UNITED NATIONS ECONOMIC COMMISSION FOR EUROPE
CONVENTION ON LONG-RANGE TRANSBOUNDARY AIR POLLUTION

International Co-operative Programme on
Assessment and Monitoring of Air Pollution Effects on Forests

MANUAL

on
methods and criteria for harmonized sampling, assessment,
monitoring and analysis of the effects of air pollution on forests

Part X

A. Monitoring of Air Quality

updated: 05/2000

and

B. Assessment of Ozone Injury

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Contents

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Index

A. Monitoring of Air Quality

1 Introduction

It is important to assess ambient air quality at the intensive monitoring plots for two reasons. Firstly, air pollutants may cause adverse effects to forest trees and forest ecosystems via direct effects, and secondly, the knowledge of pollutant concentrations in the atmosphere will improve estimates of dry deposition to the forest plots.

Measurements of air pollution deposition to forest trees are mandatory in the Intensive Monitoring Programme, and a large number of sites measuring throughfall and wet deposition have been established throughout Europe. However, throughfall measurements provide insufficient information to provide reliable estimates of dry deposition of nitrogen compounds, and additional modelling procedures must be carried out. Air pollutants of interest for this purpose are nitrogen dioxide, ammonia and other gaseous and particulate nitrogen species.

The ambient air pollutants of interest for direct effects on vegetation include ozone, nitrogen dioxide and sulphur dioxide. Of these, ozone is of primary interest due to its phytotoxicity at ambient concentrations, and widespread occurrence in Europe, particularly in the Mediterranean area.

To gain a better understanding of air pollution in forests and its effects on forest ecosystems, information on ambient air quality in forests must be collected in addition to deposition estimates. Measurements recommended within the framework of the Intensive Monitoring Plots of ICP Forests have the following objectives:

- to produce information on ambient air quality in forest ecosystems
- to gain a knowledge of the spatial and temporal distribution of gaseous pollutants
- to estimate the risk of direct effects on forest ecosystems

Today, ozone is only measured at some of the forest plots. Measurements are made using active monitors near the ground or on meteorological towers at canopy level. It is recommended to proceed with these ongoing measurements. However, at most forest sites, active monitoring of air pollutants is not feasible, and other types of measurements must be made.

To add to the information on the direct effects of air pollution on forest trees, the extent of visible and invisible injury to forest trees and plants should be assessed with the following objectives:

- to determine the extent of damage to forest trees and sensitive ground vegetation species as a direct response to air pollution
- to confirm the relationships between the concentration of specified gaseous pollutants and foliar injury

2. Assessment of ambient air quality

2.1 Methods

To gain knowledge of exposure to air pollution at specific sites, data on ambient air quality must be collected. Options for providing these data include the following:

- modelling, including the interpolation of monitoring data from near-by sites
- real time air quality monitoring and other active monitoring methods
- passive sampling

Ambient air quality at the site can be estimated by modelling or by interpolation of monitoring data from near-by sites. In most areas, however, representative local monitoring stations, that could provide the necessary data, are not available. For this reason, there is also a lack of sufficient information to enable the use of most modelling approaches.

Real time air quality monitoring will provide the most detailed information, as a result of its high temporal resolution. However, such measurements are costly, and because of the necessary infrastructure requirements, these active monitoring sites are generally scarce in background areas. As mentioned in section 1, where such measurements are being made it is recommended that they are continued.

Passive sampling for compounds such as ozone, sulphur dioxide, nitrogen dioxide and ammonia has proved to be a suitable method in many areas. This is particularly so in remote sites, where the availability of a power supply is often limited, and accurate determinations of ambient air concentration can be achieved at relatively low cost. The disadvantage of passive monitoring is the low temporal resolution (from one week to one month, mainly dependent on the magnitude of air concentrations).

For validation purposes, the results from passive samplers should be related to ambient air quality monitoring at a limited number of sites with similar pollution climate. This should be achieved using a combination of high-temporal resolution (such as the EMEP sites) and low-temporal resolution passive samplers.

2.2 Monitoring protocol

2.2.1 Choice of method

The choice of method depends on the need for high temporal resolution, the frequency of on-going visits to the monitoring sites and the resources available. Passive sampling is recommended as the main method within the ICP Forests programme on sites that do not currently monitor ozone using active samplers.

2.2.2 Equipment

Individual countries are free to select the type of passive sampling device that is used. However, it should be shown that both the samplers and procedure that are used, comply with measurements made using a reference method. It is recommended to run the samplers used at selected sites during the vegetation period in parallel with the EU Daughter Directive (COM 1999, 125) reference method, UV-spectroscopy and/or with an instrument run at an EMEP site in accordance to the EMEP Manual (EMEP/CCC/ Report 1/15, NILU, Norway).

For details on equipment see Annex 1.

2.2.3 Measurement period

Sampling will preferably be carried out on a 2-weekly basis. At remote sites, the measurement period can be extended to four weeks if necessary, and at highly polluted sites, shortened to one week. Measurements of ozone will be limited to the leafed period for deciduous species, but will be continued for the rest of the year for other pollutants.

2.2.4 Siting

Selection of plots for measurements
Ambient air quality monitoring must be site specific and it is recommended that monitoring should be carried out on key plots where meteorology and deposition data are available. Sites with variable exposure should be chosen i.e. sites with high exposure in addition to a few background stations.

Siting in the plot
Air pollution concentrations should be measured near, but outside the forest, in a place representative of the plot. Monitoring can be carried out in an open field, preferably where the samplers for wet deposition and the meteorological equipment are installed. In addition, where ongoing measurements are, or could be carried out at canopy level, it is recommended that such measurements are continued or initiated.

Number of samplers
Initially, the installation of duplicate samplers for ambient air quality at each site is recommended for quality assurance reasons.

Sampling height
The samplers should be placed at a height between 2 and 4 m above ground. The height should comply with the recommendations of the CEN-document (Annex I), and the inlet heights of active monitoring instruments.

2.2.5 Analytical procedure

The analytical procedure is directly linked to sample tube preparation. As mentioned in section 2.2.2, individual countries are free in their choice of choice of methodology, as long as good quality is assured. More details on analytical procedures are found in Annex 1.

It is recommended that all samplers, or at least all samplers measuring the same variable, are analysed at one laboratory per country. The laboratory should use well-defined sample handling and analytical procedures, according to national and/or European standards for good laboratory practices.

On a number of occasions, a field blank, not exposed to ambient air, should accompany the passive samplers sent out to the operators and be analysed as blind sample for quality control.

3 Validation

The passive samplers will provide data on accumulated exposure during a 14-day period and from this, the 14-day mean is calculated. The same applies for 4-week exposure periods. However, for further analysis of the effects of ozone, AOT40 is the index used in Europe and thus it is necessary to derive a relationship between the results from passive sampling and actual temporal variations in ozone concentration. AOT40 estimates can be made by using data from a near-by site, which is equipped with active monitoring devices and, which is also representative of the pollution climate. The active monitoring should be combined with a parallel passive sampler to provide the necessary relationships between long term exposure (14 days to 1 month) and exposure indices as listed in Table 1.

Table 1 Passive sampler results from the forest plot are linked to measurements made at a near-by site of similar pollution climate, where active and passive monitoring is carried out in parallel. The exposure index at the passive site is then estimated via this relationship.

Index	**Passive monitoring at the plot**	**Active monitoring at a near-by plot**	**Passive monitoring link at the site of active monitoring**
1 hour mean	-	Measured	-
24 hour mean	-	Calculated from monitoring data	-
14 days mean / half-month*	Measured	Calculated from monitoring data	Measured
4 weeks mean / month	Measured or calculated from 14-days measurements	Calculated from monitoring data	Measured or calculated from 14-days measurements
Growing season	Calculated from monitoring data	Calculated from monitoring data	Calculated from monitoring data
AOT40	Estimated from the link	Calculated from monitoring data	Related to AOT40 data from active measurements

* to comply with sampling/reporting of deposition data in the Intensive Monitoring Plots.

