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O3SWE - Ozone and the Forests of South-Western Europe

An international co-operative initiative aimed at assessing ozone levels, risk, actual and potential effects on selected forests in France, Italy, Spain and Switzerland

Final Report

AOT40 (ppb*h)
- 0 - 5000
- 5000 - 10000
- 10000 - 20000
- 20000 - 30000
- 30000 - 40000
- 40000 - 50000
- > 50000
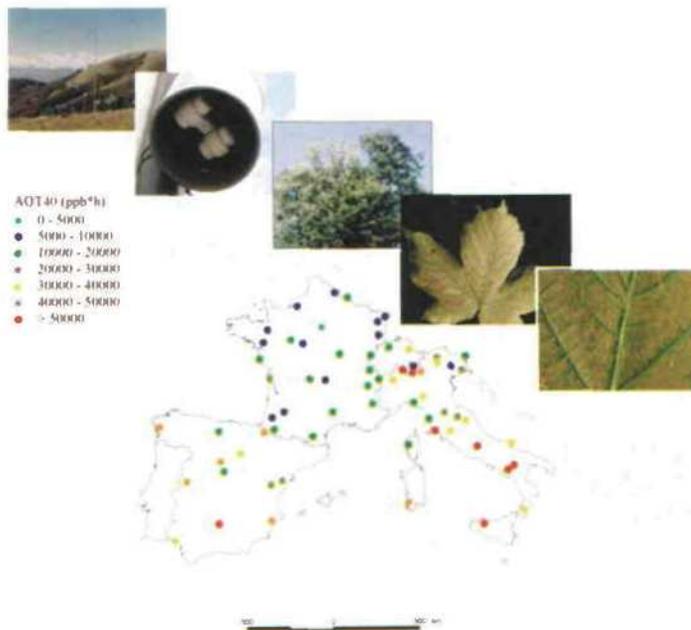
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Note

This report does not necessarily reflect the views of the authors' institutions and of the European Union and no official endorsement should be inferred.

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O3SWE - Ozone and the forests of South--western Europe: an international co-operative initiative

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Abstract. *Ozone (O3) is considered the most dangerous regional air pollutant for forest vegetation in Europe. Both exceedance of critical levels and occurrence of visible symptoms on native plants have often been reported at many sites throughout Europe. On the other hand, effects on growth and crown condition, although reported, are much less clear and documented. Overall, the knowledge relating to actual exposure and effects of ozone on forests in Europe is fragmented, and comparable large-scale information is limited. Within the framework of the EU and UN/ECE Level II forest monitoring programFrance, Italy, Spain and Switzerland have been particularly active in initiating O3 measurements by passive sampling and investigating symptoms and effects at a number of forest sites as well as at experimental sites under controlled conditions. This activity provided the basis for a new, co-operative project aimed at investigating O3 levels, risks and actual and potential effects at intensive monitoring plots in South-Western Europe. The project considers 83 sites spread over 1600 km in latitude (from Northern France to Southern Italy and Spain), 2000 km in longitude (from Western Spain to Eastern Italy) and between 5 and 1900 m a.s.l. The main tree species in this project include Fagus sylvatica L, Picea abies (L.) Karst., Abies alba Mill., Pinus cembra, P. halepensis, P. mugo, P. pinea, P. pinaster, P, sylvestris, Pseudotsuga menziesii, Quercus cerris, Q. ilex, Q. petraea L, Q. robur, Q. suber. This paper provides an overview of the project background, aims, structure and expected results.*

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Introduction

Ozone (O_3) is probably the most important regional air pollutant that may impact forest vegetation in Europe and elsewhere, and its potential impact is likely to increase in the near future at global scale as well (e.g. Fowler et al. 1999). Current O_3 levels, model predictions and evidence from a series of field and experiments, seem to support the above concern (Fowler et al. 1999; Ashmore et al. 2002; NEGTAP 2001). Mapped exceedances of the previous critical level AOT40 10 ppm*rf[1] (O_3 accumulated over threshold 40 ppb = 0.04 ppm, Fuhrer et al. 1997) suggest that a considerable proportion of the European forest area is to be considered at risk (Hettenling et al. 1996). However, these data are not easy to interpret in terms of effects: for example, while the occurrence of visible O_3 injury seems to be widespread (Innés et al. 2001; EC and UN/ECE 2003), effects on growth are much less certain (e.g., Spiecker et al. 1996; Braun et al. 1999; Ferretti et al. 2002). Despite these interpretation problems, "the lack of ozone data" was acknowledged as "a serious limitation" for the EU Intensive Monitoring (Level II) database (De Vries 2000, p. 27). In addition, it represents a considerable scientific and political shortcoming in a program aimed at investigating air pollution effects on forests and expected to provide input for the environmental decision-making process. Actually, besides the obvious connections with potential effects on forests, O_3 data are relevant to other issues which were the subject of important political agreements, like tropospheric chemistry changes and regional ozone formation (see e.g. the CLRTAP multi-pollutant, multi-effect directive; the UN Biodiversity Convention; the EU Habitat Directive; the EU acidification strategy, the UN/ECE CLRTAP, the EU Air Quality directive) (De Vries 2000, p. 19). For these reasons, since 1995, the EC included O_3 measurements in the Programme for Intensive Monitoring of Forest Ecosystems (Regulation EC n. 1091/94 as implemented by Reg. EC n. 690/95), and first projects, based on O_3 passive sampling, were co-funded starting in 1996. From 2001 onwards, a number of National Programs included O_3 monitoring surveys as routine analysis. Recently, within the CLTRAP International Co-operative Programme on Assessment and Monitoring of Air Pollution Effects on Forests, an official test phase was initiated to promote and evaluate both the feasibility of O_3 measurement by passive sampling and foliar symptoms assessment at European scale. However, activities at national level began much earlier: in particular, France, Italy, Spain and Switzerland have been measuring O_3 concentrations by means of passive samplers for some time (see below). The project presented in this report represents a cooperative initiative between the National Focal Centres (NFCs) of these four countries. This group reflects the importance attributed to O_3 in these countries (southwest Europe is a target area for O_3 - Bussotti and Ferretti 1998, Bussotti and

Gerosa 2002), acknowledged also by the ongoing activity at national and international level: these four countries organized all the training and intercalibration courses held since 2000 under the auspices of the EC and UN/ECE Level II program; Spain holds a leading position within the Ambient Air Quality group of the UN/ECE Expert Panel on Deposition; France has the largest coverage of passive sampling; in Switzerland a number of projects have been and are being developed on visible symptoms, O_3 modeling and effects on forests - see Fuhrer 1995, 1996; Fuhrer et al. 1997; Vanderhayden et al. 2001; Innés et al. 2001; Novak et al. 2003; in Italy ozone monitoring by passive samplers has been carried out since 1996 on a weekly basis at all Level II plots and a national report has been produced recently (Ferretti et al. 2003b)

On the basis of this past and current activity, the project presented in this report is an attempt to improve our knowledge on ozone and forests in Europe: levels, exposure and effects were investigated through routine monitoring activity, modelling and statistical analysis at a number of forest sites located in France, Italy, Spain and Switzerland. The project was co-operatively prepared and implemented by several institutions of the four countries involved; it is co-funded by the EU as part of the Italian National Program for monitoring of forest ecosystems in 2002.

Ozone effects on forest ecosystems[3]

While a first report of field damage on forest trees by O_3 was made in 1961 (Berry and Ripperton 1963), O_3 had been identified as "a menace to agriculture" since 1959 (Rich 1964). As scientific research devoted increasing attention to the topic, many excellent reviews discussing the effects of O_3 at cellular, biochemical, physiological, morphological and ecosystem levels became available.

