; Miller *et al.*, 1994; Chappelka *et al.*, 1997; Skelly *et al.*, 1997; Chappelka and Samuelson, 1998). Typical symptoms described in the literature include red, brown, purple or black stipple on the upper sides of sun-exposed leaves of broad-leaved trees (Brantley *et al.*, 1994; Skelly *et al.*, 1987; Flagler 1998), and chlorotic mottling of older needles in conifers.

Considering the widespread evidence of O<sub>3</sub> injury for American species, the scarcity of published information of confirmed visible symptoms of ambient O<sub>3</sub> injury to European species is rather surprising (see Davison and Barnes, 1998). Several studies have reported injury under experimental conditions (e.g. Günthardt-Goerg *et al.*, 1996, 1997), but confirmed records of O<sub>3</sub> injury under ambient field conditions are extremely limited. In a study reported by Skelly *et al.* (1998a, 1998b), several species showing symptoms typical of O<sub>3</sub> injury were grown in ambient and filtered (ca. 50% O<sub>3</sub>) air in southern Switzerland. It was possible to reproduce under experimental conditions the

symptoms recorded in the field, and reducing the ambient level of O<sub>3</sub> by ca. 50% delayed the onset of symptom development. O<sub>3</sub> injury has been confirmed on a number of native Swiss species, including ash (*Fraxinus excelsior*), beech (*Fagus sylvatica*), red-berried elder (*Sambucus racemosa*), silver birch (*Betula pendula*), wayfaring tree (*Viburnum lantana*), black mulberry (*Morus nigra*), buckthorn (*Rhamnus catharticus*) and osier (*Salix viminalis*) (Skelly *et al.*, 1998b). O<sub>3</sub>-induced visible injury has not been documented on any coniferous species in southern Switzerland, but elsewhere in southern Europe there have been a number of records of O<sub>3</sub>-like symptoms on Aleppo pine (*Pinus halepensis*), Arolla pine (*P. cembra*), Austrian pine (*P. nigra*) and Greek fir (*Abies cephalonica*) (e.g. Gimeno *et al.*, 1992; Velissariou *et al.*, 1992, 1996; Dalstein *et al.*, 1997). In at least one of these species (Aleppo pine), the visible injury has been confirmed in experimental studies as being caused by O<sub>3</sub> (Gimeno *et al.*, 1992; Anttonen *et al.*, 1998).

The severe nature of visible injury to plants in southern Switzerland is particularly interesting. A combination of factors probably influences the occurrence of such injury. Firstly, O<sub>3</sub> concentrations are high, and six-month, daylight AOT40 values are often in excess of 30 ppm.h (BUWAL, 1997; Staffelbach *et al.*, 1997). These cumulative values are accompanied by hourly peak concentrations of between 150 and 200 ppb. Secondly, southern Switzerland is unusual in having high amounts of summer rainfall, a characteristic of its insubric climate (Frei and Schär, 1998) which extends along the southern edge of the Alps, from Mont Blanc in the west to the Slovenian border in the east. Thirdly, although this remains to be confirmed, radiation intensity is high in southern Switzerland in comparison to the northern side of the Alps. In most other parts of Europe, this particular combination of conditions is not apparent. For example, in many parts of the Mediterranean region, the highest concentrations of O<sub>3</sub> occur in mid-to late summer, when most plants are suffering severe drought and might therefore be expected to experience lower O<sub>3</sub> doses than is at first apparent. The response of trees to drought stress in the Mediterranean region varies, with some species [e.g. Holm oak (*Quercus ilex*), Downy oak (*Q. pubescens*), Manna ash (*Fraxinus ornus*), and Eastern hornbeam (*Carpinus orientalis*)] exhibiting reductions in stomatal conductance and photosynthesis during the driest months, while other species (e.g. Phillyrea (*Phillyrea latifolia*) and Turpentine tree (*Pistacia terebinthus*)) show no clear responses (Tretiach, 1993; Damesin and Rambal, 1995; Radoglou, 1996). The effects are likely to vary with local water availability (see Rico *et al.*, 1996), and this will make any risk presented by O<sub>3</sub> in the Mediterranean difficult.

#### 4. Discrepancies between observed patterns in forests and the AOT40 critical threshold

The AOT40 critical threshold is intended to provide "an environmental standard of threshold to minimise the effects of O<sub>3</sub> on sensitive receptors, but does not seek to quantify the impacts of exceeding the critical level under field conditions" (Fuhrer *et al.*, 1997). The maps of AOT40 exceedances (e.g. Figure 1) and risk assessments that have been undertaken to date (e.g. Figure 3) suggest that substantial effects on European forests should be apparent. While visible injury seems not to be widespread,

it would be logical to expect to find evidence of growth reductions amongst European trees. This has not been the case, and the available data actually suggest that growth rates of forests in several parts of central Europe are currently increasing (Spiecker *et al.*, 1996). Several possible reasons for this have been proposed, including increased nitrogen deposition, fertilisation by CO<sub>2</sub>, a more favourable climate, better forest management and an increasing length of the vegetation period.

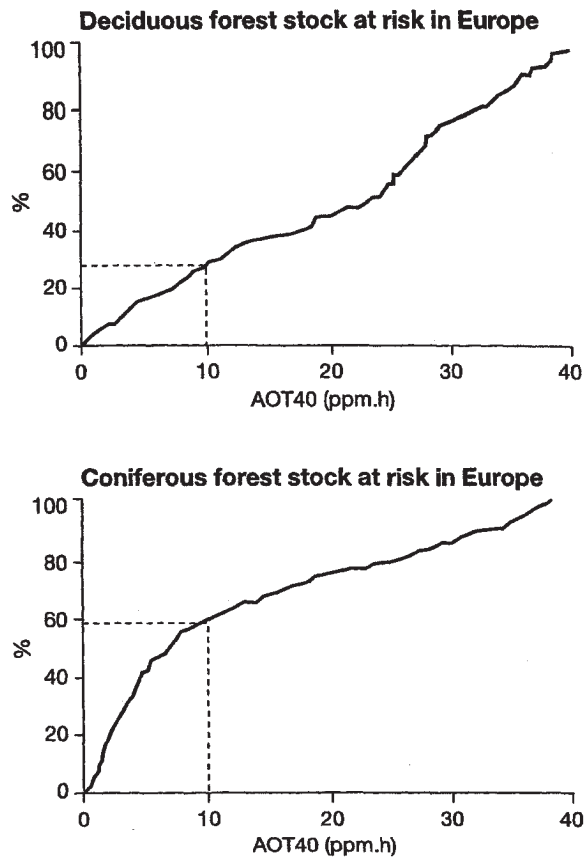


Fig. 3. Deciduous and coniferous forests at risk as judged by exceedance of the AOT40 critical level in 1990. The dotted lines indicate the proportion of forest area that lies below the AOT40 exposure threshold. The proportion of deciduous forest at risk (72%) is much greater than the proportion of coniferous forest (41%). From Hettelingh *et al.* (1996).