4 Quality assurance

Individual countries are free to select suitable sampling equipment and monitoring procedures, as long as the guidelines as stated in Annex 1 are followed. As the comparability of results is essential for the further use of data in the ICP Forests programme, as well as in all other national and European networks, a strict quality assurance system must be applied. More knowledge will be gained of the ambient air quality environment at the forest plot, including spatial and temporal variations, if the monitoring results are comparable to data produced within other monitoring networks on a national and international scale.

Regular inter-comparisons between the different samplers used and between the samplers and reference methods are necessary in order to distinguish whether there are significant differences in collection efficiency resulting from different sampling procedures.

Passive sampling is a procedure carried out on a 2-weekly to monthly basis. All steps in the procedure should be described in a national quality assurance programme, starting with sample tube preparation, continuing with sampling and maintenance in the field, transportation of the samples to the laboratory, handling of the samples within the laboratory, analysis of samples and ending with data processing, data storage and data submission to national focal centres and the central data-bank. The aim of the quality assurance is to avoid contamination and monitoring errors as far as possible. An important step in the programme is to document all actions and incidents during sampling, sample handling and analysis.

A field blank, not exposed to the ambient air, should on a number of occasions accompany the passive samplers sent out to the operators and be analysed as blind samples for quality control.

The plausibility of data submitted to the national focal centre should be verified by comparison with the normal range of concentrations measured in the same region.

To ensure consistency of the data, if monitoring sites or procedures are changed, it is recommended that parallel measurements are made for a period of time at both sites.

5 Data handling and reports

5.1 Data checks

Data should be checked with respect to quality:

- results should be checked for plausibility, in relation to available knowledge on data ranges and data variability; a comparison with other national results as regards ambient air quality is recommended as a further step in the validation of the results
- results from duplicate passive samplers should be compared

- results from passive samplers should be checked against those from active samplers
- field blanks should be analysed

5.2 Data reporting

All validated data should be sent to each national focal centre and submitted annually to the transnational central data storage. Forms for data submission are given in Annex III. The data report should include both the results and their interpretation. All important irregularities, any missing data and errors encountered in the validation should also be documented.

The data report should also include a description of the plots where ambient air quality is measured. Some of this information is already included in the description of the forest monitoring plots (longitude, latitude, altitude, tree species, etc.). Other information needs to be documented with special consideration given to relevant local conditions (exposure to local emission sources and local land use, location in relation to forest edges etc.). Measurement height above ground should be documented and reported.

A separate document, which could be an annex to the first annual report, shall be prepared to report on sampling and analytical procedures.

For ozone, relationships between AOT40 and passive sampler means should be reported.

A quality assurance report should accompany the annual data submission, describing all quality assurance results.

Annexes

Annex I: CEN document CEN/TC 264/WG11

CEN document CEN/TC 264/WG11 Diffusive samplers 1999-07-02 (CEN draft 13528 part 3)

(for copyright reasons the document not yet available in the manual.
If necessary, please contact PCC of ICP Forests)

Annex II Background: Is ozone a problem?

Outside North America and Central and Northern Europe, research has concentrated primarily on air pollutants such as fluoride, sulphur dioxide, nitrogen oxides, ammonia and ozone because they represent the most immediate problems for forest health. However, in Europe, considerable changes are occurring in the pollution climate as a result of the steps being taken to reduce the emissions of certain pollutants. The importance of sulphur dioxide as a pollutant is declining over large parts of the continent, including the Mediterranean area, and its effects on plants are being examined in fewer and fewer experiments. In contrast, nitrogen emissions have increased and remain of considerable importance, firstly because of potential ecosystem eutrophication, and secondly, because the oxides of nitrogen are an important precursor to ozone production.

Of the various pollutants present in Europe, ozone has generally been considered one of the most important for several years, particularly in the Mediterranean area. Thus, one of today's major environmental concerns is to understand the atmospheric processes that control tropospheric ozone and OH-radical budgets. Trace gas exchanges with terrestrial ecosystems and their potential effects on ecosystems are not well understood. For instance, more information on biogenic emissions (e.g. terpenes and isoprene) from forests and their potential role in ozone formation is needed, although some large EC-projects have improved our understanding of biogenic emissions in the Mediterranean area and their potential role in tropospheric O_3 formation.

As a result of photochemical processes, the deposition of nitrogen via the atmosphere seems to be increasing in some parts of Europe. Thus modifications of the N supply might directly influence carbon cycling and may lead to limitations in the supply of other major or minor nutrients, especially phosphorus.

Effects of photochemical oxidants on vegetation were first observed more than four decades ago in the greater Los Angeles basin in the US. Since then, many investigations have proved that ozone, the most prevalent and ubiquitous constituent of these gaseous pollutants has to be regarded as very phytotoxic, causing foliar injury to agricultural and horticultural crops, as well as to conifers and deciduous trees in the US. Since the early 1980s such observations have also been made in Europe.

Thus, pollution from photo-oxidants, which has been considered as one of the causes of a deterioration of the health of the European population and the vitality of ecosystems for several decades, is a major problem. Anthropogenic emissions of oxides of nitrogen and hydrocarbons, mainly released from transport activities and the use of solvents, is the main cause of many aspects of the photochemical pollution problem.

Biogenic sources of hydrocarbons are also of importance. The 4th Community Action Programme on the Environment put forward the possibility of action on ozone with a view to its harmful effects. The 5th Environment Action Programme (5EAP), issued in 1992, set emission reduction targets for ozone precursors and aimed at zero exceedances of the levels defined by the WHO guidelines (EC, 1992b), which were introduced in terms of thresholds in the current Council Directive on Air Pollution by Ozone.

Ozone is measured in a network over Europe in rural areas (EMEP sites) and in a large number of European cities. Results on the ozone situation are presented in EMEP reports, summarised in the publications of the European Environment Agency and are also available on the internet (www.nilu.no/projects/ccc/Default.htm).

Ozone precursors and ozone formation.

Photochemical pollution is derived from emissions of nitrogen oxides (NO_x, where $NO_x = NO + NO_2$) and of volatile organic compounds (VOCs) and CO in the presence of sunlight. Ozone (O_3), the major photochemical pollutant, can be transported across national boundaries. Emissions of NO_x are responsible for much of the ozone formation occurring in rural areas. In more densely populated regions, in particular close to cities, ozone formation is enhanced by VOC emissions. VOCs are mainly released from road traffic and the use of products containing organic solvents. NO_x and CO are mostly emitted from transport and combustion processes.

After emission, precursors are dispersed by wind and atmospheric turbulence. The freshly emitted pollutants mix with other pollutants, including ozone, present in background air, and a complex process of chemical reaction and continuous dilution takes place.

In the polluted boundary layer, ozone is chemically formed by the oxidation of VOCs in the presence of NO_x and sunlight. This chain reaction is initiated and carried on by reactive radicals. In the process, other products are formed such as peroxy acetyl nitrate, nitric acid, aldehydes, organic acids, particulates and many short-lived radical species. VOCs act as ‘fuel’ in the ozone formation process, whereas NO functions more or less as a catalyst, since it is regenerated in the formation process. NO also plays a key role in the regeneration of the reactive radicals, and further reaction processes.

High concentrations of freshly emitted NO locally scavenge O_3, a process leading to the formation of NO_2. Close to the sources, this process can be considered as an ozone sink. In addition, high NO_2 concentrations reduce the initial oxidation step of VOCs by forming other products (e.g. nitric acid), which prevent the net formation of O_3. Because of these reactions, a *decrease* in NO_x can lead to an *increase* in O_3 at low VOC/NO_x ratios, as is the case in cities. In this so- called 'VOC-limited regime', emission control of organic compounds is more efficient in reducing peak values of ozone pollution locally.