Molecular mechanisms of O_3 toxicity

The essential elements of the biological mechanisms of O_3 toxicity have been reported by Guderian et al. (1985). Damage produced by photochemical oxidants is the result of biochemical and physiological reactions occurring in the mesophyll. Decisive factors in causing these injuries are: the quantity of O_3 that spreads from the atmosphere to the reactive sites within the leaf and the speed at which it spreads. The flux of O_3 is a function of the chemical and physical characteristics it encounters during the transition from gaseous to liquid phase. Physical structures can hinder its flux; chemical reactions can capture O_3 and other photochemical molecules between the gaseous and the liquid phase.

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[3] *This section is based on Ferretti et al., 2003a*

The flux of O_3 in the mesophyll is considered a factor capable of predicting foliar injury, although the onset of damage does not always correlate with the quantity of O_3 absorbed. O_3 flows to the cellular sites where the injury occurs in the liquid phase. This transition through phenomena of diffusion and/or flux crosses the hydrated cellular walls, the membranes and the cytosol. Along the way O_3-consuming scavenger reactions can be triggered. In cellular solutions, a variety of organic molecules, such as unsaturated fatty acids, sulphydryls and ring compounds, are O_3-sensitive. The reaction of these compounds with O_3 produces hydrogen peroxide (H_2O_2) and free radicals, against which the cell is protected by scavenging and buffering mechanisms, such as Superoxide dismutase, peroxidase, glutathione and phenolic compounds. These processes (repair processes) are to a large extent extracellular and occur within the wall (apoplast), while the general O_3-induced defensive reactions in plants include a number of changes in the metabolic status, involving phytoalexins, cellular barriers, pathogenesis-related proteins, signal substances and antioxidative systems (Sandemann 2000). Consequently, the development of the apoplastic fraction - and therefore the density of the mesophyll - provides a substrate in which the toxic action of O_3 can be neutralized (Lyons et al. 2000; Wieser et al. 2003). The plant's capacity to activate similar detoxifying mechanisms, combined with the intensity of the O_3 flux in the leaves, explains the different degrees of sensitivity.

Ultrastructure damage

Organelle membranes, and especially chloroplast membranes, are considered the most sensitive targets to O_3 injury. The most common ultrastructure alterations are tylakoid swelling and plastoglobule production, while the stroma becomes granulated and markedly electron-dense (Sutinen et al. 1990; Selldén et al. 1996; Holopainen et al. 1996). This type of damage has been observed both in conifer needles and in broadleaf leaves. It can be detected when the symptoms are visible, but is also considered a "previsual" manifestation, i.e. it allows one to ascertain the presence of O_3 impact even without the presence of visible symptoms.

Damaged chloroplasts are followed by collapse of mesophyll cells. This is a typical hypersensitive response (HR), caused by the accumulation of ROS (Reactive Oxygen Species, see Wohlgemuth et al. 2002) and especially H_2O_2 in the cytoplasm (Oksanen et al. 2002). The HR behaviour is the result of an oxidative stress (Schraudner et al. 1997; Pell et al. 1997), which can be triggered by many abiotic agents other than ozone (drought, light), and is considered also an active response against parasite invasion (Blokhina et al. 2003). In broadleaved trees the injury mainly affects the palisade mesophyll: this is considered a specific symptom attributable to O_3, since other pollutants

tend to damage the lacunar tissue cells nearest the substomatal chambers. The collapsed cells are isolated from the healthy ones through compartmentalization processes, including the formation of a layer of callose (Gravano et al. 2003). Evans et al. (1996) found a proportionality between the percentage of foliar surface affected by visible symptoms and the percentage of collapsed mesophyll cells, although cellular damage is normally greater than visible injury.

Other alterations of the apoplastic fractions have also been described: they can be considered acclimatization mechanisms. They include thickening of the cell walls and production of pectic substances, thickening of cuticles which become imbibed in phenolic substances such as tannins or phenylpropanoids (Günthardt-Goerg et al. 1997, 2000; Vollenweider et al. 2003; Bussotti et al. 2003). These alterations enhance the role of the apoplast in the detoxifying processes. Recently Oksanen et al. (2003) demonstrated, by histochemical methods, that in tolerant genotypes of *Betula pendula* H_2O_2 accumulated in cell walls, whereas in the sensitive ones it accumulated in the cytoplasm.

Another category of alterations affects the photosynthetate transport system, with the collapse of phloem cells and the accumulation of primary starch in the chloroplasts (Günthardt-Goerg et al. 1997; Soda et al. 2000). Lastly, a great deal of attention has been devoted to the condition of outer surfaces, in relation to the action of both O_3 and other pollutants (Turunen and Huttunen 1990). It is mainly the epicuticular and epistomatal waxes of conifer needles that lose their normal fibrillar network structures, with possible consequences on the efficiency of stomatal regulation. These alterations have been considered markers useful in recognizing the effects of atmospheric pollution in the field.

Effects on physiology

According to Guderian et al. (1985) and Darral (1989), O_3 attacks the chloroplast membrane, thus hindering the photosynthesis processes connected to it. It further inhibits the carboxylic activity of Rubisco reducing the fixation of CO_2. Chlorophyll reduction caused by O_3 has been reported in several species. Chlorophyll b is more sensitive than chlorophyll a. The reduction can also occur in chronic exposure conditions or - in a previsual phase - even without the presence of symptoms. Reduced net photosynthesis in ozone-exposed seedlings was reported by Zhang et al. (2001) and Gravano et al. (2004). It can be the effect of permanent injury to the mesophyll or of a temporary stomatal restriction (stomatal conductance is inhibited by high concentrations of O_3, Minnocci et al. 1999). In this last case, the full physiological function is restored as soon as exposure ceases. An important question concerns the O_3-induced decoupling between assimilation and stomatal conductance reported for broadleaves (Matyssek et al. 1991;

Flager et al. 1994; Tjoelker et al. 1995; Clark et al. 1996; Paoletti et al. 2002), and which may also be suggested for conifers (Wieser et al. 2002).

Photosynthesis reduction also brings about a reduced production of soluble sugars and starch, which in turn reduces growth (Guderian et al. 1985); important resources are subtracted from the growth process to be used in the detoxification process. Furthermore, sugars and primary starch are retained in the leaves (probably due to the collapse of the leaves' phloem system, Wellburn and Wellburn 1994) and the transport of photosynthetates towards the roots is inhibited. Thus root growth is also reduced and the normal ratio between above- and below-ground structures is altered, in favour of the former. Reduced sugar and starch reserves in the roots can explain the alteration of the symbiontic relationship with the soil's fungine component (mycorrhizae) causing a reduction in the efficiency of mineral nutrition.

A loss of photosynthetic efficiency in individual leaves does not necessarily imply a similar loss of efficiency for the whole tree, in that certain compensation mechanisms may be set in motion (cf. Kolb et al. 1998). In fact, not all the leaves of an adult tree or of a forest stand are affected the same way. Usually it is the most exposed leaves that suffer the greatest damage. Unaffected leaves develop a greater photosynthesis efficiency, so that neither the tree's overall productivity nor its growth are jeopardized (Temple and Miller 1994). Damaged leaves undergo premature aging processes and may be shed and replaced by new, more efficient leaves. Evidence of compensation mechanisms was highlighted in *Fraxinus excelsior* leaves treated in an OTC experiment (Gravano et al. 2004). Measurements of the direct fluorescence of chlorophyll a showed that, in ozone-injured leaves, the value of the constant of photochemical de-excitation (Kn) increased. This behaviour suggests that healthy cells increased their photosynthetic efficiency to compensate the loss of photosynthesis in the collapsed ones.

