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Assessment of ozone visible symptoms in the field: perspectives of quality control

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"Capsule": *Observers need to be trained in how to assess ozone symptoms before field surveys are conducted.*

Abstract

The second UN/ECE ICP-Forests Intercalibration Course on the Assessment of Ozone Injury on European Tree Species was carried out in August 2001 at Lattecaldo (Canton Ticino, CH) and Moggio (Lombardy, I). Forty-eight experts from several European countries participated in the exercises and assessed visible symptoms of ozone injury both in open-top chambers (OTC) (Lattecaldo) and under open field (Moggio) conditions. Evaluation of the results indicated a large variability among the teams and call for adequate training of the observers prior to symptom assessment for quality assurance purposes. Highest variability was found for the species developing unclear symptoms which could be confused with senescence processes; such species should not be used in the field. The authors provide suggestions to improve the reliability of the ozone injury assessment on forest plant species.

Keywords: Ozone injury; Injury assessment; Field teams; UN/ECE ICP-Forests Intercalibration Course; Quality assurance; Level II

1. Introduction

Current levels of tropospheric ozone have been demonstrated to cause damage to forest trees, agricultural crops and semi-natural vegetation (Kärenlampi and Skärby, 1996). Ozone pollution leaves no elemental residue that can be detected by analytical techniques; therefore, visible injury on leaves and needles is the only easily detectable indication in the field. The evidence at present strongly suggests that ozone occurs at concentrations which cause visible foliar injury to sensitive plants (Innes et al., 2001). Although visible symptom expression does not include all the possible forms of injury to trees and natural vegetation (i.e. initial physiological changes, reduction in growth, etc.), observation of typical symptoms on above-ground plant parts in the field has turned out to be a valuable tool for the assessment of the impact of ambient ozone on sensitive plant species (Skelly et al., 1987; Flagler, 1998; Innes et al., 2001). During the 1990s, the potential impact of ground-level ozone on plants and human health has come into focus within the European cooperation on reductions of air pollution emissions within the United Nations Economic Commission for Europe (UN/ECE) and the European Union (EU). These findings have led to a request from policy makers to the scientific community for quantitative information concerning ozone effects. To meet this need, a specific pan-European program for the assessment, validation, and mapping of visible ozone injury on the vegetation was launched in 2000, based on the *ICP-Forests* intensive monitoring network (Level II plots, see http://www.icp-forests.org), taking into consideration both the main tree species (MTS) of each plot and the natural vegetation in light exposed sampling sites (LESS) at the forest edge. A specific manual (UN/ECE ICP-Forests Sub-Manual of Ambient Air Quality, Part II on The Assessment of Ozone Injury on European Forest Ecosystems, http://www.gva.es/ceam/ICP-forests) has been developed.

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Starting in 2000, annual UN/ECE ICP-Forests Intercalibration Courses on the Assessment of Ozone Injury on European Tree Species are held among experts from all the participating European countries. Initially, the main objectives were: (1) to reach an agreement throughout European countries in the recognition of the symptoms to the most relevant plant species; (2) to verify the quantitative evaluation of symptoms; (3) to verify comparability of results among observers within different European countries and (4) to identify the problems to be addressed that would favor the success of the program. Here, we present the results of the field exercises performed during the second UN/ECE ICP-Forests Intercalibration Course, held and organized by the Swiss Federal Research Institute for Forest, Snow and Landscape (WSL, Switzerland) in collaboration with The Pennsylvania State University (USA), the University of Florence (Italy), and Linnaea-ambiente (Florence, Italy), from 22 to 24 August 2001. The purpose of the training session was to verify the state of implementation and to improve the effectiveness of the pan-European program. The exercises were conducted at the WSL open-top chamber (OTC) research facility in southern Switzerland and on the Moggio Level II field-plot in northern Italy. The objective of the WSL exercise was the assessment and quantification of ozone induced injury on sensitive plant species as further validated under controlled OTC conditions. The aim of the Moggio exercise was to practise injury assessment under open-field conditions on branches of mature trees and on open-grown and therefore light-exposed vegetation found along the forest edge.