As an air mass moves away from an urban centre, its VOC/NO_x ratio changes due to further photochemical reactions, meteorological processes and the occurrence of fresh emissions. The concentration of NO_x decreases faster than that of VOC and consequently the VOC/NO_x ratio is amplified. At high VOC/NO_x ratios occurring in background/remote areas, the chemistry tends towards the NO_x-limited case and NO_x reductions are considered more effective in reducing reduce ozone levels in these situations.

Annex III Forms for reporting

Form with plot info on the station with the active sampler(s)

xx2000.pac

sequence 1-4	country 6-7	station 9-13	sampler_nr 15-19	compound 21-23	latitude 25-30	longitude 32-37	altitude 39-40	vegetation 42-43	Inlet height 45-48	Site classification 50-51	start_date 53-58	end_date 60-65	remarks
1													
2													
3													
4													
5													
6													
9999	99	S9999	AS999	XXX	999999	999999	99	??	99,9	99	01.01.00	31.12.00	xxxxxxxx

column		Explanatory item #
1 - 4	Sequence number	(1)
6 - 7	Country code (France = 01, etc.)	(2)
9 - 13	Station with permanent air quality measurements (Sxxxx)	
15 - 19	Number of active sampler AS001- AS999 (active sampler =ASxxx)	
21 - 23	Compound (O3, NH3, NO2, SO2, ...?)	
25 - 30	Latitude in DDMMSS	(4)
32 - 37	Longitude in (+ or -) DDMMSS	(4)
39 - 40	Altitude (in 50 m clases 1-51)	(7)
42 - 43	Vegetation at plot (1-20) (codes to be determined.....)	(99)
45 - 48	Inlet height (in 99.9 m)	
50 - 51	Site classification (code obtained from ...)	
53 - 58	Start date measurement periods (ddmmyy)	(38)
60 - 65	End date measurement periods (ddmmyy)	(38)
67 - 76	Remarks	

Note that the combination of Country code - Station code - Active Sampler number - Substance is unique (e.g. 01.S1234.AS001.NH3)

Form with information on passive sampler(s) on intensive monitoring plot and at stations

xx2000.pps

Sequence	country	plot/station	pas_samp_nr	latitude	longitude	altitude	start_date	end_date	nr_periods	Station	Active sampler	Compound	remarks
1-4	6-7	9-12	14-17	19-24	26-31	33-34	36-41	43-48	50-52	54-57	59-63	65-67	69-78
1													
2													
3													
4													
5													
6													
9999	99	9999	P999	999999	999999	99	01.01.00	31.12.00	999	S9999	AS999	XXX	xxxxxxxx

1 - 4	sequence number	(1)
6 - 7	country code (France = 01, etc.)	(2)
9 - 12	observation plot number or station number where the passive sampler is located (xxxx or Sxxxx)	
14 - 17	Number of passive sampler P001- P999 (passive sampler = Pxxx)	
19 - 24	Latitude in DDMMSS	(4)
26 - 31	Longitude in (+ or -) DDMMSS	(4)
33 - 34	Altitude (in 50 m clases 1-51)	(7)
36 - 41	Start date measurement periods (ddmmyy)	(38)
43 - 48	End date measurement periods (ddmmyy)	(38)
50 - 52	Number of measurements with passive sampler	
54 - 57	observation plot number or station number where the related active sampler is located (xxxx or Sxxxx)	
59 - 63	Number of active sampler AS001- AS999 (active sampler =ASxxx)	
65 - 67	Compound (O3, NH3, NO2, SO2, ...)	
69 - 78	Remarks	

Note that each passive sampler has a unique codes. This is either :
- the combination of Country code - Station code - Passive sampler number (e.g. 01.S1234.P001)
- the combination of Country code - plotnumber - Passive sampler number (e.g. 01.1234.P001)

Each Passive sampler is related to an active sampler using its unique code (including substance) of Form PAC (e.g. Station S1234, Active sampler AS001 and substance NH3)

Form with info on ambient air quality measurements

xx2000.aqm

sequence 1-4	country 6-7	plot 9-12	sampler_nr 14-18	start date 20-25	end date 27-32	variable_c 34-39	value 41-46	observations 48-57
1								
2								
3								
4								
5								
6								
9999	99	9999	P999	01.01.2000	31.12.2000	XXXXXXXX	99.999	xxxxxxx

column		explanatory item #
1 - 4	sequence number	
6 - 7	country code (France = 01, etc.)	
9 - 12	plot number or station number (9999 or S9999)	
14 - 18	Number of active or passive sampler 1- 999 (active sampler = ASxxx, passive sampler = Pxxx)	
20 - 25	Start date measurement periods (ddmmyy)	(38)
27 - 32	End date measurement periods (ddmmyy)	(38)
34 - 39	variable code (see table below)	
41 - 46	value	
48 - 57	Observations	

The variable codes are compound specific:

	O3 (ppb/ppbh)	NH3 µg_N/m3	NO2 µg_N/m3	SO2 µg_S/m3
mean concentration	**O3_MC**	**NH3_MC**	**NO2_MC**	**SO2_MC**
MC daytime (8 - 20)	**O3_MCd**	**NH3_MCd**	**NO2_MCd**	**SO2_MCd**
MC nighttime (20-8)	**O3_MCn**	**NH3_MCn**	**NO2_MCn**	**SO2_MCn**
max. concentration	**O3_Max**	**NH3_Max**	**NO2_Max**	**SO2_Max**
AOT40	**AOT40**			
AOT60	**AOT60**			

B. Assessment of Ozone Injury

1. Introduction

Photo oxidants with ozone as the major compound have been a concern for vegetation in Europe since the 80s. It is, however, only during the last decade that impacts of ozone have become an issue of concern in Europe. There is evidence that the ambient ozone concentrations found in Europe can cause a range of effects to vegetation, including visible foliar injury, growth and yield reductions, and altered sensitivity to biotic and additional abiotic stresses. Recent research has advanced our understanding of the underlying mechanisms of ozone effects on agricultural crops and to a lesser extent on trees and other native plant species. It can be concluded that increasing ozone concentrations not only have a negative effect on wood production (reported decreases of up to 10%), but may also lead to unstable conditions in forest ecosystems that could result in a lowered adaptive capacity to new stress in the future. Thus, long-term effects on trees may impair the function of forest ecosystems, i.e. their role with respect to water and energy balances, soil protection against erosion, vegetation cover in dry areas as well as the aesthetic appearance of the landscape. Some of the most important impacts on plant communities may be through shifts in species composition and loss of biodiversity particularly in areas with large numbers of endemic plant species with unknown sensitivity to ozone. However, much more detailed and defined site and species exposure/response research is required prior to be able to make such determinations.

Ozone pollution, unlike fluoride or sulphur dioxide pollution leaves no elemental residue that can be detected by analytical techniques. Therefore, visible injury on needles and leaves is the only easily detectable evidence in the field and is regarded as a result of oxidative stress, leading to a cascade of adverse effects. Until now experiments have concentrated on explaining the mechanisms leading to the injury observed in the experimental studies, rather than to identify and characterise the symptoms observed in the field on a regional scale. The evidence we have today strongly suggests that ozone occurs at concentrations which cause visible foliar injury to sensitive plants. Even though visible injury does not include all the possible forms of injury to trees and natural vegetation (i.e. pre-visible physiological changes, reduction in growth, etc.), observation of typical symptoms on above ground plant parts in the field – also referred to as *passive bio indication* - has turned out to be a valuable tool for the assessment of the impact of ambient ozone exposures on sensitive species in Europe. In certain parts of Europe, however, ozone-induced visible injuries can rarely be seen in needles and leaves of trees. Thus, the use of microscopy can be an appropriate tool to identify ozone responses on a cellular level under far more rigorous survey and diagnostic conditions as an optional tool[1].