Visible symptoms

In forest species, visible foliar symptoms attributable to chronic exposure to O_3 were described for the first time in the mountain pines of the south-west of the United States, a region with a Mediterranean-type climate (Fox and Mickler 1995; Miller and McBride 1999). The symptoms included chlorotic mottle, or local yellowing, degenerating into necrotic patches; in the more severe cases needle tip necrosis was also observed. The chlorotic mottle was usually associated with degeneration of the mesophyll cells (Evans and Leonard 1991; Evans and Fitzgerald 1993).

Among the woody species of European flora and the Mediterranean basin, pines appear to be among the ones most sensitive to the action of photo-oxidants. The best known symptoms have been described in *Pinus halepensis* in Spain and Greece (Barnes et al.

2000) and in Italy (Soda et al. 2000). The needles of *Pinus halepensis* develop a typical symptomatology (chlorotic mottle) similar to that described by the American researchers mentioned above.

In broadleaved trees the symptoms vary: ranging from stipples (small, punctiform necroses, ivory-white to brown-red or black in colour, which gradually merge into larger necrotic patches), to flecks (reddish spots), and reddening (leaves changing colour earlier than in normal autumn processes). These symptoms tend to affect the upper side of the leaves, in the interveinal areas. The symptomatology has been illustrated in a number of atlases and photoguides (cf. Jacobson and Hill 1970; Skelly et al. 1987; Flagler 1998; Innés et al. 2001; Sanz et al. 2001; Schaub et al. 2002) both for North America and Europe. In Europe the most widespread and evident symptoms, validated by Open Top Chamber experiments (VanderHeyden et al. 2000), were reported in southern Switzerland (Skelly et al. 1998, 1999) and in areas near the Italian border (Cozzi et al. 2000; Gravano et al. 2000). Other reports come from sites in eastern Europe (Bytnerowicz et al. 2001; Manning et al. 2002, Manning and Godzik 2004). In the Mediterranean region the majority of cases reported are in Spain (Skelly et al. 1999) where several evergreen shrubs have also been found to be sensitive. In Italy widespread symptoms have been observed in Tuscany (Bussotti et al. 2001), Umbria (Cozzi et al. 2002), Lombardy (Cozzi et al. 2000) and Piedmont (Paoletti et al. 2003) as well as on some intensive monitoring plots of the national network (Bussotti et al. 2003). The evergreen sclerophyllous species of the Mediterranean ecosystems, however, appear to be quite tolerant (Bussotti and Gerosa 2002; Nali et al. 2004).

In many cases stipples are the result of hypersensitive responses (HR) and oxydative burst (OB) processes at histological level. This means that the visual symptoms can be validated by means of microscopy (Vollenweider et al. 2003). The evaluation of reddening and early senescence symptoms is more problematic. In particular, reddening is caused by the accumulation of anthocyanins which does not necessarily imply a response in the mesophyll cells (Gravano et al. 2004) or a process of oxidative stress (Wohlgemuth et al. 2002). Anthocyanins are produced under high light conditions to reduce oxidative pressure (Steyn et al. 2002).

Many authors (cfr. Sanz et al. 2001; VanderHeyden et al. 2001; Novak et al. 2003; Manning and Godzik 2004) have proposed scales of sensitivity among different species. Nevertheless, intra-specific sensitivity may be even more pronounced than the inter-specific one. The sensitivity of an individual species is influenced by age and size (Kolb et al. 1998), by ecological conditions (Schaub et al. 2003; 2004), by the type of ecosystem (Power and Ashmore 2002) and the genotype (Paludan - Müller et al. 1999; Bassin et al.

2004). Different populations of the same species can display a very different behavior, so that comparison under field conditions is problematic.

Effects on growth and reproduction

The effects on growth were reviewed recently by Bortier et al. (2000) and Dizengremel (2001). The growth of seedlings of some important European broadleaves (birch, beech, poplar) is generally negatively affected by O_3 within one exposure season. The range of growth reduction differs by provenances (Larsen et al. 1990; Wulff et al. 1996), families (Taylor 1994) and also by clones (Pääkkonen et al. 1993).

In coniferous species, although negative effects of O_3 on growth have been reported, the responses were variable and inconsistent (Pye 1988). The results of several seasons of O_3 exposure indicate that trees show carry-over effects on growth and suggest that it is most likely that observed effects in trees reflect cumulative processes developed over several growing seasons. In the case of species with multiple flushing growth (like Douglas-fir), this characteristic contributed to the variability in the tree responses.

As previously stated, O_3 reduces root growth more than shoot growth in most species, due to an altered carbon allocation within the plant, thus leading to a reduced photosynthetate supply to roots (Darrall 1989). In a few plants, however, shoots may be more affected than roots (e.g. Kress and Skelly 1982 in loblolly pine), while in Scots pine there is no difference at all (Anttonen and Kärenlampi 1995). Reduced root growth and carbon allocation can alter the functioning of rhizosphere organisms. O_3 alone, or in combination with other pollutants (O_3+SO_2; O_3+acid deposition), not only induces quantitative alterations like reduction of the number of infected short roots and the growth rate of mycorrhizae (Kytoviita et al. 1999), but also produces qualitative changes in mycorrhizae (Diaz et al. 1996).

Information on the effects of O_3 on the sexual reproduction of forest trees is very scarce (Black et al. 2000). Direct effects of O_3 are known only on pollen germination and pollen tube growth in conifers from *in vitro* (Benoit et al. 1983) and in the field (Houston and Dochinger 1977) studies. The germination percentage and average pollen tube growth was greater in pollen gathered from the low pollution incidence area. Reduced cone production (Miller 1973) and decreased seed number and weight (Houston and Dochinger 1977) were observed in Ponderosa pine in San Bernardino Mountains, although the effects could not be specifically attributed to O_3.

Effects on crown condition

The relationship between predictive variables (site characteristics, seasonal and environmental factors) and crown condition (defoliation and discoloration) was

investigated with multivariate statistics (see the review by Seidling 2000). In particular, the possible influence of O_3 was examined both in European forests as a whole (Klap et al. 2000) and in individual countries (Switzerland: Dobbertin et al. 1997; Zierl 2002; Netherlands: Hendriks et al. 1997; United Kingdom: Innés and Boswell 1988; Innes and Whittaker 1993; Mather et al. 1995; Italy: Ferretti et al. 2003c). A study of the influence of O_3 on beech crown architecture, based on Roloffs classification, was performed by Stribley and Ashmore (2002)

In European forests as a whole, the main correlations between O_3 levels (expressed as AOT60) and crown condition (expressed as defoliation) were found in *Quercus ilex* and *Fagus sylvatica* (Klap et al. 2000). No correlations were found in the case of deciduous oaks or *Picea abies.* In *Quercus ilex* the correlation between crown status and O_3 concentration is only slightly significant, but this finding may be biased by the small number of observations carried out. The significance is much greater in the case of *Fagus sylvatica.*

As far as the national studies are concerned, the following conclusions can be drawn:

- in Switzerland the findings showed that defoliation of all species between 1985 and 1994 was greater in areas with higher levels of O_3 and SO_2, with lower winter temperatures and in sites with a lower humus content (Dobbertin et al. 1997). Zierl (2002) applied a hydrological model to Swiss forests that simulates stomatal aperture, and thus O_3 absorption. The findings support the hypothesis that O_3 contributes to defoliation, although the Author underscored the wide range of factors that may modify the response of the different species (first and foremost the effectiveness of their detoxifying processes); he also questioned the value of defoliation itself as a reliable response parameter.

- In the United Kingdom, defoliation values of Norway spruce, Scots pine, English oak, pedunculate oak and beech varied in relation to the annual levels of O_3 (Innes and Boswell, 1988). These findings were further confirmed by Mather et al. (1995) in beech and Scots pine.

