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**Forest Condition in Europe  
2016 Technical Report of ICP Forests**  
**Report under the UNECE Convention  
on Long-Range Transboundary Air Pollution  
(CLRTAP)**

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# Forest Condition in Europe

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Alexa Michel and Walter Seidling (editors)

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## 5 SPATIAL AND TEMPORAL DISTRIBUTION OF OZONE SYMPTOMS ACROSS EUROPE FROM 2002 TO 2014

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### Abstract

Ozone-induced visible foliar injury has been assessed during 2002-2014 according to ICP Forests standardized methods. This activity provided 29,809 records from 285 woody plant species, 169 plots and 19 countries. Data were evaluated for the entire period 2002-2014 as well as for 2009 only, when spatial coverage was the greatest. First results reveal that 55.0% of the assessed plots were symptomatic, and 26.0% of species developed ozone visible injury. Beech (*Fagus sylvatica*) was the species with the highest frequency of symptomatic observations (plot and years) in both 2002-2014 (40.1%) and 2009 (42.9%). The frequency of symptom reports occurred without a clear spatial pattern. In case, higher frequency of symptom occurrence seemed more common from northern Italy to North-West Germany, and towards East Europe. At country level, temporal trend analysis indicates a downward trend of mean frequency of symptomatic species for five out of six countries. Overall (all plots together), there is a slightly decreasing trend, which is consistent with the decreasing trend observed for ambient ozone concentrations. These first results demonstrate the potential of the survey on visible foliar injury to detect the potential impact of ozone on European vegetation. Further, enhanced quality control procedures are underway to aggregate the datasets and promote a more in-depth exploitation of cause-effect relationships, considering ozone symptoms, ozone concentration and measurements on forest health, growth, nutrition, biodiversity and climate undertaken at the ICP Forests plots.

**Keywords:** ICP Forests; ozone symptoms; woody species; forest edge; Light Exposed Sampling Site (LESS)

### 5.1 Introduction

Tropospheric ozone (O<sub>3</sub>) is well known to be an air pollutant causing injury to plants (Innes et al. 2001; Karlsson et al. 2007; Matyssek et al. 2007). Ozone pollution leaves no elemental residue in plant tissues that can be detected by analytical techniques; therefore, visible injury on leaves and needles is the only easily detectable indication in the field. Although visible symptoms do not include all the possible forms of injury to vegetation (i.e. physiological changes, reduction in growth, etc.), observation of typical symptoms on foliage has turned out to be a valuable tool for the assessment of the impact of ambient ozone concentrations on sensitive plant species (Skelly et al. 1987; Schenone 1993; Lorenzini et al. 1995; Bussotti and Ferretti 1998; Inclán et al. 1999; Innes et al. 2001; VanderHeyden et al. 2001; Novak et al. 2003; Benham et al. 2010). The assessment of ozone visible injury serves therefore as a means to estimate the ozone potential risk for European ecosystems, and is very relevant in the context of ICP Forests (Schaub et al. 2010b).

Starting in the year 2000, a specific pan-European programme for the assessment, validation, and mapping of ozone visible injury on the vegetation has been launched, based on the ICP Forests intensive monitoring network (Level II plots, see <http://icp-forests.net>) where also ozone concentration is

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measured (Schaub et al. 2010a; 2015). The programme considers both the main tree species (MTS) of each plot and the vegetation in Light Exposed Sampling Sites (LESS) at the forest edge. A specific manual has been developed for this purpose (Schaub et al. 2010b). Alongside, Intercalibration Courses on the Assessment of Ozone Injury on European Species among experts from the participating European countries have been implemented to promote quality assurance (QA) and quality control (QC) (Bussotti et al. 2003). QA/QC procedures are essential to ensure spatial and temporal data comparability. Participants in the UN/ECE ICP Forests programme must therefore follow the methods and QA/QC procedures described in the Manual.

