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LIFE CYCLE ASSESSMENT - OZONE INJURY IN FOREST ECOSYSTEMS

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ABSTRACT: Controlling visible ozone injury on conifer species were in locality Kopaonik – Serbia. The trials were set in accordance with methods PP 1/152 (2) (EPPO, 1997), the treatment plan was made according to a fully randomized block design. Phytotoxicity was estimated according to instructions of PP methods (1/135 (2). Intensity of injury was performed using standard statistical methods Towsend- Heuberger, the efficiency according to Abbott, analysis of variance to Duncan test and methods PP/181 (2). The differences of the disease intensity were evaluated by the analysis of variance and LSD-test. In locality Kopaonik ozone forecasts are made daily during the ozone forecast season.
KEYWORDS: LCA, forest, ozone, injury

INTRODUCTION

Vegetation is a major component of forest ecosystems. The composition, diversity, and structure of vegetation are important factors for assessing biological diversity of forest ecosystems. Vegetation is the source of primary production, plays a direct role in water and nutrient cycling, and interacts strongly with other biotic components (insects, game, etc.) being a determinant habitat for many species. Vegetation has also been identified as a specific target for the calculation of critical loads/levels.

The development of ozone-induced injury is inter- and intra-species specific, and depends apart from local ambient ozone concentrations on other environmental such as biotic and climatic factors. Due to the complex nature of the diagnosis and the given restrictions of the investment, results from the tree and vegetation assessment should be considered as semi quantitative. Ozone pollution leaves no elemental residue that can be detected by analytical techniques, as do fluorides [1] and sulfur dioxide [2]. Therefore, ozone-induced visible injury on needles and leaves is the only easily detectable evidence in the field as a result of oxidative stress, leading to a cascade of adverse physiological and morphological effects. These "ozone response variables," made at the needle, whorl, branch, tree, or stand level, and aggregated in analysis, can be an effective way to quantify ozone effects on pines [3]. Ozone response variables include microscopic and macroscopic injury to the foliage (primary effect), and injury to branches and roots (secondary effects) that can be quantified within defined levels of accuracy and precision. These "ozone response variables," made at the needle, whorl, branch, tree, or stand level, and aggregated in analysis, can be an effective way to quantify ozone effects on pines [3]. Ozone response variables include microscopic and macroscopic injury to the foliage (primary effect), and injury to branches and roots (secondary effects) that can be quantified within defined levels of accuracy and precision.

Until now, experiments have concentrated on explaining the mechanisms leading to injury observed in experimental studies, rather than to identify and characterize the symptoms observed in the field on a regional scale.

The evidence we have today strongly suggests that ozone occurs at concentrations - cumulative exposures greater than 60 ppb; [4] that cause visible foliar injury to a wide range of sensitive plants. Gaseous pollutants pass through the stomata of conifer foliage and cause direct damage to the photosynthetically active mesophyll cells, often producing a diagnostic visible injury pattern [5]. Next, degeneration of essential biological processes in the needles occur that may eventually lead to reduced crown vigor, increased susceptibility to other pathogens, and tree death [6].

Controlled exposures and field observations of ozone effects on western conifer species have confirmed that a distinct visible symptom known as chlorotic mottle typically occurs on needle surfaces [7,8]. Chlorotic mottle begins as the walls of mesophyll cells below the epidermis degrade, causing the loss of cellular contents and the subsequent degradation of chlorophyll within the cell [5,9,10]. Microscopically this condition appears as amorphous staining of cellular contents, plasmolysis of cell contents, and cell death. The degradation of chlorophyll beneath the epidermis appears on the needle surface as amorphous chlorotic blotches with diffuse borders that occur in irregular patterns, giving a yellow "mottled" appearance;

hence the terminology "chlorotic mottle". This foliar injury symptom is visibly distinct from foliar symptoms induced by other air pollutants.

Chlorotic mottle frequently appears in the one-third of the needle surface nearest the tip on 1-year-old or older needles, and progresses basipetally until the entire needle is affected [7,8].

This pattern is observed mainly in southern California. In the Sierra Nevada the mottle tends to occur randomly along the entire needle length [11,12]. The current–year's needles will show small amounts of chlorotic mottle only when summer ozone exposure levels are higher than usual and/or adequate soil moisture contributes to higher stomatal conductance and more ozone flux to needles, or both. This condition is usually the exception and not the rule for the response of current year needles to ozone exposures in the Sierra Nevada. Tip necrosis or necrotic bands can result from acute ozone exposures in fumigation experiments, but chronic field exposures typically induce only chlorotic mottling.