- In the Netherlands, O_3 was found to be one of the factors explaining both defoliation levels and foliar Mg and Al content in English oak and Scots pine (Hendricks et al., 1997).

- In Italy, crown transparency was found to increase at increasing AOT40 in the case of beech, but not in the case of Norway spruce (Ferretti et al. 2003c). However, in a recent study carried out on the beech sites of the national network CONECOFOR, Bussotti et al. (2005) reported that leaf morphology is a more suitable response-to-ozone indicator than crown transparency or defoliation. In particular, in areas subjected to high ozone levels the leaf thickness increased, thus confirming previous findings reported by Pääkkönen et al. (1997) in experimental conditions.

- In eastern Spain, a correlation between crown transparency and chlorotic mottle attributed to ozone was detected in *Pinus halepensis,* suggesting that ozone may contribute to defoliation (Sanz et al. 2000).

Interactions between O_3 and other stress factors

Drought

Stomatal activity is considered the key element determining the sensitivity of a particular species to O_3 (Guderian et al. 1985; Grulke 1999; Emberson et al. 2000: Schaub et al. 2003). Usually the prevailing weather conditions in most parts of southern Europe induce a marked reduction in stomatal conductance during the height of summer. The highest levels of O_3 experienced in the field usually coincide with the period when non-managed plants suffer the greatest degree of water deficit, and their stomata are closed. However, the behaviour of individual species varies considerably in their capacity to tolerate drought before resorting to stomatal closure. As a consequence, those species that exhibit the greatest ability to maintain, or reactivate, gas exchange under conditions of water stress, might be expected to be the most affected by O_3.

Grulke (1999) reports that genotypes of Jeffrey pine *(Pinus Jeffreyi* Grev. et Ball) differ in their sensitivity to O_3 as a result of differing stomatal responses under conditions of soil water deficit. Thus, soil moisture availability is considered an important factor modifying the impact of O_3 (Guderian et al. 1985), while, vice versa, the partial stomatal closure induced by O_3 can lead to the avoidance of drought injury (Maurer et al. 1997). The way in which the combination of O_3 and soil water deficit affects stomatal conductance is, however, much more complex than might at first be perceived: recent evidence suggests that exposure to the pollutant can impair stomatal performance under conditions of soil water deficit (Maier-Maercker 1998) and the outcomes in terms of cost/benefit analysis probably depend, at least to some extent, on the period when the stress occurs, on the plant's genotype and on the intensity of the stress (Maier-Maerker 1998).

Nutrients

An adequate level of fertilization is not a necessary prerequisite to improve O_3 tolerance (Maurer and Matyssek 1997; Maurer et al. 1997), yet in optimal edaphic conditions trees can accelerate their foliar turnover, replacing inefficient, injured leaves with new, more photosynthetically active leaves; but the overall condition of the tree suffers the effects of premature aging, since this behaviour requires a huge investment of mineral nutrients. On the other hand, a limited mineral nutrition can improve the content of secondary metabolites (Gutschick 1999) and, ultimately, the plant's antioxidant ability

to defend itself against O_3, thus delaying the premature loss of leaves. It's not certain that this is truly an advantage, since injured leaves tend to have an increased respiratory activity and a reduced photosynthetic one.

Light

Several investigations (Tjolker et al. 1995; Fredericksen et al. 1996; Günthard-Goerg et al. 1997) suggest that the effect of O_3 is more pronounced in low light or shaded environments. The mechanisms of resistance to O_3 injury in sun versus shade leaves involves both structural and physiological differences. Sun leaves are normally thicker, have denser mesophyll and higher light-saturated photosynthetic rates than shade-acclimated leaves. The amount of cell surface available to interact with O_3 is lower in shade leaves, thus limiting the effectiveness of the detoxifying processes. Furthermore, the processes whereby many scavengers are formed are stimulated by light (cf. Matyssek and Innes 1999). On the other hand, located as they are on the external part of the plant, sun leaves offer a measure of protection to shade leaves, intercepting most of the deposited O_3. Fredericksen et al. (2004) and Wei et al. (2004a, b) demonstrated that, in shade leaves of different broadleaved species, net photosynthesis is reduced more than stomatal conductance. This behaviour implies that, in ozone rich environments, the uptake of ozone in shade leaves is not balanced by an adequate production of detoxifying substances (because of the reduced net photosynthesis).

Frost

In evergreen species, the reduced sugar production caused by the inhibition of photosynthesis can influence the efficiency of osmotic regulation. Moreover, direct damage to the lipidic composition on the cell membrane may occur. This in turn can alter the hardening processes that provide a defence mechanism against frost. This aspect has been studied in *Picea abies* (Wolfenden and Wellburn, 1991) and *Pinus halepensis* (Wellbum and Wellburn 1994).

Other pollutants

The mixture of O_3+SO_2 has a synergistic effect in producing visible injury on leaves of *Pinus strobus* (Dochinger and Deliksar 1970) and on total biomass of *Pinus halepensis* (Diaz et al. 1996). Dotzler and Schutt (1990) found that fumigation with low concentrations of O_3+SO_2 affects young Norway spruce, although visible symptoms may not occur. Mixed O_3+NO_2 fumigations of forest tree seedlings have a growth-reducing effect (Krees and Skelly 1982; Mooi 1983); NO_2 applied in a sixfold ambient concentration

neither significantly compensated for a low soil N supply nor greatly modified the effect of O_3 (Günthardt-Goerg et al. 1996).

Some evidence of a compensation effect of elevated CO_2 on O_3 toxicity was provided by studies on oak (Manes et al. 1998; Broadmeadow and Jackson 2000), beech (Grams and Matyssek 1999), *Prunus serotina, Fraxinus pennsylvanica* and *Liriodendron tulipifera* (Loats and Rebbeck 1999), poplar (Schwanz and Polle 2001) and birch (Vanhatalo et al. 2001). The processes involved in the compensation of the O_3 effects are thought to be: reduction of stomatal conductance, metabolic compensation in the mesophyll (Grams et al. 1999) and the provision of increased amounts of substrate for O_3 detoxification (Allen 1990) by means of increased leaf density. Conversely, no buffering effect was seen in coniferous species as Norway spruce (Lippert et al. 1997), Scots pine (Utriainen et al. 2000) and *Pinus halepensis* (Kytoviita et al. 2001).

Pathogens and phytophagous insects

There is evidence that O_3 alters parasitism via the effects it exerts on host plants (Sandermann 2000). O_3 stress changes may either increase or decrease the susceptibility to the pest agent. The time at which the parasite infestation occurs may be not the same as the O_3 exposure, but may be delayed (memory effect, Sandermann 2000).

Generally infections by obligate pathogens, which require a healthy host for successful invasion, are decreased in intensity by O_3 exposure. Conversely, plants weakened by O_3 damage are especially susceptible to facultative parasitic fungi that can benefit from damaged host cells and from disordered transport mechanisms (Beare et al. 1999). However, several factors like tissue age, timing of exposure to O_3 and period in the disease cycle during which O_3 exposure occurs, may influence and alter this general pattern.

Direct effects of O_3 on feeding, growth and survival of insects were observed only in fumigation experiments and the results are contradictory: for example, the effect of O_3 on aphid dynamics stimulated the growth of *Phyllaphis fagi* on beech seedlings (Braun and Flückiger 1988), but had no influence on the survival, reproduction and development of the cottonwood aphid *Chaitophorus populicola* (Coleman and Jones 1988a).