The main objective of assessing ozone visible injury is to contribute to an ozone risk assessment for European forest ecosystems. In this paper, we aim at providing first, comprehensive results on occurrence of visible foliar injury over space and time in Europe. We will present findings from data collected on woody plant species at the LESS over the period of 2002-2014 across Europe and stored in the central ICP Forests database. Although data have been subjected to routine QA/QC procedures, enhanced QA/QC is yet to be implemented. Therefore, results presented here should be considered as first outcome of the evaluation procedure, and interpreted with care.

## 5.2 Materials and methods

### Sampling design and data collection

In order to assess ozone visible injury at the very site, a Light Exposed Sampling Site (LESS) has been established close to the off-plot sites of the ICP Forests Level II plots, where meteorological variables, deposition chemistry and ozone concentrations are also recorded. A LESS consists of a number of 2 x 1 m quadrates randomly selected along the forest edge. The number of randomly selected quadrates depends on the size (length) of the forest edge. Identification of ozone visible injury on woody species within the LESS quadrates has been carried out at least once during late summer and before natural leaf discoloration. Details on how to calculate the number of sampling units and conduct the assessment are outlined in Schaub et al. (2010b).

### QA and QC

Field assessments of symptoms were carried out according to the standard QA/QC procedures of the ICP Forests described by Schaub et al. (2010b). Training and intercalibration courses were organised on an annual basis across Europe between 2002 and 2010, with most of the participating countries attending. Although uncertainty and subjectivity are impossible to be eliminated, they can be controlled: early results from 11 European field crews suggest that Data Quality Limits set by Schaub et al. (2010b) were achieved in most cases (Ferretti et al. 2013). Additional symptoms validation procedures, such as microscopical analysis, were implemented on a limited number of cases (872 out of 42,329 records).

Data used in this report were extracted from the ICP Forests database on 28 October 2015 for validation purposes within the activity of the Expert Panel on Ambient Air Quality. Data completeness (number of quadrates reported vs. expected) of at least 80% was mandatory for the field survey. Thus, we assume data were complete, and we just considered all the available data. On such a dataset, however, enhanced QA/QC has been (and is still being) implemented. Plot codes, species names and codes, distinction between perennial and annual species, woody and non-woody species have been controlled. These new datasets are now being verified by National Focal Centers of the participating countries. For France, additional data will be submitted and considered for further analysis.



## Data description and analyses

Overall, the database on ozone foliar injuries (OZ\_LSS) taken into account for this work consists of 42,329 records of data, collected between 2002 and 2014. For the present evaluation, only woody species have been considered (29,809 records).

Results for both, the entire period (2002-2014) and for 2009 only, i.e. the year with the highest number of countries participating in the programme, are reported. Specific analyses focusing on the most frequently recorded species were also carried out.

For the spatial pattern representation, the number of assessed years (three classes: 1; 2-5; >5 years) and the frequency of symptomatic years (three classes: 0%; >0-50%; >50%) at plot level was calculated, both considering all woody species and only *Fagus sylvatica*. A plot was classified as symptomatic if at least one species was found symptomatic in one year.

For the ranking of symptomatic species, only species observed on at least 30 plots (2002-2014) or 10 plots (2009) were considered.

For the detection of temporal trends, only countries and plots with at least seven years of data were considered. Mean values of symptomatic species percentage at country level were used for the statistical analysis. The MAKESENS application (Version 1.0 Freeware, Copyright Finnish Meteorological Institute 2002, <http://en.ilmatieteenlaitos.fi/makesens>) was used to perform the non-parametric Mann-Kendall (Hollander and Wolfe 1999) and the Sen's (Sen 1968) tests in order to verify the null hypothesis ( $H_0$ ) of no temporal trend in the frequency of symptomatic species.

## 5.3 Results

### Overview: occurrence of ozone foliar symptoms on woody species in Europe

For woody species grown at the Light Exposed Sampling Sites (LESS), we analyzed data from 285 species on 169 plots in 19 countries (Table 5-1; see Annex III for the full account). Nineteen countries have at least one plot observed for at least one year. Longest time series were provided by Spain and Switzerland (9 years), Hungary and Lithuania (8 years), Italy and the Slovak Republic (7 years) respectively. A number of plots was observed for less than 7 years in Belgium, the Czech Republic, Germany, Greece and Serbia (5 years), Romania (3 years), Austria, Cyprus and France (2 years) and Croatia, Latvia, Slovenia and UK (1 year). The largest spatial coverage was found in 2009-2011, which was likely due to the financial contribution by the LIFE project FutMon.