2. Methods

2.1. Field teams

Forty-eight national "reference teams" represented 21 countries (Table 1). Their expertise in injury assessment and their degree of experience was variable; the first UN/ECE ICP-Forests Intercalibration Course in 2000 was considered to be the start of the field assessment activities for observing and recording ozone-induced foliar injuries for most of the countries. The teams for the different exercises were selected on a national basis and number of members varied between the different exercises. Prior to the field exercises, a 1 day training session took place at the WSL headquarters for the illustration of the symptoms and the scoring methodologies.

2.2. WSL exercise

The first field exercise was conducted 23 August 2001 at the WSL open-top chamber research facility as had been established at the Lattecaldo Cantonal Forest Nursery within the sub-Alpine region of southern Switzerland in 1995 (Innes and Skelly, 1996; VanderHeyden et al., 2001). Assessment teams were required to assess ozone-induced visible injury on thirty-three, 3-year-old seedlings of 12 different forest shrub and tree species grown in 1 out of 4 randomly selected open-plots, exposed to ambient ozone concentrations. Species observed were: *Cornus alba* L., *Crateagus monogyna* Jacq., *Clematis vitalba* L., *Fraxinus excelsior* L., *Prunus avium* L., *Populus nigra* L., *Ribes alpinum* L., *Salix alba* L., *Tilia cordata* Mill., *Tilia platyphyllos* Scop., *Viburnum lantana* L. and *Viburnum opulus* L. Ozone-induced symptoms were distinguished from other mimicking symptoms caused by drought, insects, natural leaf discoloration, or pathogens by comparing open-grown symptomatic plants with plants grown within the charcoal-filtered OTCs; i.e. plants that had been exposed to 50% ambient ozone concentrations with no or far less amounts of ozone-induced injury. Furthermore, the use of the flow chart for the diagnosis of ozone-induced injury on broadleaved plant species (published in Innes et al., 2001) was recommended in order to improve the accuracy of the assessment of typical ozone injury. The participants were asked to rate (i)% symptomatic leaves per plant, and (ii) the average injury class in% according to the Horsfall–Barratt rating system (Horsfall and Barratt, 1945). The deviation from the reference values symptom assessments conducted by the 16 teams was calculated based on the last assessment from 19 August 2001 of a series of bi-weekly observations conducted by experienced WSL/PSU research assistants throughout the vegetation period.

Table 1
List of the 21 participating countries for second UN/ECE ICP-Forests Intercalibration Course on the Assessment of Ozone Injury on European Tree Species from 22–24 August 2001 at Lattecaldo (Canton Ticino, CH) and Moggio (Lombardy, I)

Participating countries
Austria
Belgium
Czech Republic
Denmark
Finland
France
Germany
Greece
Hungary
Italy
Latvia
Lithuania
Luxembourg
Norway
Portugal
Rumania
Slovak Republic
Spain
Switzerland
United Kingdom
USA

2.3. Moggio exercise

Three different exercises were carried out at the Moggio Level II plot: the first part included diagnosis and quantification of typical ozone visible injury on leaves of *Fagus sylvatica* L. mature trees and the recognition of mimicking symptoms caused by biotic (insects and pathogens) and abiotic (drought and frost) agents. Six branchlets were sampled from the outer part of the crown of selected beech trees, according to the outlines of the ICP-Forests Manual (UN ECE, 1994). The branchlets exhibited different types of symptoms. From each branchlet 20 sun-exposed leaves (selected starting from the tip of the branchlet) were considered for the assessment. Percentage of affected leaf area of the symptomatic leaves was assessed by applying a 5% increment scale from 0 to 100%. Leaves were not pre-evaluated by a reference team. Reference values were determined as the median of all observations for each leaf. In order to rate the teams' assessments, the following parameters were applied: (1) *concordance*, i.e. the simple evaluation of presence or absence of ozone symptoms on each leaf. Ninety percent of the observations had to agree with the reference value in order to reach the level of agreement set for concordance; (2) *score*, i.e. the difference between the assessment of the team and the reference value. Ninety percent of the observations had to agree with the reference value in the range of ±10% in order to reach the expected level of agreement (Data Quality Limits, DQLs). The DQLs adopted for Moggio exercises (1 and 2) are those suggested by Tallent-Halsell (1994) and adopted in Italy in the forest monitoring programs (see Ferretti et al., 1999).