The indirect effects are connected to the changes in the physical or biochemical properties of leaves (Coleman and Jones 1988b) and trunk (Cobb et al. 1986) which affect the palatability of the foliage or change the host condition. The research conducted in the ponderosa pine forests of the San Bernardino Mountains - subjected to high levels of atmospheric oxidants from Los Angeles urban area - revealed some mechanisms of the relationships between *Pinus ponderosa* and bark beetle populations *(Dendroctonus*

ponderosae and *D. brevicormis).* It has been suggested that the reason bark beetles showed a preference for injured trees was linked to changes in resin and sapwood characteristics (reduction of oleoresin exudation pressure; quantity, rate of flux and increased propensity of oleoresin to crystallize and a reduction in phloem and sapwood moisture content) that facilitated bark beetle activity particularly in the concentration and establishment phases (Cobb et al. 1968).

Effects on ecosystem processes, patterns and dynamics

There are only a few reports in the literature concerning O_3 effects on forest ecosystem function and they deal exclusively with litter decomposition. No apparent influences of O_3-exposed litter on the decomposition process was found by Fenn (1991) and Boerner and Rebbeck (1995); on the other hand, Findlay et al. (1996) reported that early-abscised leaves from O_3-exposed *Populus deltoïdes* saplings had higher N contents and decomposed at a slower rate than leaves from control plants. In the same studies, leaves from O_3-exposed plants that abscised at the normal time had a lower N content and lower specific leaf mass than control leaves, but decomposed at the same rate as leaves from control plants. The differences in the results may be attributed to several causes, like the different incubating environments in the litterbags, the decaying environment, the effect of O_3 on the fungal populations of the decomposer community both on the leaf phyllosphere and litter layer that reduces and/or alters the composition of phyllosphere fungal populations (Fenn et al. 1989; Magan et al. 1995).

Final remarks

The literature on O_3 effects on forest trees is wide-ranging, although only a very small part of the findings are useful in understanding the natural ecological processes occurring in forests. The most important limitation lies in the fact that experiments are usually performed on young and small trees whose physiology is very different from that of adult trees (Kolb et al. 1998; Schaub et al. 2004b). Seedlings are usually considered more sensitive than adult trees due to their greater stomatal activity, but in some cases *(Quercus rubra)* the findings suggest the opposite (Kelly et al. 1995). Further problems are raised by the complex relationships between the tree and external factors (ecological conditions and interactions with other organisms) which we still know little about. It is easy to understand why, when we try to investigate one factor at a time, the findings are often contradictory. It is important to bear these limitations in mind when we attempt to interpret ecological hazards in the field.

The O3SWE project

Objectives

The project "Ozone at the intensive monitoring plots in South-Western European forests: levels, risks, actual and potential effects" is based on a similar experience carried out at national level in Italy (Ferretti et al. 2003b). The project aims to cover an important ecological, scientific and political issue not yet fully addressed by the data evaluation activity within the intensive monitoring of forest ecosystems in Europe (the so-called "Level M" monitoring system). For this reason, this project presents an opportunity to provide elements for the future implementation of the monitoring program, its evaluation strategy as well as other national and international initiatives. The project concentrates on the objectives listed below:

 (i) Provide a synoptic view of descriptors of O_3 concentration at 83 forest sites in south-western Europe (see Annex 1). Currently, most of the O_3 data at European level come from monitoring devices located in urban/sub-urban areas (e.g. de Leeuw and Bogman 2001), and a comprehensive dataset on forest sites will provide a considerable input for a better understanding of ozone levels in remote areas. This will also help in evaluating factors (e.g., geographical, meteorological) associated to O_3 concentration. The identification of the main driving factors and the relative contribution in determining O_3 concentration will provide the basis for future upscaling studies.

(ii) Estimate AOT40 values for the intensive monitoring plots in France, Italy, Spain and Switzerland starting from the data collected by passive sampling and their validation against continuous O_3 monitors (where available). This will provide a first estimate of the potential risks to forests in relation to ozone.

(iii) Explore the feasibility and provide calculated estimates of O_3 uptake by taking into account the modifying factors. Since the primary point of entry of ozone into the plant is through the stomata, the uptake of O_3 is mostly connected to the factors controlling the stomatal conductance. Different models are available (e.g. Emberson 2002; Emberson et al. 2000). Models calculate the stomatal conductance to ozone as a function of species, phenology, and environmental variables (e.g., irradiance, temperature, atmospheric and soil water content). These data are either already available for some plots or can be modelled starting from available procedures.

(iv) Explore the relationship between O_3 levels and possible response indicators at the plant community level. Data generated by the response indicators (e.g. crown condition, visible symptoms) will be subjected to statistical analysis in order to identify whether there is a consistent response.

The above analyses will provide important information for the evaluation of the potential and limitation of the current approach in 4 European countries and will help to identify recommendations as how to proceed with the data collection in the future. These elements can be useful for those countries which intend to undertake O_3-related investigations and install passive samplers in their intensive monitoring plots; it will also favour future analyses at wider geographical scales.

Project structure and partnership

The project is divided into 4 Working Packages (WPs) (Figure 1) plus the coordinating activity (carried out by the Department of Plant Biology, the University of Florence on behalf of the Italian Ministry for Policy in Agriculture and Forests). Each WP has its own tasks and leader; and work of each WP is closely related to the preceding or following WP. Responsibility is shared between project partners as a function of past experience, expertise and available time resources.
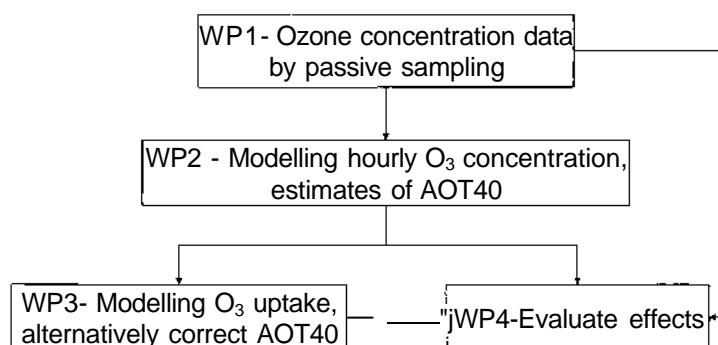
Figure 1 - Working Packages and their relationships in the O_3SWE project.

Each WP is responsible for defining data needs, sending data requests to the various project partners and deciding formats for data submission. The data flow scheme is in Figure 2: data requests proceed through the coordination body to the National Focal Centers (NFCs) of the 4 Countries and to the experts involved. Data requested are submitted following the reverse path, from experts to WPs, through NFcs and coordination.
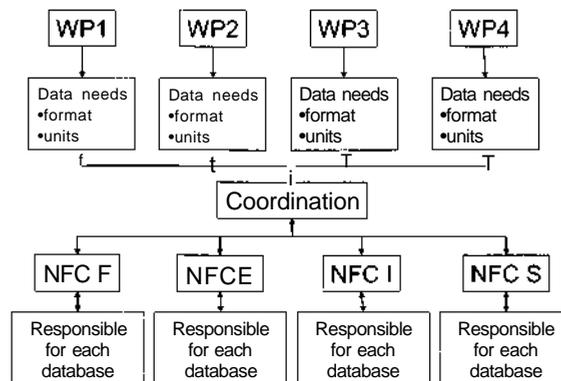
Figure 2 - Data exchange scheme.