The assessment of visible injury serves therefore as a means to estimate the potential risk for European ecosystems by ambient ozone concentrations and has to be seen in the context that ICP-Forests was intended a.o. to document the presence of environmental drivers which can affect forest condition across Europe. The concrete aims of the visible injury assessment are therefore:

- to assess the occurrence of ozone injury symptoms on main tree species on a substantial number of LEVEL II plots in Europe, in order to elucidate the distribution over space and time.

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[1] In that regard some indications can be found in the Web page, http://www.gva.es/ceam/ICP-forests/index.htm.

- to survey the natural vegetation at forest edges close to the very LEVEL II sites in order to increase the information on ozone sensitive species within native plant communities.
- to characterize the ozone concentrations at LEVEL II sites by passive sampling (see Manual on Monitoring Air Quality)

The results attributed to LEVEL II sites will be documented in a map covering Europe, characterizing hot spot zones of increased risk for European forest ecosystems by the impact of ambient ozone concentrations. However, ozone injury symptom expression is inter- and intraspecies dependent, and, apart from local ambient ozone concentration pattern, influenced by other stressors (biotic, edaphic, hydraulic, climatic). Due to the complex nature of the diagnosis and the feasible/necessary restrictions in the input of resources, results from the tree and vegetation assessment should be regarded as semi quantitative.

2. Objectives of the vegetation injury assessment

The main objective of applying the passive biomonitoring approach is to gain information on the ozone injury distribution on native sensitive plant species within the European forest ecosystems (spontaneous vegetation and tree species) in a simple, feasible and statistically sound way. The essential basis for choosing visible injury is that many plant species respond to ambient levels of ozone pollution with distinct visible foliar symptoms which can be diagnosed in the field.

The following steps are needed:

- Since visible ozone injury on coniferous trees (described as chlorotic mottling) as well as on deciduous trees (described as stippling, necrotic spots of various colorations, bronzing, or even discoloration) is known, but distinction with other abiotic and biotic symptoms is difficult, an extensive photographic documentation from field as well as from controlled experimental studies is necessary. In several places documentation already exists at various institutions in Europe. A combination and extension of this information will result in an extensive and harmonised photo-documentation for species in European forest ecosystems.
- Since the ozone sensitivity of many tree and shrub species (mostly seedlings) is known, a preliminary sensitivity-ranking list will be put together based on literature information. This list will permanently be updated with the new information obtained from the surveys.
- For each plot, a list of potentially sensitive species will be constructed on the basis of the ground-vegetation list, on the main tree species symptom assessment as well as on further plot information available from the literature and successive surveys.
- To combine the information of the ground-vegetation assessment with the information on phenological and other relevant data when it will become available within the framework of the Pan European Monitoring Programme.

3. Scope

Many plant species respond to ambient levels of ozone pollution with distinct species specific ozone visible foliar injury. These symptoms can be diagnosed in the field only after adequate training (compare chapter 6). The assessment of these symptoms is to be conducted:

- Preferably on the plots where the passive ozone sampling is carried out.
- Within *the Intensive Monitoring Plot*:
    - Mandatory, for the main tree species on Intensive Monitoring Plots
    - on leaves of the upper fully sun exposed crown,
    - every second year.
- Within *the light exposed sampling site (LESS):* Since most of the intensive monitoring plots are situated in closed forests and visible ozone injury is usually restricted to the sunlight exposed upper most crown part, a special light exposed sampling site (LESS) has to be installed in the vicinity of the open monitoring plot with passive samplers. This site serves for monitoring visible ozone at an extended number of species including, if accessible, the main tree species. The survey should be done on a yearly basis.

3.1 Assessment within the Intensive Monitoring Plots

The procedure includes the selection of main tree species for symptom evaluation at each intensive monitoring plot. The ozone symptom evaluation shall comprise:

- The assessment for visible ozone injury on main tree species that shall be conducted at least on the branches from the same 5 individual trees where foliar sampling for chemical analysis is carried out (see Part IV, ICP Forest Manual, Sampling and Analysis of Needles and Leaves).
- The samples for foliar injury should be collected every second year from the upper sun exposed crown. See ICP Forest Manual part 4, Sampling and Analysis of Needles and Leaves for further details.
- For conifers and broadleaf species different evaluation procedures are suggested (see following sections).
- An annual assessment is preferred but optional.

3.2 Assessment within the Light Exposed Sampling Site (LESS)

A LESS shall be established within the vicinity of the location where the ozone passive sampler is installed The aim of the assessment within the LESS is to provide estimates of ozone foliar injury on the vegetation at the light exposed forest edge closest to the ozone measurement device within a maximum radius of 500 m (78.5 ha). The suggested sampling scheme is a random sampling design as described in Annex I.

- The assessment is done on trees, shrubs, vines and herbs;
- Only monocotyledons are excluded from the assessment
- each field crew should be accompanied by an expert plant taxonomist. Alternatively, an herbarium should be prepared for the determination of plant species in the laboratory;
- the plant nomenclature must refer to the Flora Europaea standards;
- at the moment of the survey, the plant which has passed the phenological phase of seed ripening should be annotated in the assessment (in the box for notes).

3.3 Evaluation period

Identification and quantification of visible ozone injury for conifers and broadleaves within the intensive monitoring plot shall be carried out during the periods recommended for the chemical foliar analysis and according to gathered experience within the ICP-Forests frame work. Otherwise, it should be carried out based on the known phenology of the present species within the intensive monitoring plot:

- For conifer main tree species: October - February (see ICP Forest Manual, Sampling and Analysis of Needles and Leaves).
- For broadleaf main tree species: July - beginning of September (see ICP Forest Manual, Sampling and Analysis of Needles and Leaves).

In general, including identification of visible ozone injury on trees, shrubs and herbs[2] within the LESS shall be carried out at least once during late summer (and in early summer if feasible; see ICP Forest Manual, Sampling and Analysis of Needles and Leaves) before natural leaf discoloration sets in and senescence and/or drought leads to leaf loss.

3.4 Voucher, pictorial and sampling collection

The pictorial collection and the voucher branch/leaf samples are required for the validation of the visible ozone injury symptoms observed in the field by the evaluation teams. This collection serves as national documentation.

- During the first evaluation period, *voucher leaf samples* should be collected from each assessed species: Per symptomatic species, two symptomatic and two non-symptomatic leaves (preferably small branches). The leaves/branches should be pressed in the field between two sheets of blotting paper and cardboard and made available to the respective National Focal Centre for long term documentation (a field press is required.
- During each annual evaluation period, *pictorial samples* in form of slide photographs should be collected of two symptomatic and two non-symptomatic leaves (preferably small branches) per symptomatic species showing visible ozone injury, visible ozone like injury respectively if not confirmed yet. For each symptomatic leaf, at least two pictures of both, the upper and the lower leaf surface should be taken.

The following guidelines are strongly recommended for *quality assurance* and uniformity of the pictorial documentation:

- Pictures should be taken under full sun light exposition or with a camera equipped with a flash.
- Exposure film with the speed of 200-400 ASA is recommended.
- The same slide exposure film should be used for the same pictorial series, for all pictures if possible (colour bar reference in the picture recommended).
- Electronic pictures must have a good resolution (for example 1500 pixel per inch), JPG or TIFF format and no correction.
- The leaf sample should cover at least ¾ of the final picture area to enable proper symptom identification if possible.
- Any shading effect should be avoided.