Why should there be discrepancies between the AOT40 risk analyses and field observations? Several points can be raised about the threshold and the way in which it has been defined. It is based on observed growth reductions under experimental conditions. Leaving aside the substantial problems associated with extrapolating results from young trees growing in an experimental environment to mature trees growing in a forest situation (e.g. Fredericksen *et al.*, 1995, 1996a,b,c; Kolb *et al.*, 1997), the

approach makes the implicit assumption that timber supply is the only forest product of interest. Today, many foresters would disagree with this, although economic constraints usually mean that timber production remains the primary aim of most forestry enterprises. Moreover, the threshold of 10 ppm.h has very little scientific basis. It is too high to protect the most sensitive species from adverse impacts (Skelly *et al.*, 1998b), and appears to be too low to provide information on species which are less sensitive to O<sub>3</sub> (e.g. Norway spruce (*Picea abies*)). There may also be a number of internal and external factors that must provide some form of O<sub>3</sub> buffering capacity for trees growing in forests.

Another issue concerns the threshold level of 40 ppb. The choice of this level was based on empirical information from a small number of studies and it remains very artificial. However, the level has not been adopted in the USA (where 60 ppb is used), and in one Scandinavian study, the best fit between response parameters and cumulative exposures was obtained when a threshold of 30 ppb was adopted (Pääkkönen *et al.*, 1996). Skärby (1994) has also suggested a 30 ppb threshold to be more applicable in relation to Norway spruce (*Picea abies*). As additional studies of AOT thresholds become available (e.g. Mortensen, 1998), it should be possible to evaluate the usefulness of the current 40 ppb threshold.

There are also uncertainties about both the seasonal period (April – September) and the daylight hours used in the calculations of AOT40. For example, under high mid-day radiation conditions, typical of the Mediterranean region, the stomata of many species may narrow (Tretiach, 1993) or even close (Epron *et al.*, 1992; Inclán *et al.*, 1998). This throws serious doubts on the use of mid-day O<sub>3</sub> concentration values in the cumulative exposure indices that are calculated for some areas.

Further uncertainties surround the response parameters used in AOT40 calculations. While above-ground growth seems under certain circumstances to be correlated quite well with exposure as expressed as AOT40, other equally valid indices, such as visible injury, do not correlate well. This may be because the relationship between visible injury and growth is very variable, and may not exist for some species (Somers *et al.*, 1998). Consequently, the Level 1 AOT40 threshold must be viewed as a first approximation and there should be increased emphasis on the identification of Level 2 thresholds in future.

### 5. Potential impacts of O<sub>3</sub> on forest ecosystems in Europe

The AOT40 value was designed as a means of assessing the potential risk of O<sub>3</sub> to forest growth. It is already known that the value will not protect sensitive individuals from visible injury (Skelly *et al.*, 1998a). Effects on the forest ecosystem as a whole are much more difficult to assess. The effects of O<sub>3</sub> on wild plants have recently been addressed by Davison and Barnes (1998), whereas effects on animals are covered in Anon. (1996).

The majority of O<sub>3</sub> impact studies have concentrated on species of economic importance (commercially important trees and crops), and on response indicators of economic value (growth and yield). However, a picture is slowly beginning to emerge about the possible ecosystem-level effects of O<sub>3</sub>. O<sub>3</sub> can significantly inhibit the growth



and reproduction of the most sensitive individuals within a population (Karnosky, 1981; Berrang *et al.*, 1991). This may lead in the long-term to the loss of the most sensitive genotypes from the population (Karnosky, 1981; Berrang *et al.*, 1989, 1991). At a time when the value of genetic diversity is being increasingly realised, these losses may be important. For example, the genetic diversity of a population plays a major role in its resistance to a broad range of other stresses (Müller-Starck and Ziehe, 1991).

One widely used indicator of forest condition is crown defoliation, although this particular indicator has many problems associated with its assessment (Innes, 1990; Innes *et al.*, 1993) and interpretation (Innes, 1993; Ferretti, 1998). Crown condition varies substantially between years and in most cases correlates poorly, if at all, with O<sub>3</sub> exposure (Innes *et al.*, 1997; Klap *et al.*, 1997). Until now, O<sub>3</sub> has been shown to be an important determinant of tree health only in very special circumstances (e.g. Skelly *et al.*, 1998b) and has not been demonstrated to be a widespread cause of forest decline (Skelly and Innes, 1994; Kandler and Innes, 1995). A major difficulty in the interpretation of such studies is the extent of the information on the spatial and temporal distributions of O<sub>3</sub>. Normally, interpolated values are used, but the reliability of such values is questionable. Even if they are accurate, they may have very little relevance to the actual dose received by trees. In addition, the use of crown defoliation as a response variable is questionable because it is influenced by so many other factors. Growth is equally difficult to interpret because it too is affected by a range of different factors. Consequently, any effects of O<sub>3</sub> may be masked by other causes.

#### **6. Discrepancies between available and required knowledge on risks presented by O<sub>3</sub>**

The major difficulty associated with the analysis of the physiological status of mature forest trees is the multi-factorial impact that may bias any response to O<sub>3</sub> (Arndt and Seufert, 1990). Despite this, there is no doubt that O<sub>3</sub> poses a risk to woody vegetation (Reich, 1987; Pye, 1988), not only through acute peak concentrations, but also through chronic exposures to enhanced mean O<sub>3</sub> levels. The impacts of chronic exposures over decades are likely to have effects, but the available information is extremely limited. With the exception of a few experimental O<sub>3</sub> exposures of branches or crowns of tall trees (see 11.), most of our knowledge on the risks of O<sub>3</sub> derives from young trees exposed to controlled O<sub>3</sub> treatments (Matyssek *et al.*, 1995a). Typically, seedlings or saplings have been grown in pots in various kinds of fumigation chamber, often under non-limiting conditions except for O<sub>3</sub>. They have then been compared - under the exclusion of competition and parasites - with corresponding control plants exposed to low levels of O<sub>3</sub> or O<sub>3</sub>-free air. Using such techniques, even minor O<sub>3</sub> effects are detectable, even more so if clonal plants are used for minimizing the statistical variability in individual plant response.

Although such approaches have been valuable in revealing the principles of O<sub>3</sub> action and in showing that O<sub>3</sub> is an injurious pollutant, the findings are, *a priori*, not representative for forest sites as they are more or less biased by the nature of the exposure (Chappelka and Chevone, 1992). Given the lack of validation for forest sites and the uncertainties about the long-term acclimation and sensitivity of mature trees to

O<sub>3</sub>, any expert opinion on the risks of O<sub>3</sub> must still largely rely on findings from young trees under controlled chamber exposures.