The assessment of ozone visible injury serves therefore as a means to estimate the potential risk for European ecosystems that are exposed to elevated ambient ozone concentrations and has to be considered in the context of ICP Forests aiming among others to document the presence of environmental drivers that may affect forest condition across Europe. Specific aims are set as follows: Quantification of ozone injury occurrence on a selected number of Level II plots in Serbia – Kopaonik. Conection between selected sample and real forest for future forest is 95% for 10 years.

Life cycle assessment (LCA) models the complex interaction between a product and the environment. The International Organisation for Standardisation (ISO), provides quidelines for conducting an LCA within the series ISO 14040 and 14044. Impact assessment the effects of the resource use and emissions generated are grouped and quantified into, a limited number of impact categories which may then be weighted for importance. Air pollution can have noteworthy cumulative impacts on forested ecosystems by affecting regeneration, productivity, and species composition. Ozone in the lower atmosphere is one of the pollutants of primary concern. Ozone injury to forest plants can be diagnosed by examination of plant leaves. Foliar injury is usually the first visible sign of injury to plants from ozone exposure and indicates impaired physiological processes in the leaves.

The paper presents a model Life Cycle Assessment in the complex interaction between ozone and the environment. The main objective of research of assessing ozone visible injury on a selected number of Level II plots is to assess the effect of tropospheric ozone at the sites where ozone monitoring is performed, and to contribute to an ozone risk assessment for European forest ecosystems.

MATERIALS AND METHODS

Symptoms of ozone injury was monitored on mature stands of red fir (Picetum excelsae oxalidetosum), with standard density (about 400 plants / m2). The site on which there is a measuring station for monitoring the health situation in the IPCC project is located at 74a department, G.J. „Samokovska reka" in national park „Kopaonik". The site is located directly below the road Kopaonik Brus, slightly above the Marino sources. Stands in which the research station is classified as irregular pure spruce stands. Circuit is thick (0.8 to 0.9). Spruce trees are right, little Mouse-tailed and developed crowns, which is logical given the height at which they are located. The average density is about 690 units / ha, the average volume of 460 m 3 / ha, the current increment is 8.30 m 3 / ha, mean stand diameter is 27 cm and mean stand height is 18.8 m.

- ***Materials:***
  - *a) Container: ice box cooler, boxes.*
  - *b) Magnifying lens.*
  - *c) Labels, pencil, scotch tape*

Tools: a sharpened punching tool (diameter 0.5-1cm), sharp scalpel, cardboard, tweezers, latex gloves, Kleenex.

Solutions: fixation medium (2.5% glutaraldehyde in Soerensen buffer at pH 7.0 distributed in 1.5 ml Eppendorf vials with screw caps).

Procedures: We were excised small samples (max. 0.5-1 cm in diameter for leaves, 3-4 mm for needles) from symptomatic as well as asymptomatic needles, in order to ease and thus speed up the penetration of fixative. We immediately had diped samples into Eppendorf vials with accurate labels (1 disc/needle segment per vial only) prior to storage in the cooler. Unless samples are collected to sort out stress symptoms by different stress factors, multisymptomatic samples should be avoided by close examination of needles prior to sampling. The hand lens should also be used to select asymptomatic samples from foliage close-by with similar light exposure. When we were backed to the lab, we renewed the fixative solution. We stored the samples at 4 °C until processing for microscopical analysis.

- ***Methods***

The trials were set in accordance with methods PP 1/152 (2) (EPPO, 1997) [13], and the treatment plan was made according to a fully randomized block design. The experiment was conducted in four repetitions on basic plots consisting of 8 trees (1 x 3 m apart), 25 m^2 in total.

The estimation of needles as follows: 3x5 well-developed shoots were selected from the outer zones of each tree. The scale of values which was used to record the results of each leaf is as follows: 0 = no injury, 1 = 1 – 5% of the needles per branch show ozone symptoms, 2 = 6 – 50% of the needles per branch show ozone symptoms, 3 = 51 – 100% of the needles per branch show ozone symptoms (Table 1).

The intensity of ozone injury assessed by the method of EPPO: and time of estimation was 11/07/2009. Phytotoxicity was estimated according to instructions of PP methods (1/135 (2) (OEPP, 1997) and time of estimation was 04/07/2010. [14].