WP1 - Ozone concentration measured by passive sampling

WP 1 is responsible for collecting and evaluating data obtained from passive sampling at the various monitoring sites. Although the project will focus on the years 2000-2002, it is worth noting that the group's activity in the field of passive sampling began much earlier (Figure 3). Leader of WP1 is Spain, working in cooperation with the NFC at the Ministerio de Medio Ambiente, Direcciön General para la Biodiversidad, Madrid, and the CEAM, Valencia.
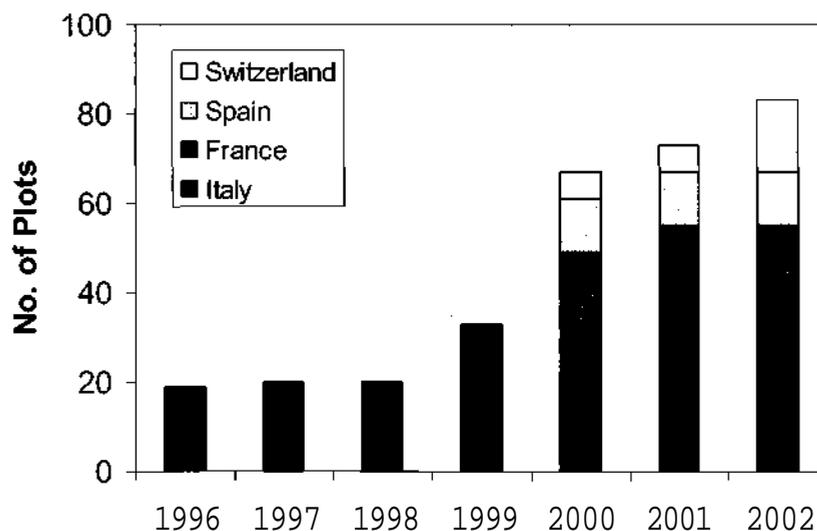
Figure 3 - Ozone monitoring by passive sampling at the Level II sites in France, Italy, Spain and Switzerland from 1996 to 2002.

WP2 - Estimates AOT40 by modelling hourly ozone concentration

Passive samplers are useful to get an idea of mean weekly/fortnightly O_3 levels, but they do not provide indication as to AOT40 values, i.e. the exposure indicator currently adopted to estimate the potential risk for forests as well as natural vegetation and crops (Fuhrer et al. 1997; Karenlampy and Skarby 1996; Karlsson et al. 2003). However, some work has been done to estimate hourly O_3 concentrations under complex terrain condition as functions of altitude and daytime (e.g. Loibl et al. 1994), as well as to estimate AOT40 values from passive sampling data (Tuovinen 2002, Krupa et al. 2003; Mazzali et al. 2003; Gerosa et al. 2003). An application study carried out in Italy demonstrated the possibility of calculating AOT40 from passive sampling data (Figure 4). Based on WP1, WP2 therefore is responsible for (i) processing validated passive sampling data to obtain hourly concentration data and AOT40 estimates and (ii) providing estimated hourly data to WP3 (flux modelling). Leader of WP2 is Italy, through the Ministero delle Politiche Agricole e Forestall, Corpo Forestale dello Stato, Servizio CONECOFOR, and the Université di Firenze, Dipartimento di Biologia Végétale.
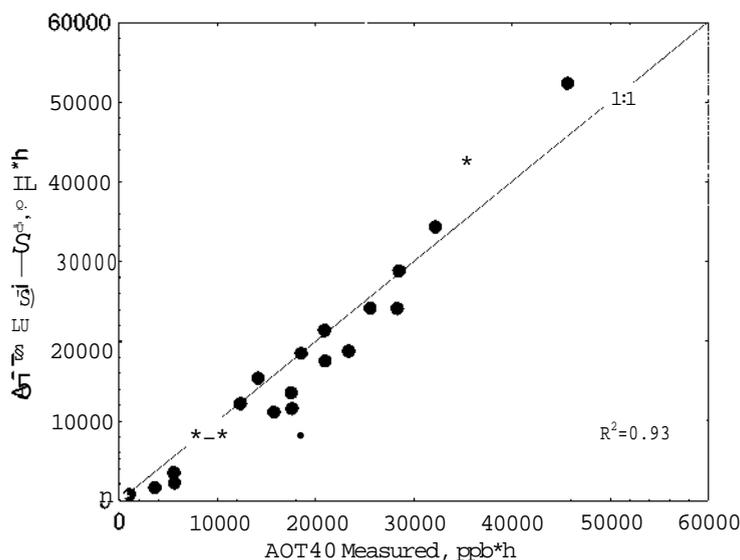
Figure 4 - Measured and Estimated AOT40 values for a number of Italian sites over the period 1996-2000 (after Gerosa et al. 2003, modified).

WP3 - Modelling ozone uptake

While AOT40 can provide a first indication of a potential risk, it is widely recognised that plant response is more closely related to the internal ozone dose (i.e. the ozone taken into the plant through the stomata), which in turn depends on a variety of ecological factors. Recently, considerable progress has been made in estimating the actual O_3

uptake by plants (Emberson et al. 1998, 2000; Simpson et al. 2000) and other exposure indices (e.g. Grünhage et al. 2001) that may provide more reliable estimates of the actual risk due to O_3. To build up on the work of WP2, WP3's task is to process O_3 and site data to obtain estimates of O_3 flux. This task is constrained by data requirements that limit the feasibility of the study as well as by the overall complexity (Ferretti and Gerosa 2002; Gerosa and Anfodillo 2003) (Figure 5). For this reason, it was decided to consider only the sites with beech as the main tree species. The WP3 is led by Switzerland, the Federal Research Institute WSL, Birmensdorf, Switzerland.
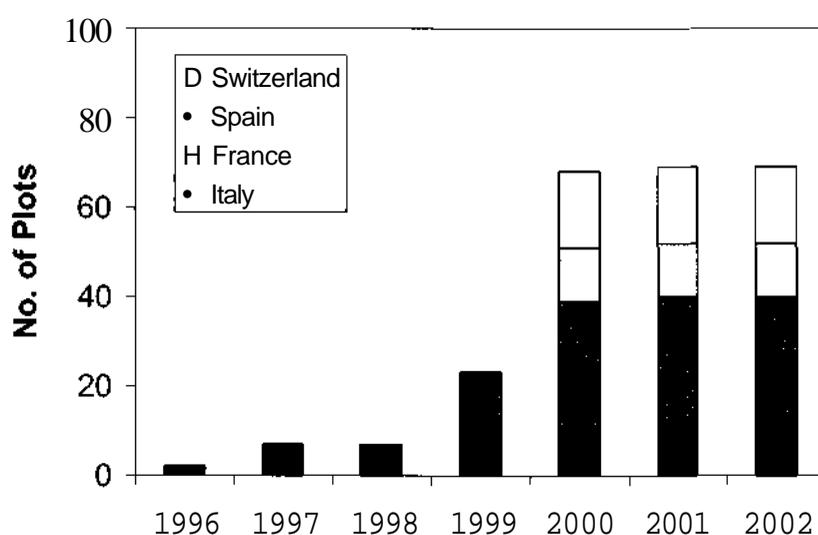
Figure 5 - *Number of co-located meteorological stations and* O_3 *passive sampling at Level II sites in France, Italy, Spain and Switzerland from 1996 to 2002.*