**7. Risk assessments based on observations of young trees under  
8. controlled chamber exposures**

To the extent that boundary layers enable O<sub>3</sub> to reach the leaf surface, the diffusive influx of O<sub>3</sub> into the plant is mainly determined by the stomatal number and aperture (Reich, 1987; Laisk *et al.*, 1989). Both stomatal density (which develops during leaf growth) and aperture are modified by chronic O<sub>3</sub> impact (Keller and Häsler, 1984; Frey *et al.*, 1996), although the available results are inconsistent in terms of the extent and direction of the response (Matyssek *et al.*, 1995a, 1998). Partial closure of stomata may occur, but often in such cases, and even in the absence of a stomatal response, leaf 'water-use efficiency' declines (i.e. photosynthetic CO<sub>2</sub> uptake *versus* transpiration rate, WUE; Sasek and Richardson, 1989; Schweizer and Arndt, 1990; Matyssek *et al.*, 1991; Lippert *et al.*, 1996; Maurer *et al.*, 1997). This effect on WUE is mainly caused by injury to mesophyll cells. The primary site of the impact by O<sub>3</sub> or its oxidative degradation products is on the walls and plasmalemma of these cells. This impact, though being 'buffered' by antioxidative defence systems in the apoplast (Polle *et al.*, 1995; 1998), may induce structural changes in the cell walls (Günthardt-Goerg *et al.*, 1993, 1997; Maier-Maercker, 1998), although being 'buffered' by antioxidative defence systems in the apoplast (Polle *et al.*, 1995, 1998). The strain on the plasmalemma may eventually lead through unknown biochemical signal transduction to chloroplast degradation and 'programmed cell death' similar to defence reactions induced by pathogenic infections (Sandermann, 1996; Kangasjärvi *et al.*, 1994). Mesophyll cell collapse and, eventually, premature leaf loss are the result (Matyssek *et al.*, 1991). In conifers, needle loss (e.g. through reduced frost tolerance) may be delayed until the growing season succeeding the O<sub>3</sub> stress event, and induced changes in the secondary metabolism may persist (Sandermann 1996). Remarkably, all other changes in the plant can be traced back to the primary site of O<sub>3</sub> action, i.e. the apoplast and plasmalemma.

Injury to mesophyll cells not only lowers the carbon gain of the leaves, but may also impede, together with the disturbance of phloem loading and transport, assimilate translocation to other tree organs (Günthardt-Goerg *et al.*, 1993; Rennenberg *et al.*, 1996; Polle *et al.*, 1998). The growth limitation of non-photosynthetic organs may be exacerbated by the enhanced assimilate demand of O<sub>3</sub>-stressed foliage for detoxification and repair, as indicated by raised respiration rate and altered primary metabolism (Maurer *et al.*, 1997; Landolt *et al.*, 1997; Einig *et al.*, 1997; Saurer *et al.*, 1995), or for compensatory new-leaf formation (Mooney and Winner, 1991; Tjoelker and Luxmoore, 1991). Often, the organ that is most limited is the root (Andersen *et al.*, 1991; Coleman *et al.*, 1996), although conclusions from short-term experiments on the long-term uptake capacity of roots for water and nutrients have not yet been clarified (Matyssek *et al.*, 1993, 1995a). C partitioning between root diameter classes may remain stable (Maurer and Matyssek, 1997), but increased fine-root production has been found in seedlings under O<sub>3</sub> stress (Kelly *et al.*, 1995). In addition, the above-ground allocation can be affected by O<sub>3</sub>: Often radial rather than longitudinal stem growth (with unknown

consequences for long-term mechanical stability) as well as lateral branching and the weight and size of individual leaves are reduced (Schier *et al.*, 1990; Matyssek *et al.*, 1992, 1993; Samuelson *et al.*, 1996). Whenever crown and root architecture are modified, plant competitiveness may be affected, because the efficiency between space occupation and resource acquisition is influenced (Küppers, 1994; Tremmel and Bazzaz, 1995). The significance for plant competition and, potentially, biodiversity, has been demonstrated in semi-natural plant communities (Nebel and Fuhrer, 1994; Barbo *et al.*, 1998), but not for woody species. The risks caused by O<sub>3</sub> through changing resource allocation should not be overlooked relative to potential limitations in plant productivity.

#### 8. Action of O<sub>3</sub> in concert with other biotic and abiotic influences

The risks highlighted above may be moderated, with simultaneous increases in uncertainty, by factors acting in concert with O<sub>3</sub> - and still, we must largely rely on findings from young plants under controlled conditions. One factor often overlooked is the interaction between mycorrhizal fungi and trees. Mycorrhizae represent a considerable sink for carbon, and both mycorrhizal and rhizospheric activities may be inhibited under O<sub>3</sub> stress by reduced carbon allocation to roots (Andersen and Rygielwicz, 1991). Nevertheless, increased fungal and bacterial biomass in the rhizosphere as well as raised root respiration have been found in *Pinus ponderosa* seedlings under environmentally relevant O<sub>3</sub> stress (Andersen and Scagel, 1997). It is uncertain if the raised metabolic activity of roots and mycorrhizae indicate enhanced nutrient uptake for assuring the maintenance of O<sub>3</sub>-stressed foliage, or if mycorrhizae become a 'burden' to trees under O<sub>3</sub> stress (cf. Mahoney *et al.*, 1985), as the plant-internal competition for assimilates required in foliage maintenance may be exacerbated. Such 'conflicts' appear to have relevance in herbaceous plants (Miller *et al.*, 1997). The observation that O<sub>3</sub> exposure of trees may cause morphological changes in their mycorrhizae while stimulating the fungal and presumably non-mycorrhizal biomass in the rhizosphere may have implications for pathogenic root infections (Duckmanton and Widden, 1994). Decline in mycorrhization under O<sub>3</sub> stress (Ericsson *et al.*, 1996) may lead to a lowered metabolic defence capacity (Gehring *et al.*, 1997) and - as a consequence - favour infections by root pathogens or even enhance the attraction to phytophagous insects (Langebartels *et al.*, 1997; Bonello *et al.*, 1993).

Interrelationships between O<sub>3</sub> impact, mycorrhization and parasite attack are apparent. Since at the biochemical level, plant response to O<sub>3</sub> impact resembles defense against pathogenic infection (Sandermann, 1996), O<sub>3</sub> may initially strengthen the trees' resistance to parasite attack, but in the long-term it may weaken it by 'over-straining' the defensive mechanisms. This pathway of O<sub>3</sub> action predisposing trees to biotic stress and eventually increasing the risk of lethal injury requires further attention.

The attraction of plants to parasite attack is basically driven by their nutritional status (Herms and Mattson, 1992) and the gradually increasing CO<sub>2</sub> concentration of the atmosphere and, in some regions, dramatically enhanced nitrogen deposition may therefore distinctly influence host-parasite relationships. The extent that O<sub>3</sub> interacts with elevated CO<sub>2</sub> and variable nutrient supply, and by this, determines tree

predisposition to parasite attack, is however unclear (cf. Manning and von Tiedemann, 1995).  $O_3$  effects on nutrition have been found to be inconsistent and may be confounded by processes of senescence and retranslocation or depend on the specific nutrient under consideration (Matyssek *et al.*, 1995a). However, both  $O_3$  and  $CO_2$  may alter C/N (Maurer and Matyssek, 1997; Hättenschwiler *et al.*, 1996) and, as a result, the balance between growth and competitiveness *versus* the capacity in parasite defence (Loomis, 1953; Herms and Mattson, 1992) - this balance being a major determinant of tree 'fitness'. In more general terms, both elevated  $CO_2$  and  $O_3$  levels have the potential for modifying the crown architecture and, *via* changing the efficiency in light use, may also affect competitiveness (Reekie and Bazzaz, 1989; Matyssek *et al.*, 1992, 1993; Hättenschwiler *et al.*, 1996). Findings from controlled experiments about high  $CO_2$  supply compensating for adverse effects by  $O_3$  on photosynthesis and growth (Volin *et al.*, 1998; Kellomäki and Wang, 1997; Olszyk and Wise, 1997; McKee *et al.*, 1997) should be cautioned in terms of ecological significance (Saxe *et al.*, 1998).