Data processing was performed using standard statistical methods (intensity of injury according to Towsend- Heuberger [15], the efficiency according to Abbott [16], analysis of variance according to Duncan test [17] and methods PP/181 (2) (EPPO 1997) [18]. The differences of the disease intensity were evaluated by the analysis of variance and LSD-test.

Table 1 – Scoring and scoring definition for visible ozone injury as it is expressed on the respective needle years for the collected branches of conifer species

Score	Frequency class (%)	Definition
0	no injury	none of the needles are injured
1	1 – 5 %	1 – 5% of the needles per branch show ozone symptoms
2	6 – 50 %	6 – 50% of the needles per branch show ozone symptoms
3	51 – 100 %	51 – 100% of the needles per branch show ozone symptoms

RESULTS AND DISCUSSION

Estimates of ozone damage was done in the manner provided for the Manual on methods and criteria for harmonized sampling, assessment, monitoring and analysis of results of impact of air pollution on forests adopted by the International cooperative program for assessing and monitoring the impact of air pollution on forests [19].Visible damage to the assimilation organs of conifer expressed ozone in the upper parts of the crown, the top of the twigs and needles.

Minimum of 3 branches per tree and five trees per plot are assessed. The evaluation is very different for deciduous trees and coniferous trees and trees from which they reap the samples in Kopaonik are dominant spruce trees. The trees are on the ground marked the permanent markings on the bark, numbered 1-195, at 3 under plot. The numbers of trees to assess damage caused by ozone are 9, 20, 54, 76, 108.

Needles in the laboratory for evaluation prepared by placing the flasks with 2.5% solution of glutaraldehyde ON (SN2) 3SNO. Microscopic changes were found chlorotic-most common symptom induced by ozone damage.

The samples were assigned to these five trees with three branches - the three clusters (1-3, 3-5 ... 15) in the form LTF2004. The number of needles per sample is 30.

Other required parameters are listed in table-form LTF2004. (Type number, dates of sampling, analysis, method of detection and other observations). Needles are grouped by category (percentage share - a result with symptoms from each branch were taken to five trees on the experimental plot).

Table 2 – LTF2004- Determination of ozone injury on conifer

Point No	Tree No	Cod of species	Latin name	Material of claster
2	9	118	P.abies	1-3
2	20	118	P.abies	4-6
2	54	118	P.abies	7-9
2	76	118	P.abies	10-12
2	108	118P	P.abies	13-15
Point No	**Date of taking samples**	**Date of analyses**	**Injury of conifer**	**Status of examples**
2	131010	191110	0,0,1 a	NR
2	131010	191110	0,1,0 a	NR
2	131010	191110	1,0,0 a	NR
2	131010	191110	0,0,0 a	NR
2	131010	191110	1,0,0 a	NR

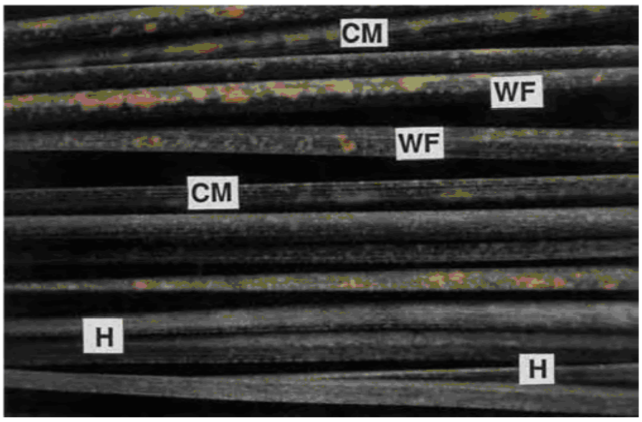

Figure 4. Three healthy (H) ponderosa pine needles (lower) are compared to those with a mixture of chlorotic mottle (CM) caused by ozone, best illustrated by the second needle from the top in which irregular chlorotic islands are visible. Weather fleck (WF) is displayed by the fourth and fifth needles from the top; note that weather fleck lesions are tan, not yellow, and borders are more irregular in shape. Weather fleck is found exclusively on the upper surfaces of needles (facing the sky). Chlorotic mottle can occur on all surfaces and is best evaluated on the lower surface where fleck is not present [22]. (Type of analyses on this figure are visible method).