The second part of the Moggio exercise included assessment of *amount* and *severity* of ozone symptoms on 10 sapling ash trees of 2–3 m in height. The evaluation was based on a USDA Forest Service scoring system (National Biomonitoring Ozone Network: http://na.fs.fed.us/spfo/fhm/ozonetrng/biozone.htm). Both parameters (*amount* = the percentage of leaflets with ozone symptoms relative to the total number of leaves on the plants and *severity* = the average percentage of leaf area of all injured leaflets) are classified according to 6 non-proportional classes: class 0 = None; class 1 = 1–5%; class 2 = 6–25%; class 3 = 26–50%; class 4 = 51–75%; class 5 > 65%. The reference injury class was determined for each tree as the median of the scoring from all teams. An assessment was considered to be accurate when at least 90% of the observations were within the range of ±1 severity class around the median.

The third part of the Moggio exercise included identification of symptoms that were typical of ozone-induced injuries on native forb and shrub species bordering a forest edge on a Level II Light Exposed Sampling Site (LESS; see http://www.gva.es/ceam/ICP-forests).

2.4. Statistics

Descriptive statistics were provided for all data-sets. The results of the WSL exercise were analyzed by species, whereas the results of Moggio exercises were analyzed by teams. In the latter, the level of disagreement between the reference values and the evaluations of each team was determined by means of the Wilcoxon sign test using the Statistica software package (Statistica 6.0, Statsoft Inc, Tulsa OK).

3. Results

3.1. WSL exercise

Results of the WSL exercise are shown in Tables 2–3 and Figs. 1–2. For the statistical analysis, only the plants rated for ozone-induced foliar injury were considered and non-rated plants were omitted as missing data. Reference values of the assessment conducted by the WSL/PSU group indicated that *V. lantana*, *F. excelsior* and *P. nigra* developed the most severe injury with respect to the percent of injured leaves per plant, as well as the average of the symptomatic leaf area (Fig. 1A and B). The same species show the highest deviation from the reference values after rating the assessment of the teams. The percentage of injured leaves per plant was underestimated by 20% for *V. lantana* and *P. nigra* but overestimated for *F. excelsior* by 10%. Except for the non-symptomatic species, *C. vitalba*, *T. cordata* and for the symptomatic species *T. platyphyllos*, the percentage of injured leaves per plant was underestimated for all species (Table 2, Fig. 2C).

Seedlings of *P. nigra* and *V. lantana* exhibited the highest average of symptomatic leaf area when applying the Horsfall–Barratt rating system for *P. serotina* Ehrh. As for the percentage of symptomatic leaves per plant, the injured leaf area of most species was underestimated except for the non-symptomatic species and *P. avium* (Table 3, Fig. 2D). Overall, the percentage of symptomatic leaves seemed to be more misjudged than the average leaf injury class per plant (Fig. 2A and B). In particular for *C. monogyna*, *P. avium* and *V. lantana*, the rating of percentage of injured leaves per plant seemed to cause more difficulties than the rating of the average affected leaf area (Fig. 2E and F).

3.2. Moggio exercises

Descriptive statistics of the visible injury assessment of canopy branchlets of *F. sylvatica* are shown in Table 4. The high values of the standard deviation and

Table 2
Descriptive statistics of the deviation (%) of the symptom assessment conducted by 16 teams from the actual % of leaves injured per plant based on the assessment of the WSL/PSU group for 12 species grown within open plots at the OTC research facility at Lattecaldo, Canton Ticino, southern Switzerland

Species	*n*	S.D.	CV%	Min.	Lower Quart.	Median	Upper Quart.	Max.
Cornus alba	32	11.26	−211	−35	−8	−5	−4	25
Crataegus monogyna	34	29.13	−202	−70	−34	−8	1	50
Clematis vitalba	29	12.66	294	0	0	0	0	60
Fraxinus excelsior	21	22.76	637	−60	0	10	10	40
Prunus avium	29	26.15	−339	−75	−25	−5	15	25
Populus nigra	32	20.36	−105	−60	−31	−20	0	20
Ribes alpinum	31	17.40	599	−17	−5	−1	5	60
Salix alba	37	16.13	−313	−40	−12	−5	0	40
Tilia cordata	34	24.63	209	0	0	0	5	80
Tilia platyphyllos	35	27.62	−546	−50	−24	0	8	65
Viburnum lantana	32	26.08	−163	−65	−40	−20	5	45
Viburnum opulus	32	21.33	−165	−50	−21	−8	0	40