Over the entire period of 2002-2014, the majority of countries reported ozone visible injury at least on one single species in one single year and on one single plot. Four out of 19 countries, i.e. Cyprus, Romania, Serbia and the United Kingdom did not observe any ozone-induced symptoms. Over all, from 169 assessed plots, 55.0% plots were found symptomatic and 26.0% of the 285 assessed species developed ozone visible injury (Table 5-2). In 2009 however, the frequency of records was lower when 15 countries assessed a total of 194 species from 109 plots, of which 12.4% species (33.0% plots) were symptomatic.

Table 5-3 provides a list based on the 10 most symptomatic species in 2002-2014 (left) and 2009 (right), and their frequency of symptom records (observations at different years and plots). For the 2002-2014 period, only species recorded at least on 30 plots and for 2009, only species recorded on at least 10 plots were considered. Among the 10 most symptomatic species in 2002-2014 and the 10 most symptomatic species in 2009, seven were in common. The ranking of frequency of symptom records shifted between 2002-2014, with the exception of beech (*Fagus sylvatica*), which was found to be symptomatic with the highest frequency during both periods.

Table 5-1. Number of assessed plots per country and year.

Country code	Country	Survey year												
		2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014
1	France				2								14	
2	Belgium					1	1	1	4	4				
4	Germany				3				18	25	11	11	11	
5	Italy			4	8	4	4	2	22	22	4	4		
6	United Kingdom			7										
9	Greece								2	3	3	3	3	
11	Spain	11	10	3		13	13	13	13	13	12			
14	Austria								6	6				
50	Switzerland			13	8	7	7	7	8		8	7	9	
51	Hungary		9	9	9	9			5	5	5	1		2
52	Romania								4	4	3			
54	Slovak Republic						1	3	8	8	8	3	3	
56	Lithuania			9			9	9	9	9	9	9	9	
57	Croatia										1			
58	Czech Republic						6	3	4	6	7			
60	Slovenia								4					
64	Latvia			1										
66	Cyprus								2	2				
67	Serbia								1	1	1	2	2	

Table 5-2. Number of countries, plots and species assessed during the entire period (2002-2014) and in 2009 only.

Informative stratum	2002 - 2014		2009	
	Tot (n)	Symptomatic (%)	Tot (n)	Symptomatic (%)
Country	19	68.4	15	60.0
Plot	169	55.0	109	33.0
Species	285	26.0	194	12.4

Table 5-3. Total number of observations and frequency of the ten most symptomatic species assessed at different years and on different plots in 2002-2014 and 2009 only. For the 2002-2014 period, only species recorded at least on 30 plots were considered; for 2009, only species recorded on at least 10 plots were considered.

Most symptomatic species	2002 - 2014		2009	
	Total number of observations (plots and years) (n)	Frequency of symptom records (%)	Total number of observations (plots and years) (n)	Frequency of symptom records (%)
<i>Fagus sylvatica</i>	237	40.1	42	42.9
<i>Rubus idaeus</i>	191	33.0	39	17.9
<i>Carpinus betulus</i>	113	24.8	20	10.0
<i>Corylus avellana</i>	160	22.5	21	23.8
<i>Cornus sanguinea</i>	58	22.4	11	27.3
<i>Sambucus racemosa</i>	30	20.0	-	-
<i>Salix caprea</i>	146	17.8	20	15.0
<i>Viburnum lantana</i>	33	18.2	-	-
<i>Rubus fruticosus group</i>	70	12.9	-	-
<i>Fraxinus excelsior</i>	118	16.9	15	13.3
<i>Acer campestre</i>	0	-	15	13.3
<i>Acer pseudoplatanus</i>	0	-	21	19.0
<i>Frangula alnus</i>	0	-	12	8.3