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[2] At least perennials should be considered, annuals are recommended but optional. In case both are considered perennial or annual condition should be indicated in the reporting forms.

- Additional pictures per species (i.e. the entire plant, parts of the crown, zoomed in leaf sections) using a macro and/or a zoom lens are strongly recommended for proper documentation and further evaluation.
- In addition, it is recommended to zoom on typical and species specific ozone symptom characteristics such as shading effect, non-symptomatic leaf veins, age effect (entire branch/plant). For ozone symptom characteristics, see chapter 4.1.
- Each slide should be labelled with species name (Latin name), date, and plot identification number.
- For each symptomatic species, the above described pictures must be made available to the respective National Focal Centre for documentation and/or further evaluation, representing each symptomatic species listed in the final data form

In cases of doubts and/or special interest, the respective Regional Validation Centre should be contacted for additional investigations such as microscopic examinations.

Seed sampling (optional): Seeds of symptomatic plants can be collected and made available to the Regional Validation Centres for the establishment of a seed bank for further validation of the ozone-like injury under controlled environmental conditions. Collected seeds should be stored, if possible in a cooled and sealed plastic bag, and tagged with species name (Latin name), date, and plot identification number. If fruits are not ripe at the time of the assessment mark the plants potentially suitable for seed collection and collect the seeds at a proper (i.e. later) time of the season. Collected seeds should be made available to National Focal Centres for storage and further use (i.e. quality assurance).

3.5 Equipment and supplies

Minimum equipment required for the assessment of ozone visible injury in the field:

- A 10x hand lens for closer examination of visible ozone injury on the plant leaves
- The respective plot maps and a compass to determine, exact location (coordinates), exposition, and elevation of the LESS
- Reference pictures to assist in symptom identification of known sensitive species: information can be found in the WebPage of the Co-ordination Centre and respective Regional Validation Centres,
- A plant press to store the leaves
- A camera (or digital camera) and slide films to take pictures
- Plastic bags for fresh sample and seed collection
- Field data sheets
- A cooler of sufficient size to accommodate storage of voucher specimens and collected seeds; equipment for microscopic sampling if required.

4. Symptom identification and injury scoring

The following recommendations should be followed for the scoring of visible ozone injury.

4.1 Symptom identification and injury scoring for broadleaf species

Visible ozone like symptoms can be identified and distinguished from symptoms caused by other biotic/abiotic factors by following the recommendations below:

1. Look for visible ozone injury on fully developed leaves that are exposed to full sunlight.
2. Symptoms are more severe on mid-aged and older leaves than on younger leaves. Older leaves are the first to develop symptoms (age effect).
3. Shaded portions of the leaves (i.e. if two leaves overlap) usually do not show any injury (shade effect).
4. Visible ozone injury normally does not go through the leaf-tissue. Visible symptoms are most likely confined to the upper leaf surface, typically expressed as tiny purple-red, yellow or black spots (described as stipple) or sometimes as a general even discoloration, reddening or bronzing.
5. Both, stippling and even discoloration only occur between the veins (interveinal) and do not affect the veins.
6. Towards the end of the growing season, foliar symptoms may progress to leaf yellowing or premature senescence. Severely injured leaves appear to senesce faster and drop sooner.

Examine visible ozone symptoms as described below, using a hand lens and the flow chart (Annex III):

- Is there any stippling?
- Is there any reddening and/or confluent, even discoloration?
- Do the symptoms, as described above, occur on the upper leaf surface only (except during late season when the injury becomes more severe and necrotic)?
- Are the symptom expressed between the veins only? Check with hand lens), are similar symptoms found on the veins and veinlets?
- Are the symptoms evenly distributed?
- Are the symptoms more developed on the older leaves (including leaflets ‘age effect’)?

If the above questions are answered affirmatively, the symptom can be considered as visible ozone injury.

Additional information will be provided on the WebPage of the Co-ordination Centre.

4.2 Symptom identification and scoring for conifer species

Visible ozone and visible ozone like symptoms for conifer species is expressed at the upper parts of the crown, in the upper side of branches and needles. For identification follow the recommendations below:

1. Chlorotic mottling is the most common symptom described for conifer

needles; it is the result of chronic exposure to ozone and can be described as yellow or light green areas of similar size without sharp borders between green and yellow zones. However, not all needles in a fascicle may be uniformly affected.
2. Chlorotic mottling frequently appears only in needles older than 1 year (second-year needles and older). That is, the observed symptom seems to increase with increasing needle age (age effect).
3. Chlorotic mottling is more distinct on light-exposed needle areas in comparison to shaded ones (shade effect).
4. It is easier to observe the mottling, if several needles are held close to each other, forming a "plane" of needles.

Examine visible ozone symptoms as described below, using a hand lens and the flow chart (Annex III):

- Is chlorotic mottling present in the current + 1 and more intensively in the current + n year needles, is the colour of the mottling yellow or light-green?
- Is the shape of the mottling areas regular with diffuse borders?
- Is the mottling evenly distributed along the entire needle, and more intense in the abaxial surface or most light exposed needle side?

If the above questions are answered affirmatively, the symptom can be considered as visible ozone injury.

Special attention has to be paid to confounding symptoms such as symptoms caused by spider mites and sucking insects. Using a hand lens helps to detect their remnants easily. Additional information about mimicking factors will be provided on the WebPage of the Co-ordination Centre.

5. Evaluation

Evaluation shall be different for broadleaf and conifer species. The following protocols are suggested.

5.1 Broadleaf trees (main tree species and others) within the Intensive Monitoring Plots

5.1.1. Evaluation for the main tree species

For the main tree species, five branches (as small as possible, but with all leaf age stages present) from each tree shall be pruned from the sun exposed portion of the upper third of the crown, simultaneously with the biannual foliar sampling for the chemical analysis of needles and leaves or according to the local symptoms phenology if possible. Once collected, a representative number of leaves per branch (i.e. approximately 30 leaves in the case of *Fagus sylvatica*) have to be examined under best light conditions and scored for occurrence of ozone-injury (yes/no).

According to the scoring system in Table 1., the percentage of symptomatic leaves per branch will be estimated and scored.

Table 1. Scoring and definition for the percentage of symptomatic leaves on a branch with approximately 30 leaves.

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

5.2 Conifer main tree species within the Intensive Monitoring Plots

Following the leaf-sampling procedure, from each tree several branches (5 branches as small as possible but having at least the first- and second- year needles) shall be pruned from the sun-exposed portion of the upper part of the crown. If this part of the tree is not accessible, use part of the branches collected for foliar analysis. Once collected and the different needle-age classes clearly identified, the needles from different age classes (at least first- and second-year needles, others are optional) have to be placed close to each other (making a “plane”, at least 30 needles if available) and examined in full sunlight. The chlorotic mottling will be scored for each needle age class (from current year (n) to 3-year old (n-2) needles) in percentage of total surface affected, and then the corresponding score (classes) for that percentage will be assigned, according to the following table. Special attention is recommended for the second-year needles (C+1).

Table 2. Scoring and scoring definition for visible ozone injury as it is expressed on the respective needle years for the collected branchlets of conifer species.

Score	Definition
0	No injury present.
1	1-5% of the surface is affected
2	6- 50 % of the surface is affected
3	51 – 100 % of the surface is affected.

A computer-generated simulation with ideal visible injury patterns and scores is available to assist with the symptom scoring in the field (Annex IV).

The final score for the harvested branches of an individual tree in the plot shall be the class corresponding to the average percentage of each year’s needle class (by averaging an assigned percentage as a mean of all needles in a whorl), while the final score for the plot shall be the class corresponding to the average percentages of all the sampled trees. The final score shall be produced per needle class; thus a species will have one score for the 1- year old needles, another for the 2- year old needles, etc.