Table 3
Descriptive statistics of the deviation of the average Horsfall–Barratt leaf injury class (%) per plant conducted by 16 teams from the actual average leaf injury class based on the assessment of the WSL/PSU group for 12 species grown within open plots at the OTC research facility at Lattecaldo, Canton Ticino, southern Switzerland

Species	*n*	S.D.	CV%	Min.	Lower Quart.	Median	Upper Quart.	Max.
Cornus alba	31	5.86	−138	−12	−6	−6	−1	13
Crataegus monogyna	32	10.82	−1237	−25	−6	−2	0	38
Clematis vitalba	29	1.27	334	0	0	0	0	6
Fraxinus excelsior	22	6.32	−221	−15	−6	−2	0	13
Prunus avium	27	12.70	317	−12	−3	0	8	44
Populus nigra	33	22.48	−199	−50	−25	−9	0	38
Ribes alpinum	32	9.61	699	−6	−3	−1	3	49
Salix alba	37	18.53	1594	−25	−12	−6	8	63
Tilia cordata	34	4.15	224	0	0	0	2	20
Tilia platyphyllos	35	11.49	−398	−19	−12	−6	0	25
Viburnum lantana	31	11.79	1740	−11	−6	−2	0	47
Viburnum opulus	33	16.41	−276	−88	−7	−4	0	15

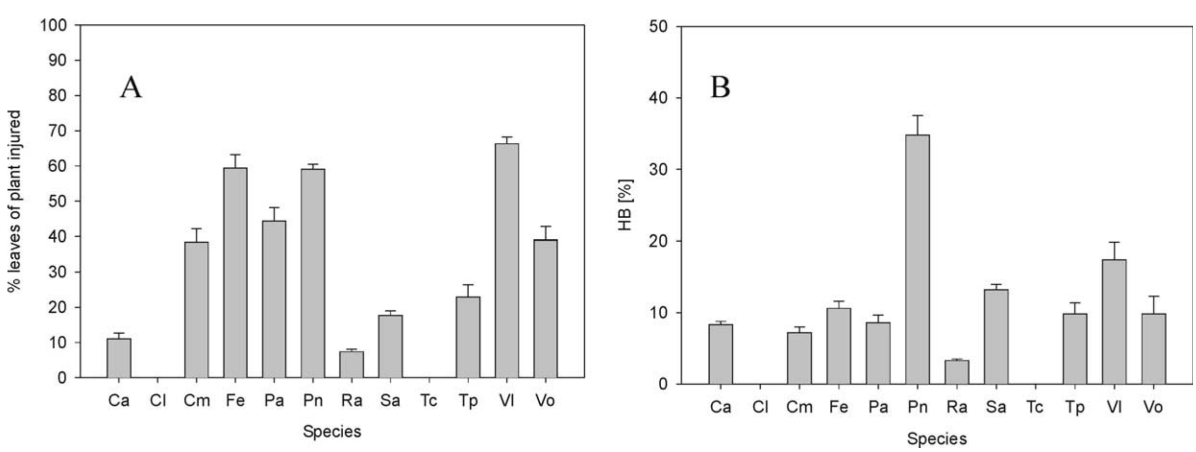

Fig. 1. Reference values for (A) percentage of injured leaves per plant and (B) average symptomatic leaf area according to the Horsfall–Barratt (HB) rating system of 3-year-old seedlings of *Cornus alba* (Ca), *Crataegus monogyna* (Cm), *Clematis vitalba* (Cl), *Fraxinus excelsior* (Fe), *Prunus avium* (Pa), *Populus nigra* (Pn), *Ribes alpinum* (Ra), *Salix alba* (Sa), *Tilia cordata* (Tc), *Tilia platyphyllos* (Tp), *Viburnum lantana* (Vl) and *Viburnum opulus* (Vo), based on the assessment conducted by the WSL/PSU group.