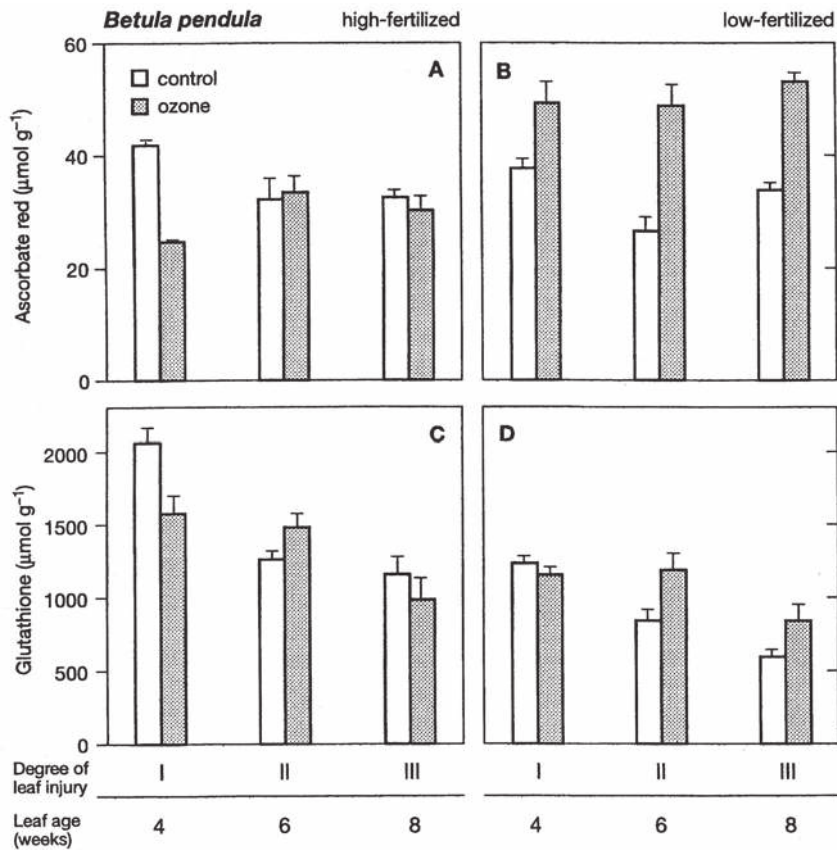


Fig. 4. Antioxidant responses (reduced ascorbate, A, B; glutathione, C, D) of birch cuttings (*Betula pendula*) grown at high or low nutrient supply (fertilization see Maurer *et al.*, 1997) to  $O_3$ -free air (control) or ozonated air ( $90/40$  nl l<sup>-1</sup> day/night). Leaves were sampled by the degree of progressive  $O_3$  injury, degree I corresponding to 4-week-old, degree II to 6-week-old, and degree III to 8-week-old leaves (redrawn from Polle *et al.* 1998).

Nutrition does affect the sensitivity in carbon allocation to  $O_3$  and may explain some of the apparent 'contradictions' found in the literature about the responses of trees to  $O_3$  (Polle *et al.*, 1998). Remarkably, high fertilization levels are not necessarily a prerequisite for enhanced  $O_3$  tolerance (Maurer and Matyssek, 1997; Matyssek *et al.*, 1997b). In young birch plants, low nutrition appeared to enhance the antioxidative defence capacity against  $O_3$  (Figure 4) and delayed premature leaf loss (Polle *et al.*, 1998; Maurer *et al.*, 1997), although the 'price to be paid' is a re-directed carbon allocation, limiting instead of stimulating root growth at low nutrient supply (Figure 5; Maurer and Matyssek, 1997; Mooney and Winner, 1991). Nevertheless, low nutrition does not necessarily prevent  $O_3$  injury, contrasting with the hypotheses of Weinstein *et al.* (1991). The extent to which nutrition may drive the turn-over of leaves and, through new leaf formation, may limit the decline of productivity under  $O_3$  stress, needs to be examined with respect to the seasonal dynamics of the different patterns in shoot growth (determinate *vs.* indeterminate; Tjoelker and Luxmoore, 1991; Laurence *et al.*, 1994; Matyssek *et al.*, 1997b). Varying nitrogen availability may differ in its effects on  $O_3$  sensitivity from responses to variable but balanced supply between the relevant macro and micro-nutrients (cf. Greitner and Winner, 1989).

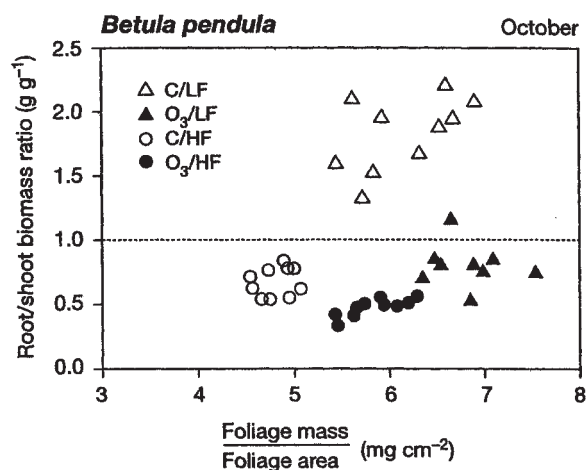


Fig. 5. Root/shoot biomass ratio in cuttings of *Betula pendula* at the end of the growing season as related to the ratio of foliage mass *versus* foliage area (one-sided); same experiment as in Fig. 6, 'C' representing control, ' $O_3$ ' ozonation, 'LF' low-fertilization and 'HF' high-fertilization treatments (redrawn from Maurer and Matyssek 1997).

Nutrition relates to the water status of trees, as a low nutrient supply in particular tends to promote high sensitivity in stomatal regulation (Schulze, 1994). The capacity of chronic  $O_3$  exposure to lower the stomatal conductance was found in birch to be most pronounced at low nutrient supply (Maurer *et al.*, 1997). Partial stomatal closure caused by low air humidity or soil drought may limit  $O_3$  uptake so that during sunny, dry days with high  $O_3$  levels the influx of  $O_3$  may be less than that under lower  $O_3$  regimes on



overcast, humid days (Wieser and Havranek, 1993). However, O<sub>3</sub> can influence the sensitivity of the stomata and, through this, of the whole plant to drought, as chronic O<sub>3</sub> exposure may lead to sluggishness in stomatal movements and to an inefficient control of the transpiration rate (Barnes *et al.* 1990a,b; Pearson and Mansfield, 1993; Karlsson *et al.*, 1995; Götzt, 1996). This change in stomatal behaviour appears to be related to altered 'mechanics' in the stomatal apparatus, possibly associated with reduced cell wall lignification, or to disturbed osmotic control of the guard cells because of membrane injury (Maier-Maercker, 1998; Heath and Taylor, 1997). Drought/O<sub>3</sub> interactions deserve greater attention in global-change research.