WP4 - Evaluation of ozone effects on vegetation

The task of WP4 is to evaluate statistical relationships between O_3 (concentration and AOT40, possibly flux) and response indicators. What response indicator should be used is a key point. Concerning the response of the ecosystems, indicators for which data are available include tree growth (estimated by basal area increment), crown transparency and visible foliar symptoms. Of these indicators, growth is available for the period 1996-1999, i.e. a period not covered by O_3 measurement. Crown transparency (defoliation) is available on annual basis: however, crown transparency is unspecific and depends on a variety of factors, including species, genetic features, site condition, soil properties, climate and weather, current and past forest management and competition for resources. These factors can be regarded as noise when attempting to evaluate O_3 effects. Foliar symptoms on trees, herbs and shrubs (Figure 6) are more specific

indicators, but they are collected mostly on a qualitative basis and are not suitable for a proper statistical analysis. Leader of WP4 is Italy, through the Ministero delle Politiche Agricole e Forestall, Servizio CONECOFOR and the Université di Firenze, Dipartimento di Biologia Végétale.
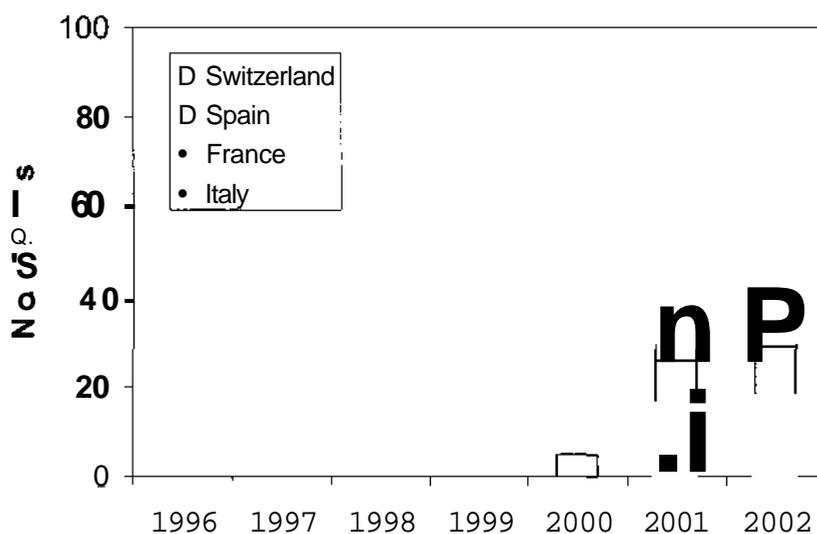
Figure 6 - Monitoring of visible symptoms at Light Exposed Sampling Sites (LESSs) close to Level II plots in France, Italy, Spain and Switzerland from 1996 to 2002.

Monitoring sites

Monitoring sites are described in Annex 1 and mapped in Figure 7. Sites cover a wide range of ecological situations and span over 1600 km in latitude (Northern France to Southern Italy and Spain), 2000 km in longitude (North-Western Spain to South-Eastern Italy) and from 5 to 1900 m a.s.l. Most represented main tree species (MTS) at the sites are European beech *(Fagus sylvatica* L.) and Norway spruce *(Picea abies* Karst.). Other MTS species include *Abies alba* Mill., *Pinus cembra, P. halepensis, P. mugo, P. pinea, P. pinaster, P, sylvestris, Pseudotsuga menziesii, Quercus cerris,* Q. *ilex, Q. petraea L.,* Q. *robur, Q. suber.* Each of these species is MTS in 7 or fewer plots (Table 1).
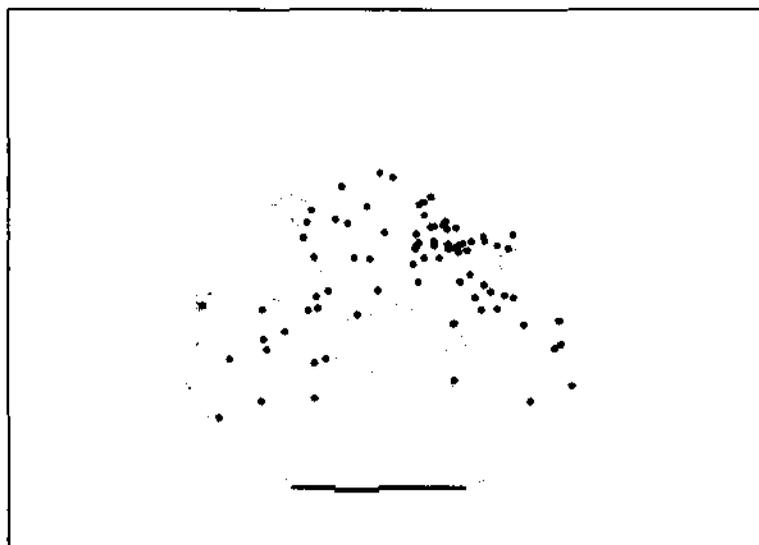
Figure 7 - Geographical distribution of the plots in the four countries participating in the project. Two additional plots from Luxenbourg (not showed in the map, see Table 1) were also considered for the evaluation of ozone concentrations.

Table 1 - Plots according the Main Tree Species (MTS) in the dominant storey for each of the the four participating countries and from Luxenbourg.

Species	Italy	France	Luxenbourg	Spain	Switzerland	Total
Fagus sylvatica	10	3	2	1	6	22
Picea abies	6	4	0	0	3	13
Abies alba	0	6	0	0	1	7
Pinus sylvestris	0	3	0	2	2	7
Quercus robur/petraea	1	4	0	1	0	6
Quercus spp.	0	0	0	0	1	1
Pinus pinaster	0	4	0	2	0	6
Quercus cerris	5	0	0	0	1	6
Quercus ilex	3	0	0	2	0	5
Mixed stand	1	1	0	0	0	2
Pinus nigra	0	1	0	1	0	2
Pinus cembra	0	0	0	0	1	1
Pinus halepensis	0	0	0	1	0	1
Pinus mugo	0	0	0	0	1	1
Pinus pinea	0	0	0	1	0	1
Pseudotsuga menziesii	0	1	0	0	0	1
Quercus suber	0	0	0	1	0	1
Total	26	27	2	12	16	83

Expected results

The expected results are directly relevant to the project objectives as they were stated in the section on aims. In general, the project aims to:

- provide a synopsis of O_3 concentration values at a number of intensive monitoring plots in forests of South-Western Europe, together with an insight on the use of

passive samplers. This is the basic information to be provided by the project and the first that will be come available.

- provide calculated estimates of AOT40 values and other exposure indices for the concerned intensive monitoring plots and to validate them against measured values (e.g. real time monitoring devices).

- explore the feasibility and provide calculated estimates of O_3 uptake by the dominant tree species at the plots by means of models (see for example Emberson, 2000; Simpson et al. 2000) and taking into account soil, atmosphere and canopy characteristics.

- explore through multivariate statistical analysis the relationships between ozone exposure (and possibly uptake) and biological (crown defoliation, symptoms on vegetation at the plot) response indicators.

- provide insight on the effectiveness of passive samplers for monitoring O_3 concentration, derive exposure indices and calculate O_3 uptake. Not only will the suitability of passive samplers be verified: the adequacy of the data collected at the intensive monitoring plots for estimating reliable risk associated to ozone will also be tested. This will provide important benefits for the future implementation of the monitoring program.

The report

The project described in this paper represents the first harmonized and coordinated attempt to provide a synoptic view of O_3 levels and effects at remote forest sites in Europe. The project uses an inexpensive technique to measure O_3 levels and explore the feasibility and reliability of methods to model O_3 exposure indices and O_3 uptake, and investigate whether effects are detectable under field condition. This report is structured according to the project steps. In section 2, Sanz et al. (2004, in this volume) provide data about O_3 concentration as measured by passive sampling and discuss reliability of measurements, mean and peak values and main geographical trends. In section 3 Gerosa et al. (2004, in this volume) report on estimation of AOT40 values starting from passive sampling data described and validated by Sanz et al. (2004, in this volume), modelling hourly O_3 concentration and providing site-related estimates of AOT40 on single-year and 3-year basis. In section 4, Schaub et al. (2004a, in this volume) use the hourly O_3 concentration modelled by Gerosa et al. (2004, in this volume), and a suite of other environmental and site parameters, to attempt flux calculation. In section 5, Ferretti et al. (2004b, in this volume) report on occurrence of symptoms and O_3 effects on crown transparency. In Section 6, Ferretti et al. (2004a, in this volume) provide a summary of major findings in relation to the objectives of the project and the expected results.

Acknowledgements

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Annex 1 - Site characteristics (continue on next page).