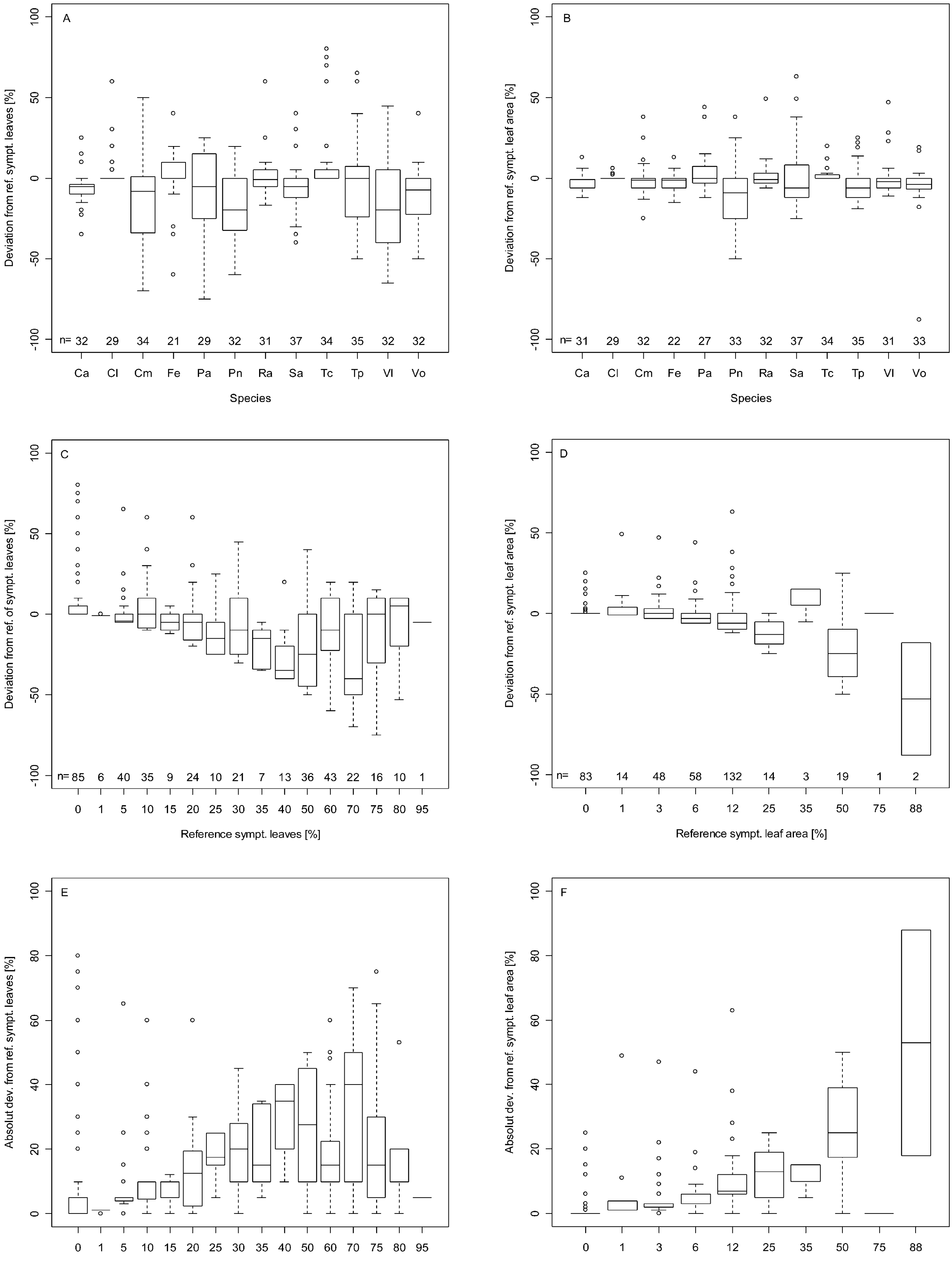

Fig. 2. Deviation and absolute deviation of the injury assessment conducted by 16 teams from the reference values based on the injury rating of the WSL/PSU group for the percentage of symptomatic leaves (A, C, E) and for the average percentage of symptomatic leaf area (B, D, F) on 3-year-old seedlings of *Cornus alba* (Ca), *Clematis vitalba* (Cl), *Crataegus monogyna* (Cm), *Fraxinus excelsior* (Fe), *Prunus avium* (Pa), *Populus nigra* (Pn), *Ribes alpinum* (Ra), *Salix alba* (Sa), *Tilia cordata* (Tc), *Tilia platyphyllos* (Tp), *Viburnum lantana* (Vl), and *Viburnum opulus* (Vo).