5.2.1 Identification of visible ozone or visible ozone-like symptoms on (small) tree, shrub, and perennial species within the LESS and (optional) the ground vegetation of the intensive monitoring plot

For the symptom assessment of small tree, shrub, and herbs species within the LESS it is recommended to apply the procedure as described in Annex I. The following information is required for each of the randomly selected quadrate (sampling spatial unit).

- The scientific name and code of the present (small) tree, shrub, and herbs species with the indication whether they show symptoms or not.
- Trees and shrubs must be assessed singularly, vines and herbs as populations;
- Estimates are therefore resulting in terms of frequency, means and totals:

- frequency of quadrates including symptomatic plants (% of forest edge vegetation area affected),
- frequency of symptomatic species (% of symptomatic species over the total number of species of the forest edge),
- mean number of symptomatic species,
- total number of symptomatic species.
- estimates should be reported with confidence intervals at a 95% probability level.

To achieve a more complete list of symptomatic species around the passive ozone sampling device in addition to the survey within the LESS, the forest edges within a radius of 500 m of the passive sampler's location can be qualitatively assessed and symptomatic species recorded. Provide both, name and code of the respective species.

Record soil moisture conditions within the LESS and the optional subplots according to the Table 3. If conditions vary markedly across the site, make a note on the result sheets and mark it on the map. Samples and pictures of each injured species should be collected in accordance of section 3.4.

Table 3. Code and definition for the classification of the soil moisture conditions within the LESS and subplots.

Code	Definition
1	Wet or damp (riparian zones and wet or damp areas along a stream, meadow or bottom land)
2	Moderately dry (grassland or meadow, and North or East facing slopes)
3	Very dry (exposed rocky edges)

6. Quality assurance

A standard guide to visible ozone injury in conifer and broadleaf species is about to be established. It will include individual descriptive (diagnostic) sheets for various species with ozone-like symptoms, and confining symptoms, and phenologically related information. A web site from the Co-ordination Centre will be available with all the above mentioned information, and with the possibility of adding new data on symptom descriptions and verifications from the participating countries.

National Focal Centres will collect information and co-ordinate national efforts, including the documentation of injuries found on new species. Designated Regional Validation Centres will serve as locations for up-to-date information by means of web site, etc. and support efforts for quality control, provide assistance in clarification of dubious cases, and recommend further detailed investigations (e.g. microscopy), and provide seed bank for the storage of seeds collected from new plant species found during surveys for controlled validation experiments.

6.1. Training

The national field teams should be trained in visible symptom identification, quantification of foliar injury symptoms and sampling. In this sense, field teams will be trained within their respective countries by the persons who attended the intercalibration courses and who were tested in data collection procedures, and for their skills to recognise visible ozone injury and to discriminate against mimicking symptoms.

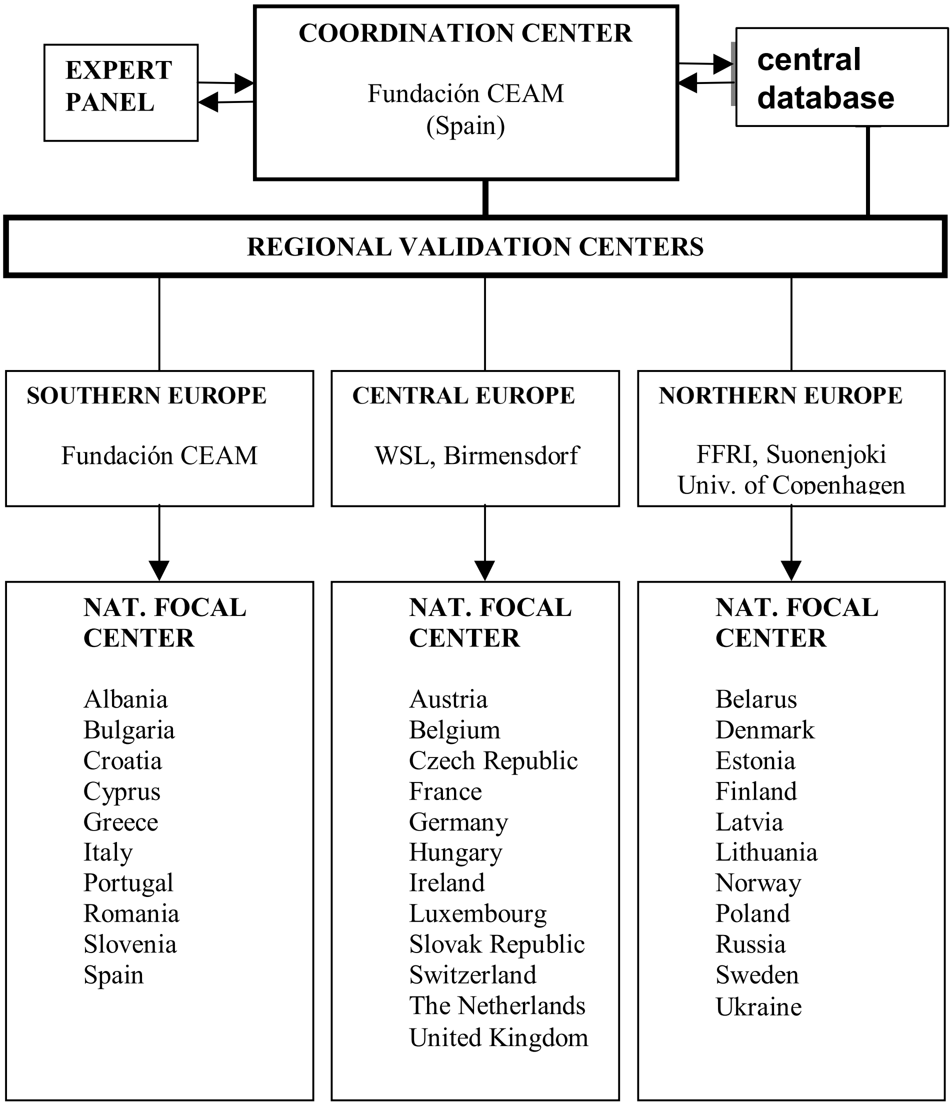


Figure 2. Organigram of the ICP-Forests Working Group on Air Quality conducting the Assessment of Visible Ozone Injury on European Forest Ecosystems.

7. Data handling and reports

Data must be submitted in electronic format, using the forms that will be provided by the Co-ordination Centre as quickly as possible but in the calendar year following the observations at the latest. The responsible of the database will inform the National Focal Centres about the different methods of electronic data submission.

8. Recommended references

The following publications (papers, books, reports, and WebPages) provide general information on bio-indication and symptom identification of air pollutant effects, in particular for ozone effects. They are recommended as a guide and further information.

Brace, S., Peterson, D.L., and Bowers, D. (1999). A guide to ozone injury in vascular plants of the Pacific Northwest. USDA Forest Service Pacific Northwest Research Station. General Technical Report PNW-GTR-446.

Campbell, S., Smith, G., Temple, P., Pronos, J., Rochefort, R. & Andersen, C. (2000). Monitoring for ozone injury in West Coast (Oregon, Washington, California) Forest in 1998. USDA. General Technical Report PNW-GTR-495.

Flagler, B. (1998). Recognition of air pollution injury to vegetation: A Pictorial Atlas. Second Edition. Air & Waste Management Association. Pittsburgh.

Hanisch, B. & Kilz, E. (1990). Monitoring of Forest Damage. Spruce and Pine. Verlag Eugen Ulmer Stuttgart / A + C Black, London / Arts Graphiques Européens, Le Plessis.

Hartmann, G., Nienhaus, F. und Butin, H. (1995): Farbatlas Waldschäden. Diagnose von Baumkrankheiten. Auflage 2. Ulmer, Stuttgart, 288 S.