Site name	Site code	ID	Country	X_UTM31	YJJTM31	Elevation	Main Tree Species
CHP40	3	F_3	France	192684	4850210	20	*Quercus robur*
CHP59	6	F_6	France	555052	5558191	160	*Quercus robur*
CHS 35	16	F_16	France	165340	5346413	80	*Quercus petraea*
CHS 41	17	F_17	France	370829	5270208	120	*Quercus petraea*
CPS77	30	F_30	France	480482	5367093	80	*Mixture Q. robur/Q. petraea*
DOU71	36	F_36	France	583510	5216343	520	*Pseudotsuga menziesii*
EPC08	37	F_37	France	630776	5534613	435	*Picea abies*
EPC63	41	F_41	France	498675	5067127	950	*Picea abies*
EPC74	44	F_44	France	758868	5125070	1210	*Picea abies*
EPC87	46	F_46	France	409743	5072771	750	*Picea abies*
HET30	57	F_57	France	544650	4884948	1300	*Fagus sylvatica*
HET54a(1)	59	F_59	France	781235	5378663	315	*Fagus sylvatica*
HET64	63	F_63	France	204592	4784138	300	*Fagus sylvatica*
PL 20	69	F_69	France	982362	4695709	1030	*Pinus nigra var. laricio*
PM40c	74	F_74	France	261535	4881568	150	*Pinus pinaster*
PM72	75	F_75	France	302108	5292082	160	*Pinus pinaster*
PS44	80	F_80	France	141075	5276735	40	*Pinus sylvesths*
PS 67a	84	F_84	France	845839	5421738	150	*Pinus sylvesths*
PS 76	86	F_86	France	338630	5480680	50	*Pinus sylvesths*
SP05	90	F_90	France	775606	4932253	1150	*Abies alba*
SP 11	93	F_93	France	428035	4746511	1010	*Abies alba*
SP25	94	F_94	France	763848	5208549	960	*Abies alba*
SP38	96	F_96	France	745616	5034699	1130	*Abies alba*
SP57	98	F_98	France	805107	5392585	350	*Abies alba*
SP68	100	F_100	France	808518	5317389	650	*Abies alba*
PM 17	101	F_101	France	178760	5075345	15	*Pinus pinaster*
PM 85	102	F_102	France	119959	5187210	5	*Pinus pinaster*
MAR1	MAR1	I_15	Italy	1317116	4843146	775	*Quercus cerris*
ABR1	ABR1	M	Italy	1379671	4687873	1500	*Fagus sylvatica*
BAS1	BAS1	I_2	Italy	1590724	4576177	1125	*Quercus cerris*
BOL1	BOL1	I_3	Italy	1146045	5194121	1740	*Picea abies*
CAL1	CAL1	I_4	Italy	1652713	4336554	1100	*Fagus sylvatica*
CAM1	CAM1	L_5	Italy	1555534	4551031	1175	*Fagus sylvatica*
EMU	EMU	L_6	Italy	1070581	4977306	200	*Quercus petraea*
EMI2	EMI2	I_7	Italy	1149799	4916195	975	*Fagus sylvatica*
FRI1	FRI1	I_8	Italy	1289628	5125979	6	*Mixed deciduous*
FRI2	FRI2	I_9	Italy	1312959	5203737	820	*Picea abies*
LAZ1	LAZ1	1_10	Italy	1227983	4780052	690	*Quercus cerris*
LIG1	LIG1	I_11	Italy	1014322	4937027	1290	*Fagus sylvatica*
LOM1	LOM1	I_12	Italy	1008695	5142505	1190	*Picea abies*
LOM2	LOM2	I_13	Italy	1052602	5114174	1150	*Picea abies*
LOM3	LOM3	I_14	Italy	1004468	5104910	1250	*Fagus sylvatica*
PIE1	PIE1	I_16	Italy	894908	5071686	1150	*Fagus sylvatica*
PUG1	PUG1	I_17	Italy	1579995	4712729	800	*Fagus sylvatica*
SAR1	SAR1	L_18	Italy	981363	4370679	700	*Quercus ilex*
SIC1	SIC1	I_19	Italy	1416162	4247330	940	*Quercus cerris*
TOS1	TOS1	I_20	Italy	1100743	4844671	150	*Quercus ilex*
TOS2	TOS2	I_21	Italy	1135325	4775890	30	*Quercus ilex*
TOS3	TOS3	I_22	Italy	1190470	4878749	1170	*Fagus sylvatica*
TRE1	TRE1	I_23	Italy	1153333	5169429	1775	*Picea abies*
UMB1	UMB1	I_24	Italy	1265834	4856571	725	*Quercus cerris*
VAL1	VAL1	I_25	Italy	808875	5071162	1740	*Picea abies*
VEN1	VEN1	I_26	Italy	1225747	5143691	1100	*Fagus sylvatica*
05Ps	5	E_5	Spain	-455191	4798873	1630	*Pinus sylvesths*
06Qi	6	E_6	Spain	-362169	4156204	750	*Quercus ilex*
10Ppa	10	E_10	Spain	-301235	4491965	65	*Pinus pinea*
11Qs	11	E_11	Spain	148029	4770214	455	*Quercus suber*

Annex 1 - continued

Site name	Site code	ID	Country	XJJTM31	YJJTM31	Elevation	Main Tree Species
15Fs	15	E_ 15	Spain	184917	4469966	900	*Fagus sylvatica*
22Pn	22	E_22	Spain	184300	4269471	1410	*Pinus nigra*
25Ph	25	E_25	Spain	-117761	4249353	760	*Pinus halepensis*
26Qi	26	E_26	Spain	14486	4652121	610	*Quercus ilex*
3OPs	30	E_30	Spain	-114362	4775198	1100	*Pinus sylvestris*
33Qpe	33	E_33	Spain	-107088	4603762	1150	*Quercus petraea*
37Ppr	37	E_37	Spain	249241	4492727	800	*Pinus pinaster*
102Ppr	102	E_ 102	Spain	-87120	4545697	260	*Pinus pinaster*
Alptal	1	CH_1	Switzerland	940407	5236820	1160	*Picea abies*
Beatenberg	2	CH_2	Switzerland	863205	5169360	1511	*Picea abies*
Bettlachstock	3	CH_3	Switzerland	846930	5248378	1149	*Fagus sylvatica*
Celerina	4	CH_4	Switzerland	1028536	5154597	1871	*Pinus cembra*
Chironico	5	CH_5	Switzerland	945174	5151426	1365	*Picea abies*
Isone	6	CH_6	Switzerland	986438	5129997	1220	*Fagus sylvatica*
Jussy	7	CH_7	Switzerland	761197	5136898	501	*Quercus robur*
Lausanne	8	CH_8	Switzerland	778721	5160952	807	*Fagus sylvatica*
Nationalpark	10	CH_10	Switzerland	1076516	5165910	1899	*Pinus mugo*
Neunkirch	11	CH_11	Switzerland	932163	5279322	582	*Fagus sylvatica*
Novaggio	12	CH_12	Switzerland	946448	5123207	950	*Quercus cerris*
Othmarsingen	13	CH_13	Switzerland	917257	5260304	484	*Fagus sylvatica*
Visp	14	CH_14	Switzerland	867868	5141376	695	*Pinus sylvestris*
Vordemwald	15	CH_15	Switzerland	869317	5251695	480	*Abies alba*
Schaenis	16	CH_16	Switzerland	989398	5244179	733	*Fagus sylvatica*

(1) stand destroyed by the 1999 storms; the plot is still part of the French network
Note: two sites from Luxenbourg (Codes: HET L1 and HET L2; main tree species: Fagus sylvatica.) considered
by WP1 are not listed.

Annex 1 - continued

Site name	Site code	ID	Country	XJJTM31	YJJTM31	Elevation	Main Tree Species
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