Table 4
Descriptive statistics of the visible symptom assessment on canopy leaves of *Fagus sylvatica* (CV = coefficient of variation, i.e. [SD/Mean]×100)

Branchlet	Injured leaf area (%)			Number of injured leaves				Number of discordant teams
	Median	Mean	SD	Median	Mean	SD	CV%	
1	8.21	10.55	7.25	5.00	6.39	5.20	81.44	6
2	0.00	2.38	3.70	0.00	1.35	2.96	218.55	1
3	7.78	11.23	10.84	9.00	8.71	5.27	60.46	6
4	15.45	23.25	17.02	16.00	12.25	7.80	63.69	9
5	15.00	15.79	13.55	12.00	10.95	7.80	71.26	15
6	8.75	11.83	12.86	1.00	3.27	4.88	149.28	6

Number of discordant teams: the teams which made an evaluation significantly different ($P > 0.05$) with respect to the reference value of injured leaf area (Wilcoxon sign test).

the coefficient of variance indicate a great dispersion of the data. The level of reliability of the data for each team, based on the comparison of the assessment and the respective reference value is shown in Fig. 3. For most branchlets the previously determined level of agreement for concordance and score was not reached when evaluating the teams' symptom assessment. Only for branchlet two (control sample without symptoms) could a relevant degree of agreement be shown with 13 teams reaching a level of agreement above 90%, and with only one team fully discordant, with respect to the reference values according to the Wilcoxon sign test. The results from the injury assessment of ozone-induced symptoms on *F. excelsior* sapling trees (amount and severity) are shown in Fig. 4. Most of the teams reached the reliability level. Four teams for *Severity* and two for *Amount* carried out an assessment significantly ($P < 0.05$) different from the reference values, according to the Wilcoxon sign test.

The results of the LESS exercise are shown in Table 5. Visible symptoms typical of ozone-induced injury were recognized mainly on *F. sylvatica*), *Laburnum alpinum*, *F. excelsior*), *Rubus* spp., and *Rosa canina*. Some teams recognized symptoms also on herbaceous species which are not considered for these statistics.