### Spatial pattern

The spatial pattern of symptoms (all species) over the period 2002-2014 across Europe against the estimated seasonal mean ozone concentrations (see Schaub et al. 2015 for details) is shown in Figure 5-1. The map distinguishes the plots with respect of the number of available survey years (size of dots), and the frequency of survey years when the plot was found symptomatic (color of dots). A higher number of survey years (>5 years) was available for plots in Spain, Switzerland, Northern Italy, Hungary, the Slovak Republic and Lithuania. The frequency of symptom reports occurred without a clear spatial pattern. Higher frequency of symptom occurrence (red dots; >50%) seemed to be more common from northern Italy to North-West Germany, and towards East Europe. Interestingly, plots in regions with high ozone levels (e.g. central and southern Italy) showed no symptoms; and plots in regions with low ozone concentrations (e.g. South-West Germany, Lithuania and Latvia) showed frequent symptom occurrence. However, the majority of the plots seems to show a frequency of >0-50% symptom occurrence, with seasonal background ozone concentrations around 20-60 ppb. Figure 5-2 shows the same data for beech only. Also in this case, no clear pattern is obvious.

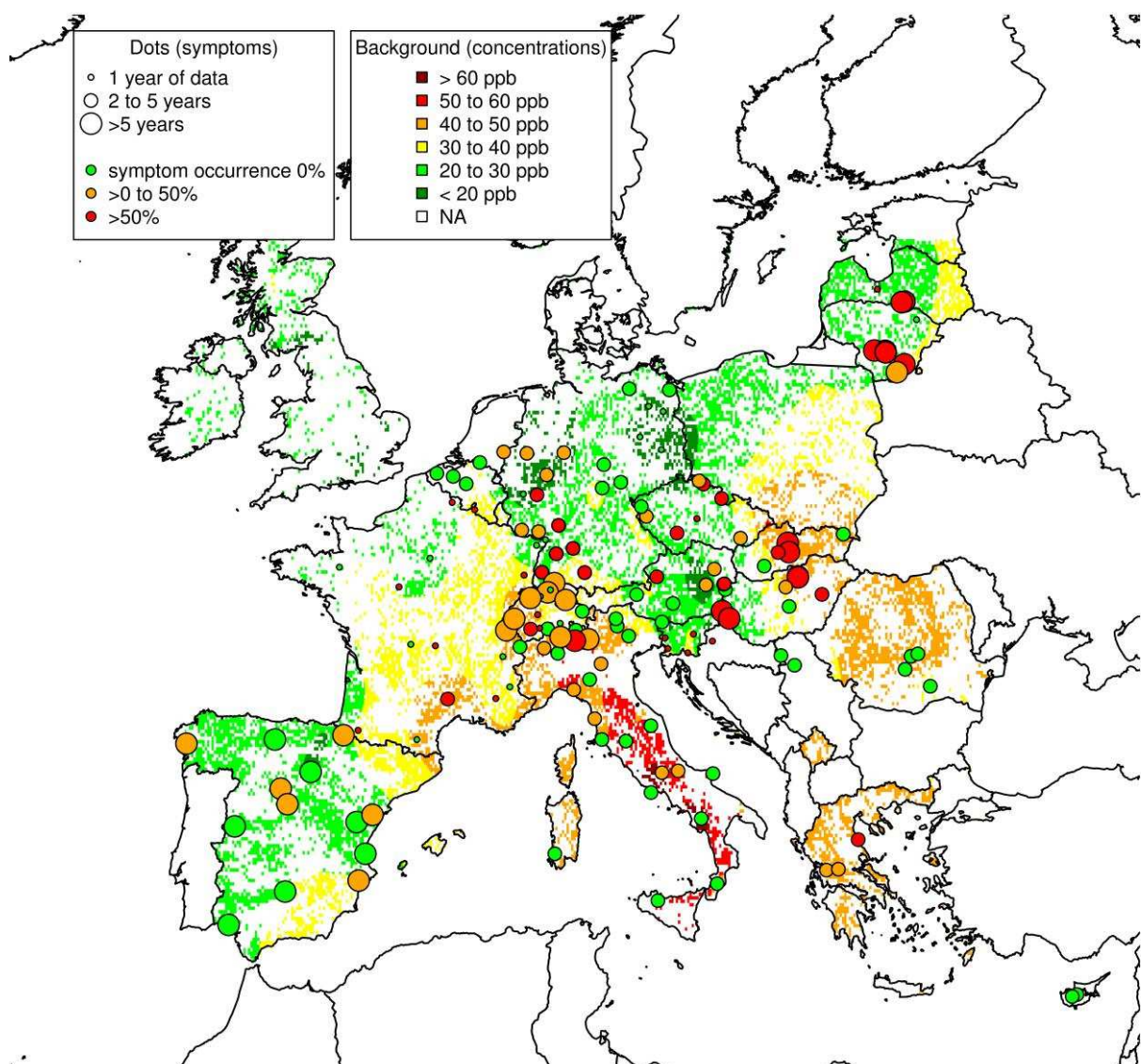


Figure 5-1. Spatial distribution of April – September mean ozone concentrations (ppb) from passive samplers on 203 plots and 20 countries during 2000-2013 and ozone symptom occurrence on 169 plots and 19 countries during 2002-2014. For ozone symptoms, dot size represents temporal data coverage (small = only 1 year; medium = 2-5 years; large > 5 years) and color represents frequency of symptom occurrence (green = 0%; orange = 0.1-50%; red = >50% of years measured were symptomatic).