Another important factor determining O<sub>3</sub> sensitivity is irradiance, as not only high but also low light conditions can promote O<sub>3</sub> injury (Volin *et al.*, 1993; Oren and Schoeneberger in Matyssek *et al.*, 1995a). Low-light conditions are relevant in the shade and at dawn and dusk, and stomata may remain open at night, facilitating O<sub>3</sub> uptake (Tobiessen, 1982; Matyssek *et al.*, 1995b). Although stomata may be less open under limiting light conditions, the lower rate of O<sub>3</sub> uptake can result in greater effects on production and carbon allocation to the stem and root (Figure 6) than higher rates of O<sub>3</sub> influx associated with non-limiting irradiance (Matyssek *et al.*, 1995a, 1995b). The lack of light seems to limit the detoxification capacity (Menser, 1964; Foyer *et al.*, 1991). This may have implications for the emphasis given in AOT40 calculations to daylight conditions. High O<sub>3</sub> sensitivity of shade leaves (Tjoelker *et al.*, 1995) may relate also to their higher ratio of mesophyll surface area *versus* projected leaf area (Matyssek *et al.*, 1995a). Implications for light gradients in canopies or scenarios of high O<sub>3</sub> regimes at night are imaginable. The state of knowledge on temperature/O<sub>3</sub> interaction, in particular the influence of O<sub>3</sub> on frost hardiness, has been dealt with comprehensively by Skärby *et al.* (1998).

Given the multi-factorial impacts at field sites and their capacity to exacerbate, weaken or even mask the action of O<sub>3</sub>, it is clear that we are far from being able to quantify the risks imposed by O<sub>3</sub> on forest stands. This is emphasized when potential interactions between O<sub>3</sub> stress and the global-change scenarios are considered as well as the resulting consequences for nutrient availability and predispositions to biotic stress. These gaps in understanding are aggravated by the fact that current knowledge about the action of O<sub>3</sub> on trees is derived mainly from exposures of young plants under artificial conditions and are, therefore, not necessarily meaningful for mature individuals under stand conditions.

### 9. Ontogeny and the sensitivity of trees to O<sub>3</sub>

Although ample information exists on the responses of woody plants to O<sub>3</sub> (see 7. and 8.), most of this knowledge derives from young trees, which are uncertain surrogates for mature trees (Kelly *et al.*, 1995). One cause of different sensitivities to O<sub>3</sub> between ontogenetic stages and between species may be related to contrasting allocation patterns (Laurence *et al.*, 1994). Young trees typically possess a high proportion of foliage to total biomass, enabling high annual increments in stem growth, but also representing a large surface area vulnerable to O<sub>3</sub> attack. In contrast, the respiratory demand for maintaining the high proportion of non-green biomass may be high in mature trees

(Waring and Schlesinger, 1985) and may be increased by O<sub>3</sub> defense demands so that reductions in net primary production and relative growth rate may be exacerbated. As plant defense against O<sub>3</sub> must

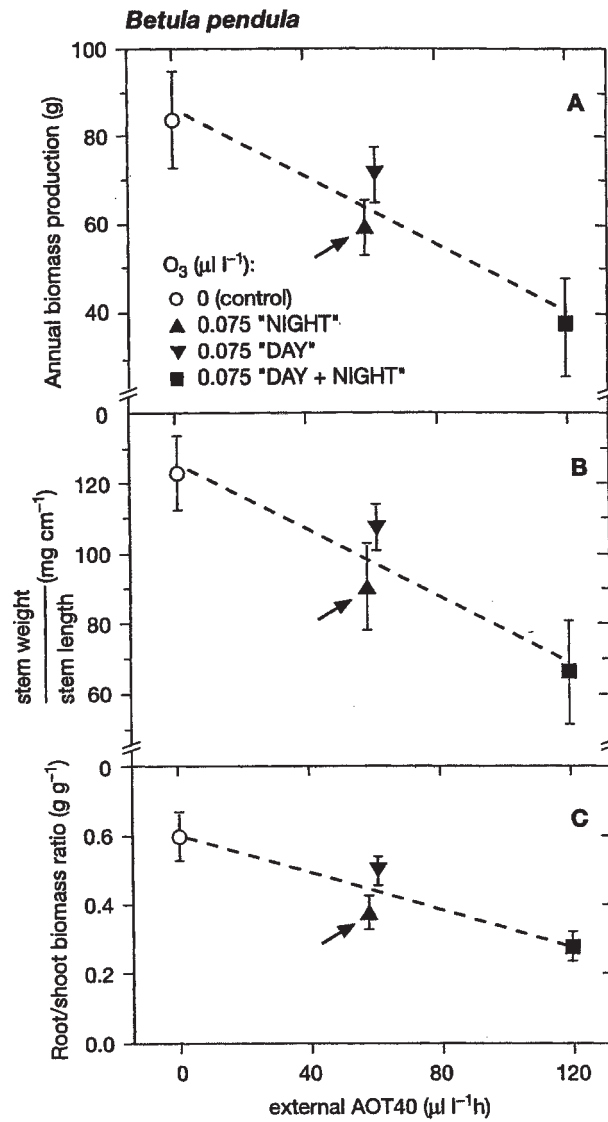


Fig 6. The effect of O<sub>3</sub> exposure on the annual whole-plant biomass production (A) and allometric relationships (B, ratio of stem weight vs. stem length; C, root/shoot biomass ratio) in cuttings of *Betula pendula* by the end of the growing season. Plants were exposed to O<sub>3</sub>-free (control) or ozonated air (75 nl l<sup>-1</sup>), the ozonation being applied each day either during daylight hours (7:00 hrs through 19:00 hrs, 'DAY'), or during the night including dawn and dusk (19:00 hrs through 7:00 hrs, 'NIGHT'), or throughout the entire daily course ('DAY + NIGHT'; arrows highlighting the 'NIGHT' treatment; redrawn from Matyssek *et al.* 1995b).

be fuelled from the internal resource pool, age-dependent 'conflicts' during ontogeny together with the demand in allocation for substrate and energy by other plant processes may affect O<sub>3</sub> tolerance (Herms and Mattson, 1992; Sandermann, 1996). Also, in mature trees rather than unshaded young individuals in experiments, large proportions of the crown can consist of shade leaves that - given the uncertainty about their O<sub>3</sub> sensitivity - may substantially contribute to the carbon gain, in particular, of shade-tolerant species (Schulze, 1970). Moreover, the control of carbon allocation between primary and secondary metabolism (which is responsible, for example, for O<sub>3</sub> defense) is affected by the resource availabilities governing the competitive interactions between plants as well as host-parasite relationships. There is increasing awareness (particularly in relation to tree responses to air pollutants or global-change scenarios) that both competition for resources and ontogenetic stage may determine the susceptibility of woody plants to environmental stress (Manning and von Tiedemann, 1995; Arnone, 1996; Groninger *et al.*, 1996; Skelly *et al.*, 1997).