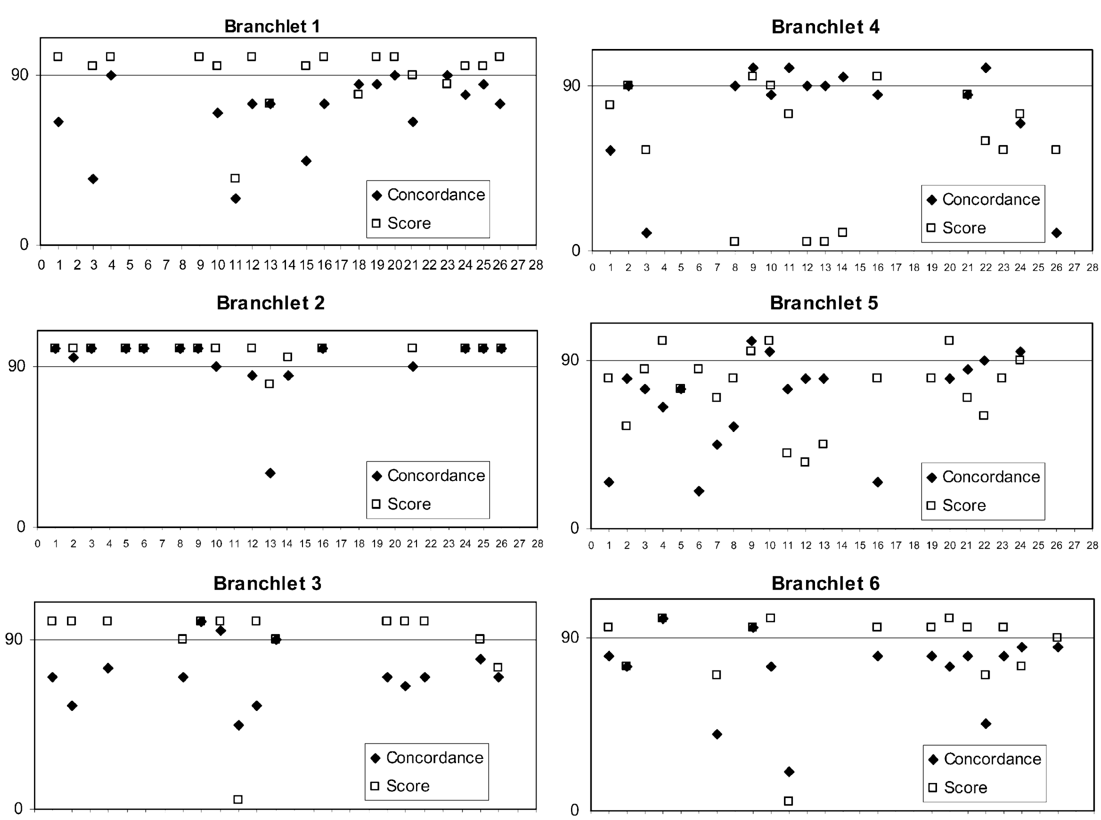

Fig. 3. Visible symptom assessment on leaves of six branchlets of selected *Fagus sylvatica* canopy trees. Level of agreement (0–100%) reached for each branchlet by each team (1–27).

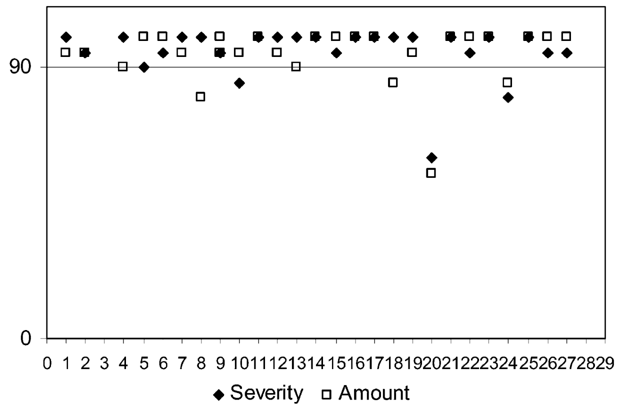

Fig. 4. Assessment of ozone-induced visible symptoms on 10 Fraxinus excelsior sapling trees. Level of agreement reached for each tree by each team (1–29).

Table 5
Results of the assessment of visible ozone-induced symptoms on herbaceous species on the Level II LESS plot

Species	Number of records Recorded	Doubtful
Fagus sylvatica	12	4
Laburnum alpinum	11	
Rubus spp.	9	
Fraxinus excelsior	8	
Rosa spp.	5	
Salix spp.	2	
Acer pseudoplatanus	1	
Petasites spp.	1	2
Centaurea spp.	1	1
Rumex spp.	1	
Potentilla spp.		1
Ribes spp.	1	
Geranium spp.		1
Stachys spp.		1
Gentiana spp.	1	

4. Discussion and conclusions

The results of the Lattecaldo exercises demonstrate that the percentage of visible injury per plant and leaf area is usually underestimated. In particular, the high-injury classes tend to be misjudged more often than the lower-injury classes. The standard deviation of the team rating from the reference values would increase with an increasing percentage of symptomatic leaves and leaf area (Fig. 2E and F). The correlation between absolute deviation and the reference values of the percentage of injured leaves shows an increasing misjudgment towards the range of 50–70% of injured leaves, whereas the deviation decreased with increasing injury classes when higher than 70% (Fig. 2G). For the average of injured leaf area, there is a consistent trend of increasing deviation from the reference values with increasing injury class (Fig. 2H).

The Horsfall–Barratt rating system with pictorial examples for each injury class per leaf area for *P. serotina* were used (Horsfall and Barratt, 1945). This may be one reason for the very accurate rating of *P. avium* among the teams. Within a range of ±5% the rating of the most accurate group (Romania) correlated with 88% with the reference values, followed by Lithuania and Latvia (76%), Slovak Republic (70%), Finland (67%), and Czech Republic (56%). The high score of agreement with the reference values not only shows that a trained person can obtain higher skills in the assessment of visible ozone-induced injury, but also that the applied Horsfall–Barratt rating system and the flowchart for the diagnosis of ozone-induced injury are valuable tools to achieve accurate results for symptom assessment on broadleaved species.