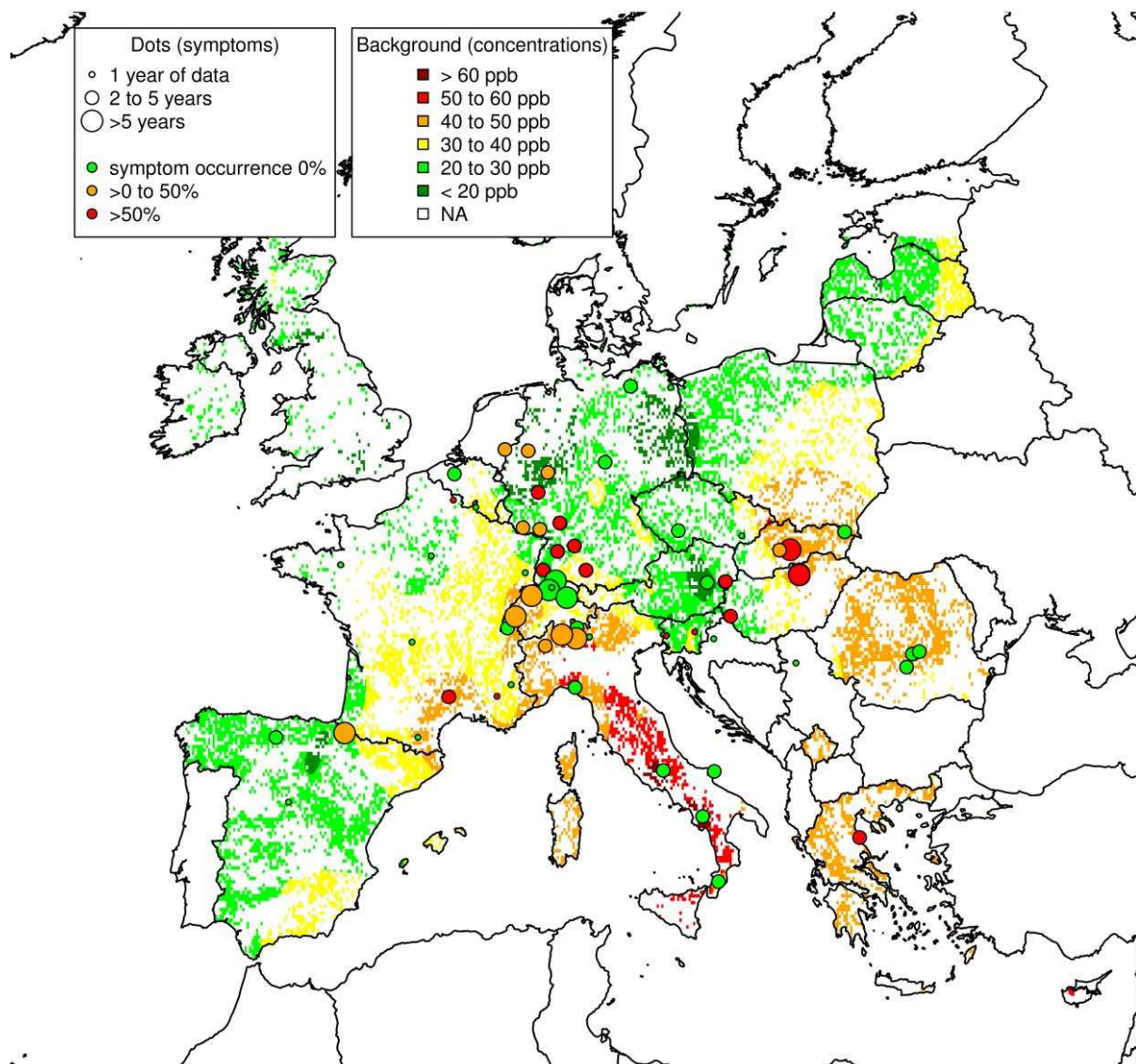


Figure 5-2. Spatial distribution of April – September mean ozone concentrations (ppb) from passive samplers on 203 plots and 20 countries during 2000-2013 and ozone symptoms occurrence for *Fagus sylvatica* on 74 plots and 15 countries during 2002-2014. For ozone symptoms, dot size represents temporal data coverage (small = only 1 year; medium = 2-5 years; large > 5 years) and color represents frequency of symptom occurrence (green = 0%; orange = 0.1-50%; red = >50% of years measured were symptomatic).

### Temporal pattern

Mean frequency of symptomatic species per country and year is reported in Figure 5-3. The Mann-Kendall test (Table 5-4) indicates a downward trend of mean frequency of symptomatic species for 5 out of 6 countries, which is significant only for Hungary. When data are processed on the basis of individual plots (Figure 5-4), there is a slightly decreasing trend that is consistent with the decreasing trend of ambient ozone concentrations reported by Schaub et al. (2015).