If a high sink strength for carbon in mature trees is caused by the maintenance respiration requirement of non-green biomass, then a feedback to increasing photosynthetic capacity of the leaves is imaginable (Stitt, 1991). A comparison between seedlings and mature individuals of Red oak (*Quercus rubra*) revealed higher photosynthetic rates in older trees (Hanson *et al.*, 1994; Figure 7). However, photosynthesis of the mature trees was also more susceptible to O<sub>3</sub> stress. Interestingly, the photosynthetic decline was most pronounced in the lower tree canopy (Samuelson and Edwards, 1993), consistent with the high photosynthetic sensitivity to O<sub>3</sub> of shade leaves of Sugar maple trees (*Acer saccharum*; Tjoelker *et al.*, 1995). The O<sub>3</sub>-induced decline in photosynthesis of oak trees may relate to the concomitantly higher stomatal conductance as compared with the seedlings and, because of higher O<sub>3</sub> uptake through the stomata, a resulting higher risk of injury (cf. Reich, 1987). There are observations that trees with high metabolic activity (as reflected, for example, in high photosynthetic capacity) are more susceptible to O<sub>3</sub> stress (Laurence *et al.*, 1994). However, the photosynthetic limitation by O<sub>3</sub> in *Quercus rubra* trees had no measurable impact on growth performance, although allocation was affected (Kelly *et al.*, 1995): Mature oak trees retained carbon in leaves and branches, while root growth was limited (as elsewhere found in seedlings: Matyssek *et al.*, 1992; Polle *et al.*, 1998). In the oak seedlings, however, exposure to O<sub>3</sub> led to stimulated fine-root production (which contrasts with many observations of seedlings under O<sub>3</sub> exposure, cf. Matyssek *et al.*, 1995a). Differences in the micro-climate between the forest floor and the canopy - although influencing growth - were excluded as key factors determining the different O<sub>3</sub> sensitivities in oak trees and seedlings (Samuelson, 1994).

Given the limited knowledge about the responses of mature forest trees to O<sub>3</sub>, the findings on red oak should not be extrapolated to other species. For example, an investigation on *Sequoia gigantea* revealed higher O<sub>3</sub> sensitivity in seedlings than in mature trees (Grulke and Miller, 1994). In this species, it is assumed that light interception overrules age-dependent shifts in sensitivity to O<sub>3</sub>, as low irradiance in particular may lower O<sub>3</sub> tolerance. Using grafts of *Picea rubens*, a rather O<sub>3</sub>-tolerant species, Rebbeck and Jensen (1993) found a lower O<sub>3</sub> sensitivity in scions from mature than from juvenile trees, the latter having higher stomatal conductance and, therefore, higher O<sub>3</sub> uptake capacity. When comparing trees of different ages beyond the early

juvenile stage in *Pinus contorta* and *P. ponderosa*, Yoder *et al.* (1994) found that photosynthetic performance and stomatal conductance decreased with tree age, and even questioned maintenance respiration in mature trees as a limiting factor of growth. An implication may be that old trees are less endangered by O<sub>3</sub> through reduced O<sub>3</sub> uptake. It is hypothesised that the lower stomatal conductance of mature trees is related to increases in hydraulic resistance as stem and crown extend in size with age. Several observations support this hypothesis (Kline *et al.*, 1976; Mattson-Djos, 1989; Sperry *et al.*, 1993; Waring and Silvester, 1993). Given such relationships, O<sub>3</sub> uptake (and photosynthesis) may be controlled by water rather than the carbon relations in mature trees (Waring and Silvester, 1993; Yoder *et al.*, 1994; Farquhar *et al.*, 1989). The impact of hydraulic architecture, extensive soil water consumption and the gravitational constraints of large, mature trees on the internal water status may favour hormonal signals that close the stomata (Meinzer *et al.*, 1991). However, little is known - except for the role of ethylene in promoting O<sub>3</sub> injury (Sandermann, 1996) - on the hormonal status in trees under O<sub>3</sub> stress (Lucas and Wolfenden, 1996). This gap in knowledge exists not only in relation to growth hormones, given the differential O<sub>3</sub>-induced shifts in the meristematic activities between above- and below-ground organs, but also to the stomatal sensitivity to abscisic acid (ABA) under O<sub>3</sub> exposure. Apart from potential changes in membrane and cell wall properties of the stomatal apparatus under O<sub>3</sub> stress or shifts in the nutritional status that may affect stomatal sensitivity to ABA (Heath and Taylor, 1997; Maier-Mercker, 1998; cf. Schulze, 1994), it is imaginable that O<sub>3</sub>-induced dysfunction in phloem transport may result in enhanced ABA levels in the foliage (Neales and McLeod, 1992) that contribute to stomatal closure.

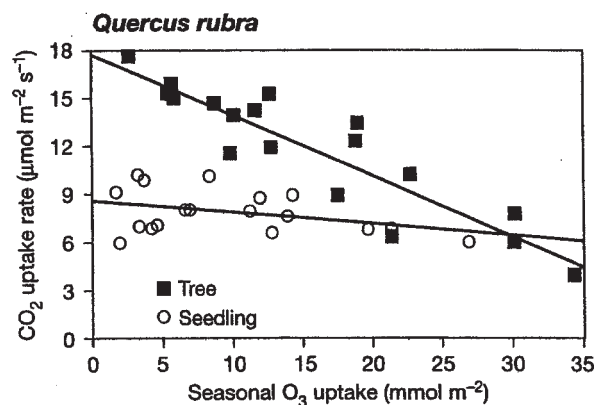


Fig. 7. Photosynthetic CO<sub>2</sub> uptake rate as related to the seasonal O<sub>3</sub> uptake of leaves in seedlings and mature trees of *Quercus rubra* (redrawn from Hanson *et al.* 1994).

The extent to which ontogenetic stages differ in O<sub>3</sub> tolerance cannot be resolved at present, but age-dependent differences in O<sub>3</sub> uptake, anatomy and detoxification, as well as in injury repair, appear to be of paramount importance. These properties are under genetic control and are modified by prevailing site conditions. Thus, general

rules about the O<sub>3</sub>-sensitivity of given ontogenetic stages may not exist, but perhaps stages of high rather than low metabolic activity are prone to limitations by O<sub>3</sub>, as the interference of O<sub>3</sub> with the whole-plant carbon allocation may be most pronounced during stages of high metabolic activity (Laurence *et al.*, 1994). A similar principle has been proposed for plant interactions with biotic stress (Herms and Mattson, 1992). In contrast, low metabolic activity may enhance O<sub>3</sub> tolerance, but possibly only in the short term, as the capacity for defense may be exceeded by the chronic O<sub>3</sub> exposure (Langebartels *et al.*, 1997). There is a need to examine whether young *versus* mature trees, and species of different foliage type, flushing dynamics and allocation pattern, can be classified in terms of their O<sub>3</sub> tolerance by the above principles (Kelly *et al.*, 1995). The need to clarify 'tree fitness' (cf. 8.) under field conditions is apparent, since trees, irrespective of their age, are physiologically 'challenged' whenever O<sub>3</sub> is taken up through open stomata.