Innes JL, Skelly JM, Schaub M (2001). Ozone and broadleaved species. A guide to the identification of ozone-induced foliar injury. Ozon, Laubholz- und Krautpflanzen. Ein Führer zum Bestimmen von Ozonsymptomen. Birmensdorf, Eidgenössische Forschungsanstalt WSL. Bern, Stuttgart, Wien; Haupt. 136. ISBN 3-258-06384-2.

Jacobson, J.S. & Hill, A.C. (1970). Recognition of air pollution injury to vegetation: A Pictorial atlas. Pittsburgh: Air Pollution Control Association.

Miller, P.R., K.W. Stolte and D. Duriscoe (eds.) (1996). Methods for monitoring ozone air pollution effects on western conifers. USDA General Technical Report PSW-GTR-155.

Sanders, G. & Benton, J. (1995). Ozone Pollution and Plant Responses in Europe. An illustrative guide. UN ECE Convention on Long-Range Transboundary Air Pollution. Nottingham. (ONLY CROPS).

Skelly, J., Davis, D., Merrill, W., Cameron, A., Brown, H.D., Drummond, D.B. & Dochinger, L.S. (1987). Diagnosing Injury to Eastern Forest Trees. A manual for identifying damage caused by air pollution, pathogens, insects and abiotic stresses. USDA-Forest Service, Forest Response Program. Agricultural Mailing Room , Pennsylvania State University, University Park, PA. USA 16802. 122 pp.

WebPages of interest

http://www.gva.es/ceam/ICP-forests/index.htm. Co-ordination Centre, Pictorial Atlas. Fundación CEAM, Valencia, Spain.

http://www.ozone.wsl.ch. Ozone injury database. Swiss Federal Research Institute WSL, Birmensdorf

Annexes

Annex I: Procedure for the establishment of a Level II LESS

The procedure is as follows:

1. Identify an area (A) (500 m radius) centred around the ozone measurement site (M) (Fig. 1a).
2. Identify all the light exposed forest edges within A (Fig. 3a).
3. From those, choose the forest edges closest to M (Fig. 3b).
4. Measure the length of the selected forest edges and virtually identify a 1 m width area along them. You now have an *x* m long and 1 m width transect. (Fig. 3b).
5. Consider how many possible 2 x 1 m not overlapping quadrates fit into the selected forest edge area. To do this, just divide it by 2. The rectangular shape (with the longer size along the forest edge) is more effective given the nature of the forest edge. The total number of non-overlapping quadrates is our target population.
6. Select your sampling quadrates, which will constitute the respective LESS:
    a. On a paper, number all the possible not overlapping quadrates. For practical reasons, start from the point closest to M and label each quadrate assigning a code 0, 2, 4, 6, 8, 10..., n which means the distance of the beginning of each quadrat from the beginning of the selected forest edge (very useful for planning the field work) (Fig. 3c).
    b. Extract randomly the *n* non overlapping quadrates (see Table 1. for sampling density) and compile a list. Replace any extraction, i.e. put again the extracted number in the "basket": if you extract again the same number, repeat this step until you "draw" a different number.
7. At the end you will obtain a list of *n* codes. Each code is a 2 x 1 m quadrate within the LESS; the codes will give you the distance of the beginning of each quadrate of the LESS from the beginning of the selected forest edge. Now you can go in the field and install your LESS (Fig. 3d).

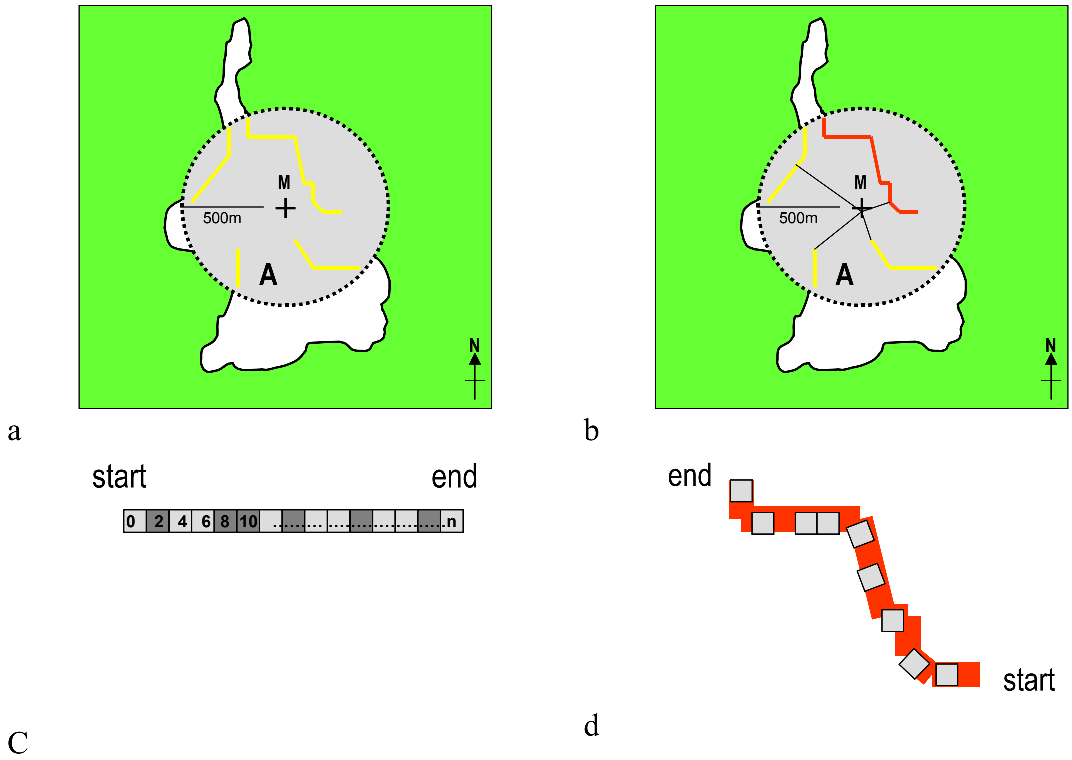


Figure 3. LESS establishment and the selection process of non overlapping quadrates within a light exposed forest edge.

Cases of special interest:

- If there is no forest edge at all, the assessment cannot be conducted. Site discarded. If there is a forest edge beyond the 500 m limit. Site discarded. See above.
- For special cases in which less than 100 m of light exposed forest edge are available for the survey within the 500 m radius, smaller sizes and correspondingly less non overlapping quadrates can be considered (according to Table 4.), and this particularity should be reported.