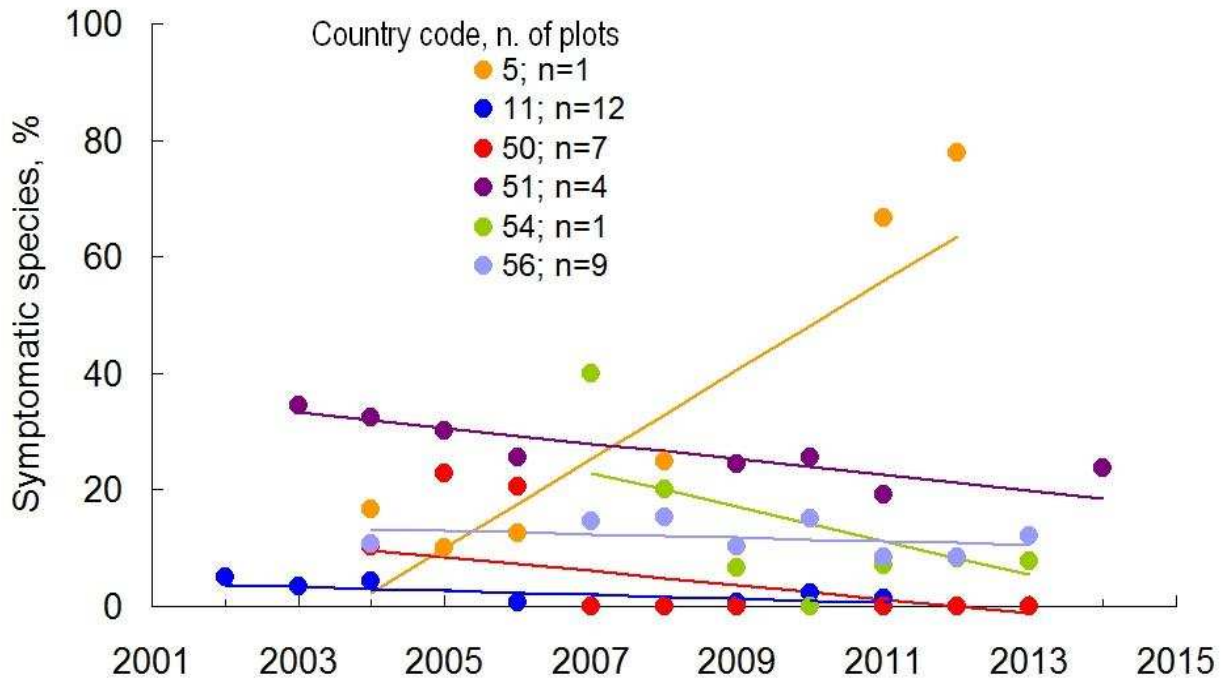


Figure 5-3. Temporal trend of the mean frequency of symptomatic species per country. For each country, only the plots with at least seven years of data have been considered. The legend reports the country codes and the correspondent number of plots.

Table 5-4. Makesens statistics (S Mann-Kendall test) to detect and estimate trend in time series 2002-2014 of frequency of symptomatic species in six European countries with at least seven years of data. Plots may vary from year to year. Ns, not significant; \* P<0.05; \*\* P<0.01.