The results from the Moggio exercises showed a great deal of variability and uncertainty of symptoms on the *F. sylvatica* canopy trees. Difficulties became apparent when the teams were assessing and rating bronzing and stippling. Moreover, the assessment was not performed under ideal (bright) light conditions. Insufficient light conditions may have caused some confusion in distinguishing symptoms caused by biotic and/or abiotic factors other than symptoms induced by ozone. The Wilcoxon sign test (see Table 4) demonstrated that when the symptomatic leaf area was greater, the differences between the reference value and the assessment of each team increased. In other words, the results of the assessment were better when the symptoms were absent or minimal; a result that further confirms the Lattecaldo results as well. For the symptom assessment on the LESS, the different plant responses to ecological conditions such as edaphic conditions and inter- and intra-specific species competition for light and water, etc. were significant factors and as such they caused further disparities within the results the assessment. Many herbaceous species are potentially sensitive to ozone and may be used as bioindicators; however, the limited knowledge of the species-specific plant response to environmental site conditions such as light, soil moisture content and vapor pressure deficit, in combination with elevated ambient ozone concentrations, make an accurate risk assessment for symptom development difficult. The best results were obtained on *F. excelsior* when applying the USDA–Forest Service scoring system. In this case, the good performance was determined from the choice of the sample trees (all tree were similar for age and height) along with the clear unequivocal manifestation of the typical ozone-induced foliar symptoms.

Quality assurance (QA) is an important issue for long term environmental monitoring programs (Cline and Burkman, 1989; Millers et al., 1994). This is especially true for data originating from different countries and collected from several field crews having to be processed in a common database at a European level. The knowledge of the internal coherence and reliability of the data is a crucial issue for their use and interpretation as supporting information for environmental policies. Standard operating procedures (SOPs) are currently adopted in programs for monitoring ozone injury on vegetation. Nevertheless, when a program is in its early stages, and the relative activities are not fully implemented, many questions remain open and need to be correctly addressed. From this point of view the experience of the second UN/ECE ICP-Forests Intercalibration Course on the Assessment of Ozone Injury on European Tree Species can provide useful information to improve the part of the program concerning the recognition and evaluation of symptoms.

In contrast to the USDA–Forest Service program (where only a few selected species are considered as bioindicators), the European project considers all the species present in the plots (Main Tree Species in the Level II plots and Light Exposed Sampling Sites), a practice that may induce many problems. Because of the large floristic variability among the plots, there is an inherent lack of knowledge about the reproducibility (in controlled and semi-controlled conditions) of the symptoms for most of the species that are present, in particular for the herbaceous species. Moreover, the variability of symptoms in some important species (e.g. *F. sylvatica*) and the occurrence of mimicking factors are still not fully considered and solved. Other problems, not considered in this paper but relevant for the implementation of the program, concern the best time period in the season for the assessment and the selection of the sites to have a spatial representativeness.

We suggest that the European ozone monitoring program consider a close cooperation with the symptom validation activities conducted in OTCs or fumigation chambers (Skelly et al., 1999; VanderHeyden et al., 2001), as well as with studies investigating symptoms on a microscopical level (Günthard-Goerg et al., 2000; Gravano et al., 2003). We also strongly suggest involving the contributions of plant pathologists and entomologists in recognizing other biotic and abiotic causes of the observed foliar symptoms. We recommend selection of a limited number of species that are widespread across Europe, according to the different climatic and ecological regions, which show unambiguous and easily recognizable symptoms of ozone-induced foliar injury. The European system of Quality Assurance and Quality Control needs to be considerably improved and standardized following, for example, the USDA – Forest Service, Forest Health Monitoring, Ozone Bioindicator Survey experience with a specific training course before each late-summer assessment campaign. At present, we have reported encouraging results in the OTCs assessment (where surveyors had an immediate paragon with non-symptomatic trees, in the filtered chambers), but a substantial unhomogeneity when the teams operated under field conditions with a great variability of ecological conditions and symptoms manifestation. In order to improve training and calibration of the symptom rating, we suggest to realize a common photoguide incorporating digital picture analysis as an unbiased additional tool, in particular with respect to the increasing variety of symptom expression of different species (e.g. http://www.gva.es/ceam/ICP-forests and http://www.ozone.wsl.ch).

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