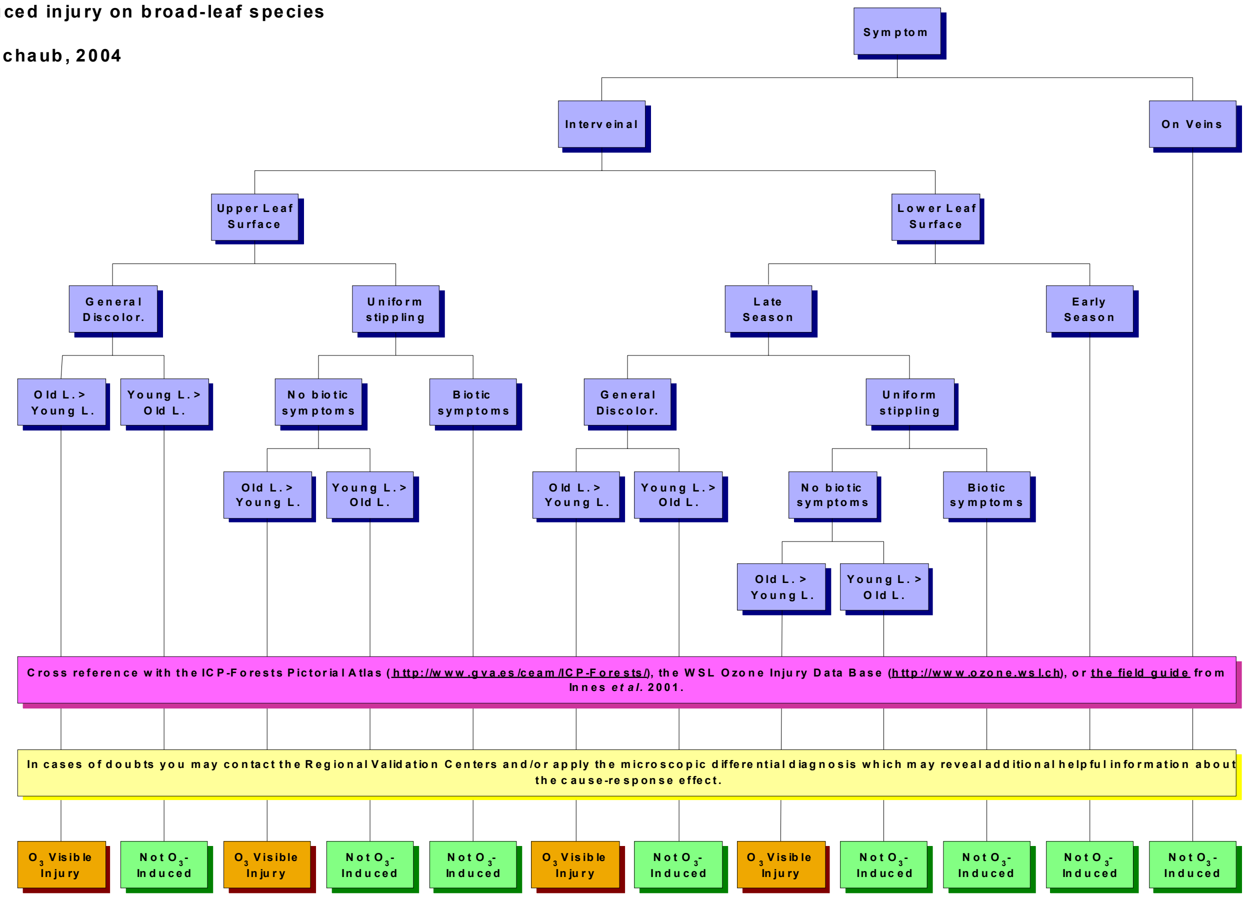
Table 4. Sample sizes at specified precision level, for different length of the selected forest edge.

Length of the light exposed forest edge.	Possible 2x1 m non overlapping quadrates	Adjusted sample size (FPC adjusted), 10% error	Adjusted sample size (FPC adjusted), 20% error
10	5	5	4
15	8	7	6
20	10	9	7
25	13	11	8
30	15	13	9
35	18	15	10
40	20	17	11
45	23	18	12
50	25	20	12
60	30	23	13
70	35	26	14
80	40	28	15
90	45	31	16
100	50	33	16
150	75	42	18
200	100	49	19
250	125	54	20
300	150	59	21
350	175	62	21
400	200	65	21
450	225	67	22
500	250	69	22
600	300	73	22
700	350	75	22
800	400	77	23
900	450	79	23
1000	500	81	23
2000	1000	88	23

Temporal fashion of LESS’s

There is no need to permanently mark the LESS if only one annual assessment is to be done. On the contrary, LESS can be permanent within a given year, e.g. between subsequent assessment over the same season. In this case, a permanent record of LESS location is useful.

Annex II: Flow Chart
(modified by Marcus Schaub, WSL)



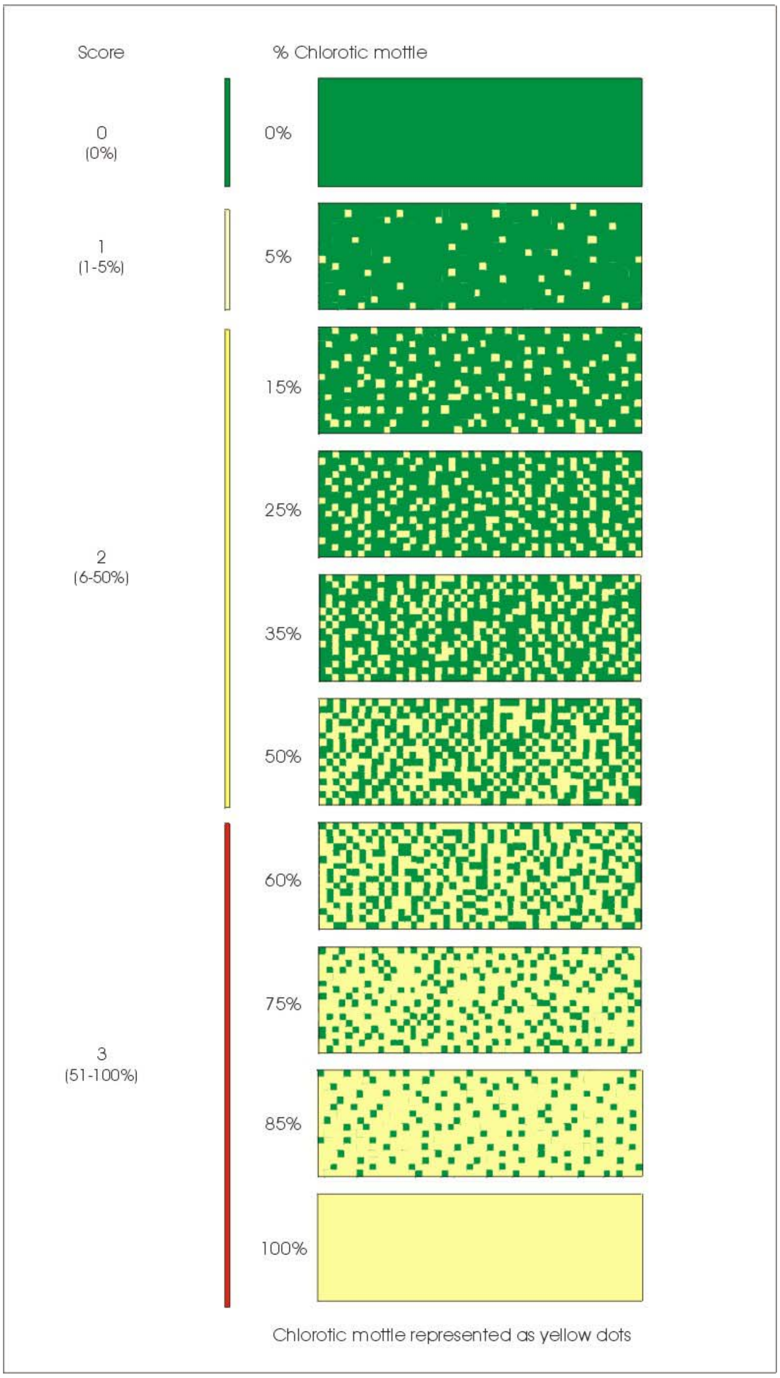
Flowchart for the diagnosis of ozone-induced injury on broad-leaf species

M. Schaub, 2004

Cross reference with the ICP-Forests Pictorial Atlas (http://www.gva.es/ceam/ICP-Forests/), the WSL Ozone Injury Data Base (http://www.ozone.wsl.ch), or the field guide from Innes *et al.* 2001.

In cases of doubts you may contact the Regional Validation Centers and/or apply the microscopic differential diagnosis which may reveal additional helpful information about the cause-response effect.

Annex III: Computer generated chart for the evaluation of chlorotic mottling
(V. Calatayud, 2000)



(V. Calatayud, 2000)