Country code	Country	No of years	No of plots	Mann-Kendall trend		Sen's slope estimate	
				S	Significance	Q	B
5	Italy	7	1	11	ns	7.639	-12.92
11	Spain	9	12	-11	ns	-0.335	3.67
50	Switzerland	9	7	-17	ns	-1.213	12.13
51	Hungary	8	4	-24	**	-1.352	34.71
54	Slovak Republic	7	1	-5	ns	-2.917	37.50
56	Lithuania	8	9	-8	ns	-0.302	13.94

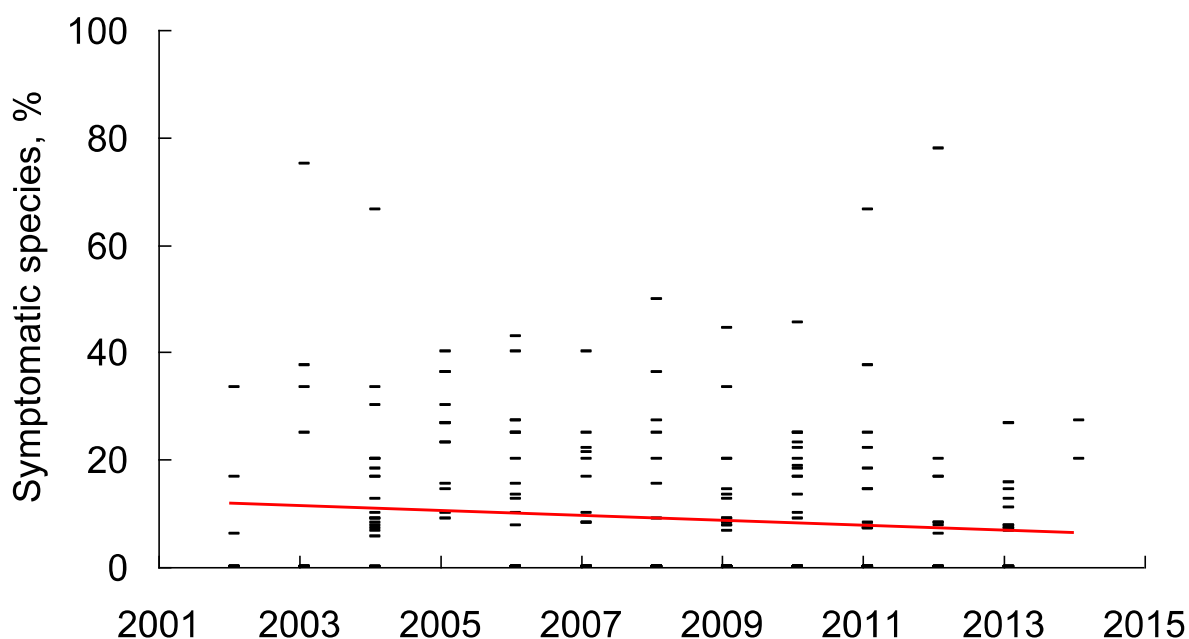


Figure 5-4. Overall temporal trend of the mean frequency of symptomatic species. Only the plots with at least seven years of data have been considered.