### 10. Unknown risks by O<sub>3</sub> in mature trees and forests

A major problem for the assessment of the risks imposed by O<sub>3</sub> to mature trees under stand conditions is the uncertainty about diurnal and seasonal O<sub>3</sub> dynamics inside canopies and the significance of potentially enhanced foliage sensitivity to O<sub>3</sub> under limiting light conditions (Tjoelker *et al.*, 1995; Matyssek *et al.*, 1995b; Samuelson and Edwards, 1993). During the first half of the day, O<sub>3</sub> concentrations underneath the canopy of a spruce stand were found to stay almost as high as above, whereas during dusk and at night, the concentrations were distinctly lower underneath the canopy (Matyssek *et al.*, 1997b). The factors determining the variability of O<sub>3</sub> flux from the free atmosphere into the different layers of canopies and the deposition of O<sub>3</sub> onto leaves need to be analysed (Fuentes *et al.*, 1994). Pleijel *et al.* (1996) found the O<sub>3</sub> concentration at night to be higher inside than outside a spruce stand, when the wind velocity was low. Apart from the O<sub>3</sub> impact on shade foliage under limiting light, the overall constraint by O<sub>3</sub> on canopies at night appears to be important, as the nightly O<sub>3</sub> impact may increase in the proximity to urban centers (Lefohn and Jones, 1986) and with altitude (Sandroni *et al.*, 1994; Beyrich *et al.*, 1996). Advection from urban areas to the rural surroundings and the lack of nitrogen oxides may result in high O<sub>3</sub> concentrations in forests at night, and incursions of O<sub>3</sub> from high atmospheric layers may be an important factor at higher altitudes (Zaveri *et al.*, 1995).

The extent to which stomata may be open under light limitation is debatable, particularly at night. However, stomata can open without light stimulation (Tobiessen, 1982), and such a behavior may be favored by warm and humid conditions (Lösch, 1979; Schulze and Hall, 1982). In addition, stomata may stay open in darkness as a result of the sluggishness in stomatal regulation associated with leaf senescence (Field, 1987; Benecke *et al.*, 1981) or as a consequence of chronic O<sub>3</sub> exposure (Barnes *et al.* 1990a,b; Keller and Häslar, 1984; Götz 1996). Stomata that were open at night have been found in conifers and angiosperm trees (Wieser and Havranek, 1993; Matyssek *et al.*, 1995b), as well as in herbaceous plants (Goknur and Tibbitts, 1984; Aben *et al.*, 1989). Winner *et al.* (1989) found that night-time exposure to O<sub>3</sub> reduced production in turnip plants, and similar findings have been reported for birch (Matyssek *et al.* 1995b).



However, the capacity for O<sub>3</sub> uptake must be related to the boundary layers inside canopies and around leaves, as aerodynamic resistances can strongly impede the O<sub>3</sub> influx into plants, even though the stomata may be open (Pleijel *et al.*, 1996). O<sub>3</sub> flux into the different canopy layers and its significance for shade crown sensitivity, as well as O<sub>3</sub> impact during night-time, may depend strongly on the extent of canopy closure and the crown architecture of single trees under particular stand conditions. So again generalisations are not possible about O<sub>3</sub> dynamics and the related risks at the canopy level. Case studies for the specific structural features of individual forests are required, especially for stands with unevenly aged and tall trees or mixed forests with tree species contrasting in crown shape or foliage type. Such stand structures are being increasingly favored as forestry moves towards a more ecologically-based management paradigm (Bode, 1997).

In addition to the uncertainties about spatial and temporal variations of O<sub>3</sub> regimes in canopies and the consequences for the O<sub>3</sub> sensitivity of different foliage types, it is not known whether chronic O<sub>3</sub> exposure induces a decline in stomatal aperture in mature trees as observed in young trees, and thus, whether effects on the water balance of whole stands and the 'water-use efficiency' of the stand production are to be expected. Although mature trees may not be limited by chronic O<sub>3</sub> exposure to a higher extent than by other site factors – given the increased productivity of forests presently observed in many parts of Europe – O<sub>3</sub> can act on the carbon allocation in the trees, as defense costs against O<sub>3</sub> need to be covered by the assimilate pool. The resulting differential shifts in growth patterns between the organs, particularly if exacerbated by disturbed assimilate transport, rather than effects on the overall production, appear to be critical for long-term stand development, because competitiveness is affected whenever crown or root architecture become altered. Competition between plants is exerted *via* the occupation of space, exclusion of neighbours and efficient sequestration of resources. Therefore, changes in crown and root allometry and in the associated cost/benefit balances between the investment of resources in, and gains from, organ structure may be the 'pathway' through which O<sub>3</sub> most effectively acts on the stability and biodiversity of forests (cf. Küppers, 1994; Tremmel and Bazzaz, 1995). O<sub>3</sub>-induced changes in organ differentiation and turnover (e.g. of roots and leaves), associated with raised respiration and its consequences for the net primary production (especially in mature trees), may be most significant under chronic O<sub>3</sub> stress. Moreover, changed allocation may promote susceptibility to shortages in water and nutrient supply (through potential long-term limitations on root development) and the predisposition to parasite attack (Langebartels *et al.*, 1997). O<sub>3</sub> effects on fruiting and natural recruitment of seedlings also need to be clarified (Chappelka and Chevone, 1992).

Although many forests in Europe are not 'natural' and are subjected to management, the impact of O<sub>3</sub> on resource fluxes in the ecosystem, through modifying competitive interactions, may be a determinant of the stability of managed mixed-species plantations and forests. Knowledge about the long-term mechanisms of O<sub>3</sub> impact on forests appears to be a pre-requisite for silvicultural measures that may compensate - to some extent at least - for the stress caused by O<sub>3</sub>. However, such information does not yet exist, and information from controlled O<sub>3</sub> exposures in chambers with young trees still requires validation under specific O<sub>3</sub> regimes in 'real' forest stands.

### 11. Requirements for a quantitative risk assessment of the O<sub>3</sub> impact on forests

Risk assessment of O<sub>3</sub> impacts on mature trees has made use of cuvettes or bags for exposing branches in tree crowns to defined O<sub>3</sub> regimes (Houpis *et al.*, 1991; Havranek and Wieser, 1994; Musselman and Hale, 1997). This approach has provided valuable insights into the mechanisms underlying O<sub>3</sub> uptake under conditions of air or soil moisture deficit in mature trees (Wieser and Havranek, 1995, 1996). Tolerance to chronic O<sub>3</sub> exposure and biochemical acclimation was indicated in *Larix decidua* and *Picea abies*, especially under the persistently elevated O<sub>3</sub> levels encountered at higher altitudes (Polle *et al.*, 1995; Sandroni *et al.*, 1994). The results of such approaches are subject to the micro-climatic bias introduced by branch enclosure or, perhaps, biochemical interaction with non-fumigated branch and crown parts (Musselman and Hale, 1997; Matyssek *et al.*, 1997a), even though 'net carbon fluxes' may largely indicate carbon autonomy of branches (Stitt and Schulze, 1994; Dickson and Isebrands, 1991; Matyssek and Schulze, 1988). Large 'open-top' chambers are also influenced by micro-climatic bias, even though whole mature trees can be exposed to O<sub>3</sub> fumigation (e.g. Hanson *et al.*, 1994).