Foliar injuries resulting from biotic agents may appear on needle surfaces, confounding diagnosis of ozone injury. Chlorotic and necrotic spots or blotches caused by sucking insects, such as aphids and pine needle scale, may sometimes closely resemble ozone chlorotic mottle. The most common confounding pests are fungi [20], and chewing (needle weevil) or sucking (scale or aphids) insects [6,11]. Chlorotic mottle can be differentiated from injuries brought about by these diseases and insects by in-hand observation of the color and pattern of the symptoms on the needles. Careful observation may reveal the presence of fungus fruiting bodies. Close inspection of chlorotic islands of tissue (aided by a hand lens) often reveals a distinct necrotic point at the center of the discolored area, where the insect penetrated the epidermis with its piercing mouth parts.

In many conifer species, foliage longevity can be measured by counting nodes on branches back from the branch tip to the oldest whorl, with each node separating a annual whorl of needles or needle fascicles corresponding to one year of growth. Reduction in needle longevity is recognized as an indicator of air pollution stress for these species when other factors leading to accelerated abscission of needles are taken into account. Foliage longevity is related to other factors, most particularly the elevation at which a tree grows [22], which is an indication of the length of the growing season at a particular site. Interpretation of data on foliage longevity must consider other confounding factors, for example, persistent infections of needle cast fungi can lead to tree crowns that are extensively defoliated. The bole and other crown variables that are associated with growth and overall tree vigor can respond to elevated ozone exposures. Branch mortality in the lowest portion of the crown has been observed in southern California [23], leading to a decrease in vertical crown length, as measured by percent live crown [24]. Before lower branch mortality occurs, a decline in vigor in the lower crown may be observed as a reduction in needle length [23], and the production of fewer numbers of needle fascicles [26,27]. A reduction in the vertical and radial growth of stems has been documented for ozone-stressed trees in southern California and southern Sierras [28,29,30], Cone and seed production can also be reduced by ozone stress in ponderosa pines [6]. Oleoresin exudation pressure, yield, and rate of flow were all substantially reduced in oxidant-injured ponderosa pines in southern California, while the crystallization rate was observed to increase [31]. The moisture content of phloem and sapwood were found to be reduced, as well as a reduction in phloem thickness. These phenomena have been associated with susceptibility to cambium damage from the heat of fire and successful attack by bark beetles [31, 32].

Monitoring of ozone injury to plants by our Institute has expanded over the last 1 year. Ozone damage to forest plants is classified using a subjective three-category biosite index based on expert opinion, but designed to be equivalent from site to site. Ranges of biosite values translate to no injury, low or moderate foliar injury (visible foliar injury to highly sensitive or moderately sensitive plants, respectively), and high or severe foliar injury, which would be expected to result in tree-level or ecosystem-level responses, respectively. Based on the tabular results of the Level II - Kopaonik can be monitored data of the ozone damage to forest in the next few years. Because that our research is only one year, monitoring of meteorological data will be an accurate forecasting model of possible ozone depletion over the next 10 years.

What are the sources of crop-damaging concentration of ozone? The first and the most important sources result from the activities of man. Other possible sources are the ozone-rich layers of the stratosphere, and thunderstorms.

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 (ICP Forests) prepared Manuel on methods and criteria for harmonized sampling, assessment, monitoring and analysis of the effects of air pollution on forests and in Part VII are Assessment of Ozone Injury [19], and their opinion is that based on methods we were used significant changes within 10 years are a 95% significance level for individual plots.

In NIVO II locality Kopaonik ozone forecasts are made daily during the ozone forecast season for each of several areas. Each forecast is a simple yes or no prediction for the question: Will ozone levels reach or exceed a target level for a particular area? Our meteorologists use a set of criteria from historic meteorological data, ozone measurements, and ozone prediction models to make these predictions.

CONCLUSIONS

- In Europe, ambient ozone levels are high enough to cause visible injury in native species. Assessment of visible injury is a feasible way to detect the impacts of this pollutant in forest plants and to identify potential risk areas. Ozone-induced visible injury has been incorporated in monitoring programmes, and it is surveyed at a pan-European scale under the protocols of ICP-Forests and FutMon (Life+) project.
- Conifers are presented on categories in percentage. Results with symptoms are shown from each branches from 5 trees from experimental plate. The peaces of conifers were estimated for 5 trees from 3 clasters (1-3, 3-5...15).
- Chlorotic mottle caused by ozone injury was on the toop of the tree, in first part of the conifer.
- Minimum 3 brances by tree and 5 trees by parceles are controled. Evaluation is different to leaves and conifers, and the experiment was made on red fir. In locality Kopaonik- Rtanj.

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