## 5.4 Discussion and conclusions

Ozone is the only air pollutant occurring in remote areas at concentrations that may cause visible injury on plants. Over the period 2002-2014, the assessment of ozone-induced symptoms on woody plant species at Light-Exposed Sampling Sites (LESS) nearby selected ICP Forests Level II plots reveals that visible injury attributed to ozone occurs every season on numerous plots and plant species across Europe. In fact, the 29,809 records from 169 plots in 19 countries, with 285 assessed species provide evidence that ozone still occurs at levels which are harmful to forest vegetation. Moreover, preliminary results demonstrate the complexity of the interactions between ozone exposures and forest ecosystems across Europe. As a matter of fact, symptoms were frequent even on plots with seasonal ozone background concentrations of 20-30 ppb. On the opposite, no or infrequent symptoms were found on plots with seasonal ozone background concentrations exceeding 50 ppb. A range of intermediate situations also occurred.

Among the symptomatic species, *Fagus sylvatica* turned out to be the species with the highest frequency of symptom occurrence, during both periods, 2002-2014 and 2009 only. VanderHeyden et al. (2001) compared 16 common woody species with each other and developed a sensitivity ranking, based on ozone visible injury development under ambient ozone concentrations. They found that among the symptomatic species, *Viburnum lantana* was the most sensitive one, followed by *Fraxinus excelsior*, *Frangula alnus*, and *Fagus sylvatica*. Novak et al. (2003) also found *Viburnum lantana* to be the most sensitive species, followed by *Fraxinus excelsior* and others, which are not included in Table 3. It must be noted, however, that for some species (e.g., *Fagus sylvatica*, *Rubus* sp.), identification of ozone symptoms in the field can be confounded by various factors (see Bussoti et al. 2003; 2006). Therefore, further QA/QC checks and training are necessary to gain a better insight.

The discrepancy between spatial distribution of seasonal ozone concentrations, frequency of symptom occurrence, and species specific sensitivity may be explained by the influence of internal and external

factors affecting the sensitivity of an individual plant to ozone. External phenomena affecting ozone sensitivity include both a range of factors that influence gaseous uptake rates in the leaf and the characteristics of the ozone regime. Nutrition, water availability, temperature, atmospheric and soil humidity, wind speed, and incident light levels are all known to affect ozone uptake (Sandermann et al. 1997). These factors interact in a complex fashion to determine whether or not the leaf will develop symptoms of injury, making the experimental simulation of ozone exposures extremely difficult.

Leuzinger et al. (2011) postulated that the larger the spatial perspective of estimating water use under elevated CO<sub>2</sub>, the smaller the response compared to the control scenario – often being conducted under experimental conditions. Here, we may face a similar phenomenon. Although much is known about the mechanistic understanding of plant-ozone interactions under experimental conditions, the actual effects on forest ecosystems in the real world is less certain (e.g. Bussotti and Ferretti 2009).

In combination with the measurement of ozone concentrations at the very forest sites, the assessment of ozone visible injury across Europe and in different forest ecosystems can be valuable to evaluate the risk for vegetation and to document spatial patterns, temporal variability, and trends of ozone effects. The results presented here, originated from the first comprehensive, European-wide evaluation of the data collected by the participants to the ICP Forests, and must be considered with caution due to some pending issues in terms of QA/QC checks. With this limitation in mind, however, results demonstrate that the survey on ozone visible injury can provide important indication for ozone risk assessment. Given the extended spatial and long-term coverage as well as the concurrent measurements of ozone concentrations and several other variables on forest health, growth, nutrition, biodiversity and climate, the potential of the ICP Forests ozone symptom dataset is unique. Enhanced QA/QC are being performed, new perspectives (e.g., survey restricted only to sensitive species or bio-indicators) and follow-up studies are being designed to study spatial and temporal trends and the relationship between ozone, ozone-induced symptoms, tree health and growth.

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