Such micro-climatic bias can be overcome by using 'free-air' fumigation systems that avoid branch enclosure. Such a system releasing O<sub>3</sub> to leaf clusters on several sun and shade branches was constructed within in the canopy of an *Acer saccharum* stand (Tjoelker *et al.*, 1994, 1995). The study revealed enhanced O<sub>3</sub> sensitivity in photosynthesis of the shade foliage. Employing 'free-air' fumigations to entire crowns can assure the experimental O<sub>3</sub> regime to induce whole-tree responses. With the exception of a number of 'free-air' O<sub>3</sub> fumigation experiments in agricultural crop plants (Manning and Krupa, 1992; Musselman and Hale, 1997), the few 'free-air' O<sub>3</sub> fumigations on trees have been restricted mainly to young plants, grown in experimental plots, with small crown dimensions (Wulff *et al.*, 1992; McLeod *et al.*, 1992). O<sub>3</sub> responses of these plants have, to some extent, resembled findings from similarly-aged individuals under chamber conditions (Pääkkönen *et al.*, 1993; Wulff *et al.*, 1992) and highlighted the importance of genetic variation in O<sub>3</sub> sensitivity. In possibly the most extensive study conducted to date (the Liphook experiment) few significant effects of O<sub>3</sub> were found on *Picea abies* (Wellburn *et al.*, 1997). So far, 'free-air' fumigations of large, ageing trees have been restricted to other air pollutants such as SO<sub>2</sub> (Hendrey *et al.*, 1992) or used to clarify impacts of rising CO<sub>2</sub> levels (Lewin *et al.*, 1994).

At present, a 'free-air' O<sub>3</sub> fumigation study is being set up by scientists from the University of Munich in a 40 to 60-year-old mixed beech/spruce forest (*Fagus sylvatica/Picea abies*; Matyssek *et al.*, 1997b). In this case study, O<sub>3</sub> is released into the canopy through a tubing system, with exposures controlled by feedbacks from on-line monitors. In addition, the weekly O<sub>3</sub> exposure in the different crown parts is assessed by passive samplers (Werner, 1992). The canopies of the about 27 m tall trees are accessible *via* scaffolding in sun and shade crowns. Groups of five beech and spruce trees each are exposed to '2 x ambient' O<sub>3</sub> levels up to a maximum of 150 nl O<sub>3</sub> l<sup>-1</sup>, tracking diurnal and seasonal courses in ambient O<sub>3</sub> concentrations above the canopy. Corresponding groups of spruce and beech trees in ambient air serve as 'controls'.

Impacts of O<sub>3</sub> are based on a broad spectrum of biochemical, (eco)-physiological and structural analyses in this interdisciplinary project, providing data for biophysical and ecophysiological models required for 'Level 2' critical levels development (cf. Ollinger *et al.*, 1997). 'Free-air' fumigations appear to be the 'ultimate choice' for approaching a quantitative risk assessment of the chronic O<sub>3</sub> exposure of mature trees and forest stands and for scaling O<sub>3</sub> effects from the tree to the stand level.

## 12. Conclusions

The O<sub>3</sub> levels currently prevailing in Europe do not seem to pose an acute danger to mature trees and forests and are unlikely to lead to widespread dieback and break-down in the short-term. However, regional exposure conditions, such as those in southern Europe, may be conducive to O<sub>3</sub> injury. At a European level, judgement of the effects of chronic exposure to the persistently elevated O<sub>3</sub> levels is difficult, because the database available to achieve a long-term and quantitative risk assessment is still incomplete. Although partly reliable maps showing the distribution of O<sub>3</sub> at a regional scale are available, meso-scale information about diurnal and seasonal O<sub>3</sub> regimes in stands is scarce. Most importantly, knowledge about the physiological effects of O<sub>3</sub> on trees is not representative for the environmental conditions at forested sites, as most experiments deal with young trees, often grown for short periods of time in isolation inside fumigation cabinets. This biases exposure conditions and minimises the complex biotic and abiotic interactions which are typical of the field. Although such experiments have been valuable for revealing the 'principles' of O<sub>3</sub> action on woody plants and for indicating the broad spectrum of risks presented by O<sub>3</sub>, these findings lack ecological significance as long as validation at forest sites is missing.

Due attention must be given to the three atmospheric components that alone or in combination represent the greatest risks to the long-term stability of forests, namely elevated O<sub>3</sub> and CO<sub>2</sub> concentrations and high nitrogen deposition. Impacts of these three factors relate to changes in allocation patterns, with subsequent and ecologically meaningful effects on 'tree fitness', i.e. competitiveness and predisposition to different kinds of stress. 'Free-air' fumigations of canopies with O<sub>3</sub> can approach a quantitative risk assessment in forest stands. Of course, such approaches can only be undertaken locally and are valid primarily for the case study under consideration. However, conducting studies of this kind for a transect of representative forest types throughout Europe would provide a 'realistic' database for urgently needed mechanistic modelling approaches towards a quantitative O<sub>3</sub> risk assessment (cf. Ollinger *et al.*, 1997) and an integration of O<sub>3</sub> impact across the different geographical and ecological scales. A broad array of plant parameters would contribute to adequate definitions of Level 2 'Critical Levels for Ozone'. Quantitative risk assessment is necessary for achieving an environmental legislation that optimizes and reconciles, on a sound scientific basis, ecological *versus* economic demands.

Even though an ecological risk assessment for O<sub>3</sub> impacts on forests still needs to be accomplished, in many regions of Europe O<sub>3</sub> regimes prevail today that are known from controlled fumigation experiments to have the potential to adversely affect tree growth. For chronic O<sub>3</sub> stress, it seems that the O<sub>3</sub> impact on the plant-internal resource

allocation rather than on the extent of productivity may be significant for the trees' fitness in the long term. Such a pathway of O<sub>3</sub> action may eventually lead to losses in the genetic diversity. It needs to be examined to what extent catastrophic break-down of stands caused by disease may arise from predispositions relating to chronic O<sub>3</sub> exposure, and also changes in competitiveness are relevant to managed forests. Knowledge about such 'pathways' of O<sub>3</sub> impact may be important for 'buffering' O<sub>3</sub>-induced constraints by silvicultural measures, and such a view appears to be relevant as no prognosis exists today that would predict, for the decades to come, a decline in the O<sub>3</sub> regimes to pre-industrial levels. Despite the requirements for increased knowledge of the actual risks of O<sub>3</sub> to mature trees and forests, the information available today about the action of O<sub>3</sub> on trees does provide arguments for a responsible, precautionary policy that may prevent adverse effects of O<sub>3</sub> on the forests of the 21<sup>st</sup> century.

### Acknowledgements

We are grateful to Doris Pichler and Verena Fataar for producing the diagrams, and to Michèle Kaennel for her help with the literature search.

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